12th National and 4th International Biotechnology Congress of the Islamic Republic of Iran

دوازدهمین همایش ملی و چهارمین همایش بین المللی بیوتکنولوژی جمهوری اسلامی ایران

المؤتمر الدولي الرابع و المؤتمر الوطني الثاني عشر حول التقنيات الاحيائية
في الجمهورية الاسلامية الايرانية

خلاصه مقالات

Abstracts Book

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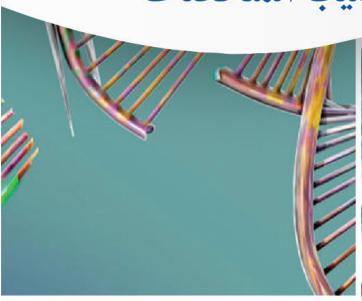

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انجمن بیوتکنولوژی جمهوری اسلامی ایران

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IN THE NAME OF GOD

4th International and 12th National Biotechnology Congress of the Islamic Republic of IRAN

August 22 – 24, 2021

www.biotechcongress.ir

Preface: The 4th International and the 12th National Biotechnology Congress of Iran

Biotechnology congress is held every biannually to acquaint researchers and domestic producers with the latest achievements of Iran and the world. This congress held by effort of the Biotechnology society of the Islamic Republic of Iran and the support of governmental and non-governmental institutions, private sector and international organizations, universities, Related scientific centers and other associations.

This great scientific event, by holding eleven national and three international successful congress and with the presence of domestic and foreign researchers has always been able to provide a forum for scientists from various fields of biotechnology and strengthen interdisciplinary approaches.

The other goal of the Biotechnology Congress has been encouraging students and professors to submit scientific papers and facilitate to publish their findings to promote science. In addition, in order to apply biotechnology to resolve the challenges and needs of society, it has presented biotechnology solutions to national and military policymakers, by inviting decision makers to discuss current issues in symposiums and specialized meetings and in the form of final resolutions.

The 4th International and the 12th National Biotechnology Congress of Iran, according to the routine of previous periods and based on the explained goals, held virtually on September 2021 with the slogan "1400 century: Biotechnology for food safety and health" under the Corona pandemic

A distinctive feature of this congress is the efforts of the Scientific Committee to explain the role and importance of biotechnology and its impact on the preparation of vaccines, identification and diagnosis of COVIID-19 disease and the entry of the biotechnology industry in the OTC market.

In addition, according to the impact and relevance of biotechnology in all trends, including industry, environment, agriculture, medical sciences and even humanities and law, therefore the scientific committee has been trying to select the congress branches based on the needs of the world and the country and define specialized committees.

The 4th International and the 12th National Biotechnology Congress of Iran in the keynote speeches section, hosted 23 international and national lectures and in the branch lectures section there were 24 speakers.

Generally, the purposes of the Fourth International Congress and the Twelfth National Biotechnology Congress are:

1. Introducing the latest research achievements on biotechnology
2. Creating interaction and scientific symposium between researchers & Biotechnology thinkers and Facilitate knowledge transfer and promote science
3. To draw the attention of the country's officials (politicians, legislators and members of the government) to the importance of biotechnology and Its stunning progress in recent years for the development and progress of the country
4. Introducing the power and experience of Biotechnology society of Iran with about half a century of experience as a non-governmental structure and consulting in policy-making, law-making and macro-biotechnology planning
5. Holding training workshops and specialized meetings

Head of the Congress message

In The Name Of the Almighty God

It is with pleasure to announce that the 4th International and 12th National Congress of the Iranian Biotechnology Society (IBS) is being held in year 2021. This biannual congress is coincided with COVID-19 pandemic and the big role that Biotechnology sector has played so far from diagnosis to vaccination to treatment for the health of earth's inhabitants.

During preparation for the congress, we sadly lost one of the greatest biotechnologist in Iran, naming Professor Behzad Ghareyazie in June 2021. Behzad was indeed one of the key player in the Iranian biotech arena particularly in the field of agricultural biotechnology. Professor Ghareyazie was the chair of the scientific committee of this congress and his loss due to COVID-19 is even more and great loss to us all at the Iranian Biotech Society; may God bless his soul and grant his family peace. We are going to announce creation of Professor Ghareyazie's Foundation during this congress.

We have gathered one of the most comprehensive program for participants to use and enjoy. We have several great scientists around the world from North America to Europe to Asia. Several key speakers are from prominent scientists from Iran. We have also participants from several countries. All of the keynote lectures are provided in English with several specialized lectures in the afternoon (Iranian time, -3.5 hours GMT). We have also several Workshops, specialized round table discussions (via internet), virtual poster presentations and announcing the winner of this year's Big Influencers in the Field of Biotechnology in Iran. This prize is given figures who have made big impacts in the field of biotechnology in Iran. 1.3 prize for those over 35 years of age and one to young under 35 years.

Last but not list we have to thank and acknowledge several key players of this congress. First of all late professor Behzad Ghareyazie (chair of the scientific committee), Dr. Azadeh Shooshtari, vice of the scientific committee, Dr. S Ebrahim Seifati, chair of the organizing committee, valued members of the scientific and organizing committees, board members of the IBS, members of the reviewing committees,

speakers and presenters at this congress and many more.

We hope that you will enjoy the content of this congress and wish you all a safe and happy life.

Sirous Zeinali
Head of the Congress
Head of the Iranian Biotechnology Society

The Memorial of Scientific Chair

In the Name of Allah

The Iranian Biotechnology Society has organized the Fourth International and the Twelfth National Congress of Biotechnology of the Islamic Republic of Iran in 2021. The Scientific chair of this congress was the famous Iranian scientist professor Behzad Ghareyazie until COVID-19 took this great scientist from us. A loss that is heavy and devastating to all us active in the field of biotechnology in Iran. Profes sor ghareyazie, founder of the Agricultural Biotechnology Research Institute of Iran (ABRII) and president of the Biosafety Society of Iran, passed away on June 6, 2021, due to COVID-19. He was an authoritative scientist who used his skills and abilities to promote genetic engineering and GMO both at the level of applied and theory. The production of transgenic rice resistant to Stemborer (Bt Rice) for the first time by this great scientist brought him worldwide fame and honor as well as international

recognition. These achievements has supporters and opponents in Iran as well. The late president of the Biosafety Society of Iran has consistently fought against pseudoscience and technophobia. Behzad Ghareyazie advocated the use of knowledge and skills of researchers and technologists for the progress and development worldwide. He was very active internationally as Iranian representatives in meetings and scientific gatherings as well as decision making processes. During his professional life, he used all legal tools for the advancement and development of technology in the country, especially in the field of biotechnology. He did not cease self-judging in the court of history. The Biotechnology Society of Iran hopes to be a worthy heir to his valuable heritage by honoring his memory and advancing his lofty goals for the development of technology in the country and worldwide. May his memory be cherished and his soul be in peace and his path be followed by those who have love of people and humanity as a whole.

The IBS is creating a foundation in his memory as Professor Behzad Ghareyazie Foundation. This foundation is aiming to promote molecular biotechnology particularly genetic engineering in different fields especially in agricultural biotechnology.

Scientific committee

The Executive Chair of Congress Message

In The Name of God

First of all, I thank God for providing me another chance to serve Iran's scientific society, especially scientists and scholars of Biotechnology. International Biotechnology Congress of Islamic Republic of Iran is one of the Iran's biggest scientific events and epitome of the diligent scientists', experts' and researchers' latest findings and achievements in this field. Regarding the significant speed of progress and its border crossing nature, Genetics and its related fields including biotechnology has privileged position among all sciences. Along these fast developments, appropriate presentation of new findings and providing grounds to transfer these findings to practical areas are of particular importance. My colleagues in congress executive department and I have considered this privileged position and did our best to provide best grounds and facilities which deserves honorable researchers of the field.

Despite receiving great enthusiasm and motives from all participants from Iran's biotechnology society and also foreign experts and researchers of the congress, we spent very hard and sad moments during preparation of the congress due to the loss of Professor Behzad Ghareyazie, our honorable teacher, expert and scientist. Although it has been a great loss for Iran's biotechnology society, his major heritage and teachings guides us i.e continuous effort to promote Iran and fighting with misunderstandings and regressionism in spite of frequent difficulties and troubles. We hope the success of the congress which is the result of his continuous efforts may keep his memory alive and we will be able to follow and continue his way in scientific competition of our country.

At the end, I appreciate all the honorable attendants for their effective role in holding this glorious congress. There have certainly been shortcomings and deficiencies for which we have to apologize. Certainly, by applying these valuable experiences, we will try to provide more competent services in future events.

We also appreciate the ceaseless efforts of all responsible personnel in holding the congress, especially my colleagues in different working groups of the congress executive committee who worked continually tolerating difficulties specially limita-

tions forced by the Covid-19 pandemic and hold the congress as it was programmed. I hope they have benefitted the congress and gained pleasant experiences and accept our apologizes for inconveniences.

S. Ebrahim Seifati
Executive Chair

The Congress Intl. Relations Director Message

In the name of God

"The 4th International and the 12th National Biotechnology Congress of Iran" would be held online on August 22-24, 2021 in Tehran, Iran.

This great scientific event by holding eleven national and three international successful conferences and with the presence of national and foreign researchers has always been able to provide a forum for scientists from various fields of biotechnology and strengthen interdisciplinary approaches. The Event aims to provide an international forum for scientists, researchers, students, and faculties to exchange ideas and broaden their knowledge.

My hope is for this congress to allow discussion that will continue to scientific cooperation between Iranian and non-Iranian researchers. Scientific cooperation with some neighboring countries of the Islamic Republic of Iran especially the Arab Republic of Iraq is necessary and will be instrumental in maintaining the quality of such congress in the future.

I would like to express my personal gratitude to all Iraqi university presidents, researchers, faculty, and staff, whose support and cooperation have provided significant contributions to the congress continuing growth and success. I am very grateful to the congress organizing committee members especially the congress head Prof. Dr. Sirous Zeinali, as well as our colleagues for all their preparation and hard work to ensure a stimulating and enjoyable experience for us all.

Also, I am thankful to the numerous volunteers from Iraq, without whose generous contributions to this congress would not have set a record number of presentations and number of participants.

I hope you will enjoy the content, old friendships, make new friends, get new ideas, and above all, have a good time.

Dr. Mohammad Golbashy
Congress Intl. Relations Director

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Congress Agenda

12th National and 4th International Biotechnology
Congress of the Islamic Republic of Iran

دوازدهمین همایش ملی و چهارمین همایش بین المللی
بیوتکنولوژی جمهوری اسلامی ایران

Congress Agenda

4th International and 12th National Biotechnology Congress of the Islamic Republic of Iran

August 22-24, 2021

Time / Date	08:00 – 10:00	10:00 – 13:00	13:00 – 14:00	14:00 – 16:00	16:00 – 17:30	17:30 – 19:30
August 22 , 2021	Opening Ceremony	Keynote Speeches	Lunch break	Pharmaceutical Biotechnology Systems Biology and Bioinformatics Genetic Engineering, Genome Editing, Biosafety Environmental Biotechnology Animal Biotechnology Molecular Markers	Poster Hall visiting Keynote Speeches	Panel discussion on Digital Sequence Information **Director of the meeting:** Ms. Samira Kahak Panel discussion on increasing the role of biotechnology in the national economy **Director of the meeting:** Dr. Amir Meimandipour Panel discussion on biosafety **Director of the meeting:** Dr. Reza Aghanouri
August 23 , 2021	Keynote Speeches	Keynote Speeches	Lunch break	Microbial Biotechnology Genomics, Proteomics, Metabolomics Industrial Biotechnology Coronavirus & Biotechnology	Poster Hall visiting Keynote Speeches General Assembly of the Biotechnology Society	Panel discussion on The situation of genetic engineering in agriculture, self-reliance and the global economy **Director of the meeting:** Dr. Mahmoud Tavalaei International Roundtable on Covid-19 and Biotechnology **Director of the meeting:** Prof. Hamid Mobasheri Panel discussion on How to build our biotech startup **Director of the meeting:** Dr. Heidi Bagheri
August 24 , 2021	Keynote Speeches	Keynote Speeches	Lunch break	Synthetic Biology Marine Biotechnology Biotech Commercialization Law and Bioethics Tissue Culture Pharmaceutical Biotechnology Microbial Biotechnology	Poster Hall visiting Keynote Speeches	**Closing Ceremony**

Main Hall https://b2n.ir/biotechcong2021
Room 1 https://b2n.ir/biotechcong2021-01
Room 2 https://b2n.ir/biotechcong2021-02
Room 3 https://b2n.ir/biotechcong2021-03
Room 4 https://b2n.ir/biotechcong2021-04
Room 5 https://b2n.ir/biotechcong2021-05

Main Hall - August 22

Opening Ceremony	
08:00 – 08:15	**Quran recitation and national anthem**
08:15 – 09:00	**Speech by Mr. Ayatollah Ali Akbar Rashad**
09:00 – 09:15	**Speech by Dr. Sirous Zeinali (Conference Head)**
09:15 – 09:35	**Commemoration of Dr. Behzad Gharehyazie**
09:35 – 10:00	**Biotechnology Awarding**
Keynote Speeches ***Panelist:*** *Dr. Babak Nakhoda*	
10:10 – 10:50	**Grain Dispersal Evolution In Cereals** Dr. Mohammad Pourkheirandish (University of Melbourne- Australia)
10:50 – 11:30	**Genomics-Assisted Breeding In Field Crops** Susanne Dreisigacker (International Maize and Wheat Improvement Center- Mexico)
11:30 – 12:10	**Two Decades Of Research For Introducing Transgenic Sugar Beet Plants Resistant To Rhizomania Disease Based On Gene Silencing Against Beet Necrotic Yellow Vein Virus** Dr. M.A. Malboobi (National Institute for Genetic Engineering and Biotechnology- Iran)
12:10 – 12:50	**Biocatalytic Based Synthesis Of Chemical Space-An Approach For Cost – Effective Drug Discovery** Prof. Dr. M. Iqbal Choudhary (University of Karachi- Pakistan)
16:05 – 16:45	**Imaging Of Immune Responses** Dr. Mohammad Rashidian (Harvard Medical School – United States)
16:45 – 17:25	**Cold active enzymes and their potential industrial applications** Prof. Krss Rao (Acharya Nagarjuna University - Guntur, India)

Room 1 - *August 22*

Pharmaceutical Biotechnology
Panelist: *Dr. Eskandar Omidinia*

14:00 – 14:15	**Production of Nanobody-Based Anti-PSMA CAR T Cells by Lentiviral Vectors** Mahdie Jafari
14:15 – 14:30	**Apoptotic and Antiproliferative Effect of Rutin Nanoencapsulated in Human Breast Cancer Cells MCF-7** Akram Firouzi-Amandi
14:30 – 14:45	**Generation and Characterization of a Camelid Diabody against Placental Growth Factor for Targeting Angiogenesis** Abolfazl Nikooharf
14:45 – 15:00	**HTERT-molecular Targeted Therapy of Ovarian Cancer Cells via Folate-Functionalized PLGA Nanoparticles Co-loaded with MNPs/siRNA/wortmannin** Somayyeh Ghareghomi
15:00 – 15:15	**Expression and Intein Mediated Purification of P28-IL24 and P28-M4 Fusion Proteins for Targeted Cancer Therapy** Elahe Khodamoradi
15:15 – 15:30	**Doxorubicin-loaded Chitosan- Montmorillonite- Carbon Quantum Dots Hydrogel Nanocomposite for the Treatment of Breast Cancer** Fatemeh Yazdian
15:30 – 15:45	**Physicochemical Characterization of MiRGD peptide Nanocarrier for Delivering Anti-angiogenic sFLT01 gene to Retinal Pigment Epithelial Cells** Somayeh Piroozmand
15:45 – 16:00	**Preparation, Evaluation and In vivo Assessment of Nanofibers Containing Herbal Extracts with Antibacterial agents to Prevent and Control Bacterial Infection with Staphylococcus aureus** Mahboubeh kabiri
17:30 – 19:30	**Panel discussion on Digital Sequence Information (DSI)** Ms. Samira Kahak

Room 2 - *August 22*

Systems Biology and Bioinformatics *by: Dr. Zarrin Minuchehr*
Panelists: *Dr. Vahid Shariati, Dr. Najaf Allahyari Fard, Dr. Hossein Safarpour*

14:00 – 14:30	**Single-cell Transcriptomics in Cancer: Computational Challenges and Opportunities** Dr. Hossein Safarpour
14:30 – 14:45	**Identification of Drought Tolerance Genomic Regions in Foxtail Millet** Fateme Ioni
14:45 – 15:00	**Meta-Analysis of Microarray Data to Iidentify Drought Stress Responsive Genes in Maize** Narges Safari
15:00 – 15:15	**High Expression of CDK1 and NDC80 Predicts Poor Prognosis of Bladder Cancer** **Seyedeh Sajedeh Mousavi**
15:15 – 15:30	**In silico Analysis of the Interaction of cLF.arm Engineered Recombinant Peptide with LPXTG Surface Protein in *Corynebacterium bovis* Bacteria of Bovine mastitiss** Romina Mirzadi
15:30 – 15:45	**NSP1 Inhibition Effects on Human 40s Ribosomes in Comparison to Intermediate Carrier Animals** Afagh Bapirzadeh
15:45 – 16:00	**A Bioinformatics Screen Uncovers Involving Pathways of Transcribed Ultraconserved Regions (T-UCRs) in Gastric Cancer** Anis Khalafiyan
16:00 – 16:15	**Genomine; Cloud-based Platform for NGS Data Analysis** Fahimeh palizban
17:30 – 19:30	**Panel discussion on increasing the role of biotechnology in the national economy** Dr. Amir Meimandipour

Room 3 - August 22

Genetic Engineering, Genome Editing, Biosafety *by: Dr. Kasra Esfahani, Dr. Ali Hatef Salmanian, Dr. Ebrahim Seifati, Dr. Mokhtar Jalali Javaran*
Panelists: *Dr. Kasra Esfahani, Dr. Ali Hatef Salmanian, Dr. Ebrahim Seifati, Dr. Mokhtar Jalali Javaran, Dr. Masoud Tohidfar*

14:00 – 14:30	**Do TransgenicFood Products Have a Higher Nutritional Value?** Dr. Kasra Esfahani
14:30 – 14:45	**Genetic Engineering of Rice to Change Root Structure Improve Nutrient Uptake, Improve Yield and Drought Tolerance** Dr. Motahhareh Mohsenpour
14:55 – 15:20	**The Editing of FAD2-1 gene using CRISPR/Cas9 system to Increase Oleic Acid Content in Safflower Plants** Amin Neycee
15:20 – 15:40	**Role of Hydroxymethylglutaryl-coenzyme A (HMG-CoA) Reductase 1 in Nodule Development of Soybean** Dr. Ali Izadi Darbandi
17:30 – 19:30	**Panel discussion on biosafety** Director of the meeting: Dr. Reza Aghanouri

Room 4 - August 22

Environmental Biotechnology *by: Dr. Nayer Azam Khoshkholghsima*
Panelists: *Dr. Seyed Abbas Shojaosadati, Dr. Seyed Morteza Zamir, Dr. Habibollah Younesi, Dr. Ali Ebadi*

14:00 – 14:30	**Two-liquid Phase Trickling Bioreactors for Sustainable Waste-gas Treatment** Dr. Seyed Morteza Zamir
14:30 – 15:00	**Microbial Fuel Cells: Into the Next Century** Dr. Habibollah Younesi

15:00 – 15:20	**Molecular Identification of Some Isolates of Fungi Isolated from Al-Barakia Wastewater Treatment Plant** Dr. Nihad Habeeb Mutlag
15:20 – 15:40	**New Strategy to Increase Oil Biodegradation Efficiency by Selecting Isolates with Diverse Functionality and No Antagonistic Interactions for Bacterial Consortia** Ali Ebadi
15:40 – 16:00	**Comparison of Polyhydroxybutyrate production with external potential application at Cathode and Anode poles compared to Control(Blank) with inexpensive whey substrate** Fariba Gholami

Room 5 - *August 22*

Animal Biotechnology *by: Dr. Fazlullah Afraz* ***Panelist:*** *Dr. Mohammad Hossein Banabazi*	
14:00 – 14:30	**Effect of Caralluma tuberculata on Regulation of Genes Related to Carbohydrate Metabolism** **"** Prof. Dr. Nawazish-i-Husain Syed (University of the Punjab, Pakistan)
14:30 – 15:00	**Prevalence of Sarcocystis Infection in Slaughtered Sheep and Goats in Duhok Province/ Iraq** Prof. Dr. Shivan Nawzad Hussein (University of Duhok, -Iraq)
15:00 – 15:30	**Effects of New kisspeptin Neuropeptide on Gonadotropin Secretion in Goldfish (Carassius auratus)** AbdolMajid Valipoor
15:35 – 16:00	**Transcriptome analysis of the laying hen magnum reveals differentially expressed genes involved in egg formation** Zahra Pezeshkian

Keynote Speeches
Panelist: *Dr. Manouchehr Vossoughi*

08:30 – 09:10	**The Biotechnology In The Era Of COVID-19 Pandemic** Dr. Mahdi Taghadosi (Kermanshah University of Medical Sciences, Kermanshah, Iran)
09:10 – 09:50	**Developing And Rolling Out Diagnostic Tests For Emerging Infectious Diseases Laboratories Network: The Iranian Laboratory Network For COVID-19** Dr. Kayhan Azadmanesh (Pasteur Institute of Iran, Iran)
09:50 – 10:30	**Towards The Generation Of A GMP Compliant Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial Cells For The Treatment Of Age-Related Macular Degeneration: The First Pre-Clinical Study For Safety And Efficacy In Iran** Dr. Hossein Baharvand (Royan institute, Iran)
10:40 – 11:20	**The Role Of Medical Biotechnology In Health Promotion In Iran** Dr. Fereidoun Mahboudi (Pasteur Institute of Iran- Iran)
11:20 – 12:00	**National Programs For Prevention Of Genetic Disorders In Iran, 20 Plus Years Of Practice** Dr. Sirous Zeinali (Pasteur Institute of Iran- Iran)
12:00 – 12:40	**Predicting Anticancer Drug Response Using Machine Learning Approaches** Dr.Changiz Eslahchi (Shahid Beheshti University – Iran)
16:05 – 16:45	**Technology Transfer', 'Knowledge Exchange' And 'Commercialization'. A History And Lessons Learned.** Dr.John Fraser (Retired from Florida State University – United States)
16:45 – 17:25	**An Optimal Amplicon Based Genotyping By Sequencing Panel In Sunflower** Dr.Farhad Ghavam (Chief Science Officer (CSO) at Eurofins Bio Diagnostics Inc. - United States)

Room 1 - *August 23*

Molecular Markers *by: Dr. Ghasem Mohammadi-Nejad*
Panelists: *Dr. Mehrshad Zeinalabedini, Dr. Hossein Ramshini, Dr. Ghasem Mohammadi-Nejad*

14:00 – 14:30	**Marker Assisted Selection for Developing Melon Cultivars Resistant to Virus and Aphids** Dariush Tafrihi
14:30 – 15:00	**Gene Expression Analysis of Some Key Flavonoids Synthesis Genes on Vitis *vinifera* cv. Syrah under Regulated Deficit Irrigation Condition** Hamideh Arab Bafrani
15:00 – 15:30	**Diversity of AvrStb6 Affecting Gene for Gene Relation Between Zymoseptoria tritici and Wheat** Leila Ebrahimi
15:30 – 16:00	**Identification and Isolation of Stress related Transcripts in Olive and Probable Mechanisms of Stress in this Plant** Fateme Razeghi Jahromi
17:30 – 19:30	**Panel discussion on The situation of genetic engineering in agriculture, self-reliance and the global economy** Dr. Mahmoud Tavalaei

Room 2 - *August 23*

Microbial Biotechnology *by: Dr. Fatemeh Tabandeh*
Panelists: *Dr, Seyed Abbas Shojaosadati, Dr. Saeed Mirdamadi, Dr. Parvin Shariati*

14:00 – 14:25	**In silico Investigation of Alpha, Beta and Gamma Carbonic Anhydrases as Catalysts of CO2 bio Mineralization Processes** Dr. Ozlem Tastan Bishop
14:25 – 14:35	**Production and Optimization of Pullulan from Sugarcane Residues by Yeast-like Fungus *Aureobasidium pullulans*** Raziye esfandiar

14:35 – 14:50	**Iogas Production by using Codigestion Method of Sugarcane Bagasse with Marine Algae** Marjan Mollaaghaei Kollai
14:50 – 15:00	**Investigation of Bio-cement Performance for Granular Soils in Order to Optimize the Values Used in Road Construction Operations** Amin Maleka

Panelists: *Dr. Zahra Khodabandeh, Dr. Kianoush Khosravi-Darani, Dr. Kianoush Khosravi-Darani*

15:10 – 15:20	**Probiotics, Post-biotics and their Derivatives: Opportunities and Challenges** Dr. Saeed Mirdamadi
15:20 – 15:50	**Rapid Detection and Quantification of Bacteriophage Contamination of Lactic acid Bacteria in Yogurt** Seyede Mehri Javadi
15:30 – 15:40	**Factors Affecting the Growth of Lactobacillus Plantarum on Non-alcoholic Malt Waste in Solid State Fermentation** Nooshin Baz Sefidpar
15:40 – 15:50	**Optimization of Coating Formulation to Increase the Survival of Probiotic Bacteria During One-week Storage in Room Conditions** Parisa Khodadadi
17:30 – 19:30	**International Roundtable on Covid-19 and Biotechnology** Prof. Hamid Mobasheri

Room 3 *- August 23*

Genomics, Metabolomics and Proteomics *by: Dr, Mohammad Reza Ghaffari*
Panelists: *Dr. Parviz Moradi, Dr. Mehrshad Zeinalabedini, Dr. Seyed Abolghasem Mohammadi, Dr. khalil Zaynali Nezhad*

14:00 – 14:20	**Application of Genetic and Genomic Data in Applied Research of Halophyte Plants: Salicornia, Exploration of a Halophyte Plant against Climate Changes** Dr. Mehrshad Zeinalabedini

14:25 – 14:45	**Epigenome Engineering for Boosting Crop Production** Dr. Ali Mohammad Banaei-Moghaddam
14:50 – 15:10	**An Overview on Haploid Inducer Lines Production via Centromere Engineering: History and Perspective** Dr. Raheleh Karimi-Ashtiyani
15:15 – 15:35	**Metabolically Engineered Rice Biomass and Grain using Genes associated with Lipid Pathway Show High Level of Oil Content** Dr. Ali Izadi Darbandi
15:35 – 16:00	**Metabolomic Profiling of Resistance and Susceptible Peanut (*Arachis hypogaea* L.) Genotypes in Response to *Cercospora arachidicola* infection** Dr. Zahra Ghorbanzadeh
16:00 – 17:30	**General Assembly of the Biotechnology Society**

Room 4 - August 23

Industrial Biotechnology *by: Dr. Manouchehr Vossoughi*
Panelist: *Dr. Manouchehr Vossoughi*

14:00 – 14:45	**An Introduction to Full Scale Application of Aerobic Granular Sludge Processes** Dr. Sirous Ebrahimi
14:45 – 15:25	**Application of Nanobiotechnology for Defeating Antibiotic Resistant Bacteria** Dr. Ghasem Najafpour
15:25 – 15:45	**Isolation and Molecular Identification of Endophytic Fungi in Saffron (*Crocus sativus L.*)** Farzaneh Taheri
15:45 – 16:00	**Optimization of Xanthan Gum Production by Sugarcane Molasses Broth using Plackett-Burman Design** Abbas jafari jaid
17:30 – 19:30	**Panel discussion on How to build our biotech startup**

Room 5 - *August 23*

Coronavirus and Biotechnology *by: Dr. Majid Tebianian*
Panelists: *Dr. Kayhan Azadmanesh, Dr. Morteza Taghizadeh, Dr. Reza Jafari, Dr. Mehdi Shabani, Dr. Majid Tebianian*

14:00 – 14:20	**the Review of Recombinant Razi COVO Pars Vaccine Production** Dr. Mojtaba Nofeli
14:20 – 14:30	**Investigating the Interaction of Antimicrobial Peptides, Specifically Probiotic Bacteriocins, on Spike Proteins of SARS-CoV2 Virus** Bahar Saadaie Jahromi
14:30 – 14:40	**Microfluidic System for Detecting COVID 19 Virus using Nano Molecularly Imprinted Polymers** Saman Zafarian
14:40 – 14:50	**Effect of hsa-miR-4735 on nsp2 gene in SARS-CoV-2 virus** Shokoofeh Ghaemi
14:50 – 15:00	**Molecular Cloning and Sequences Analysis of the Receptor-Binding Domain (RBD) and S1/S2 protease Cleavage Site of SARS-CoV-2** Seyed Amir Hossein Mohammadzadeh Hosseini Moghr
15:00 – 15:10	**Molecular Basis of Angiotensin Converting Enzyme-2 Receptors in Severe Iraqi Patients with Covid-19 Pandemic and its Relations with Smoking** Fadhil Jawad Al-Tu'ma
15:10 – 15:20	**Investigation of Differences in the Sequence of the Corona virus (COVID-19) Spike (s) gene in Iran and Comparison of a Sample of the Iranian Spike (s) gene Sequence with other Countries** Milad Toloi
15:20 – 15:30	**Secondary Metabolites of Myxobacteria as a Source for anti-SARS-CoV-2 Drug Discovery** Romina Hajihassani
15:30 – 15:40	**CORONAVIRUS-2 "CELL CYCLE AND POSSIBLE THERAPEUTIC TARGETS"** Dr. Falah Abbas Mohammed Salih
15:40 – 15:50	**Quantitative Structure–activity Relationship Study of some papain-like Protease (PLpro) Inhibitors** Elham zamani

Main Hall - August 24

Keynote Speeches	
Panelist: *Dr. MohammadAli Malboobi*	
08:30 – 09:10	**Applications Of Mycoprotein In Immunity (Food And Humans)** Dr.Kianoush Khosravi Darani
09:10 – 09:50	**Process Development For Caffeine Degradation In Effluents & Food Products** Sathyanarayana n. Gummad (Department of Biotechnology- India)
09:50 – 10:30	**Non-Ionizing Radiation As A Tool For Modern Biotechnology** Prof. Sinerik Ayrapetyan (UNESCO/UNITWIN Network- Armenia)
10:40 – 11:20	**Marine Bacterium As Potential Protective Agents For Oyster Aquaculture** Dr. Jamshid Amiri Moghaddame (E.V.Hans-Knöll-Institut- Germany)
11:20 – 12:00	**Classification And Regression Trees For Tissue Culture Data Analysis** Dr. Melekşen Akın (Horticulture at Iğdır University – Turkey)
12:00 – 12:40	**Resource Recovery From Wastes Using Biotechnology** Dr.Mohammad J. Taherzadeh (University of Borås- Sweden)
16:05 – 16:45	**The Challenges Of Creating A Tech Transfer Ecosystem In An Emerging Economy** Ashley J. Stevens (Past President of University Technology Managers - United States)
16:45 – 17:25	**Non-Ionizing Radiation-Induced Stimulation Of Bull Sperm Motility** Dr. Naira Baghdasaryan (UNESCO/UNITWIN Network- Armenia)
Closing Ceremony	
17:15 – 17:35	**Lecture by Dr. Hamidiyeh**
17:35 – 17:55	**Speech by Executive Director**
17:55 – 18:20	**Appreciation of the best article, speaker and poster**
18:20 – 18:30	**Read the conference statement**
18:30 – 19:00	**Speech by Conference Chair**

Room 1 - August 24

Synthetic Biology *by: Dr. Safa-ali Fatemi* ***Panelists:*** *Dr. Sareh Arjmand, Dr. Najaf Allahyarifard, Dr. Tahmineh Lohrasbi*	
14:00 – 14:20	**Machine Learning Applications to Design and Optimize Synthetic Biological Systems** Dr. Amir Pandi
14:20 – 14:35	**Design of a Nucleic Acid Circuit for Intelligent Detection of Biomarkers using DNA Strand Displacement Process** Fatemeh Jafari-Mohammadabadi
14:35 – 14:50	**Potential of a Novel Metagenome-derived Laccase with Stable Performance in Biorefinery of Lignocellulosic Biomass** Dr. Shohreh Ariaee Nejad
14:50 – 15:05	**Evaluation of the Ligand Independent Activation of Anti CD19 SynNotch Receptor by Dual Luciferase Assay** Yasaman Asaadi
Marine Biotechnology *by: Dr. Mohammad Pourkazemi, Dr. Mohammad Reza Kalbassi, Dr. Saeed Keyvanshokooh* ***Panelists:*** *Dr. Sareh Arjmand, Dr. Najaf Allahyarifard, Dr. Tahmineh Lohrasbi*	
15:10 – 15:20	**Marine Biotechnology in Iran, a high priority issue for investment and strong consideration sector for extension** Dr. Mohammad Pourkazemi
15:20 – 15:40	**Marine Toxins and their Bioactivity: How TTX can Control Tumor Growth** Dr. Saber Khodabandeh
15:40 – 15:55	**Optimization of flow cytometry method to identify chromosom Manipulation Individuals in Rainbow Trout (*Oncorhynchus mykiss*)** Hajar Sadat Tabatabaie Pozveh
15:55 – 16:15	**Single Nucleotide Polymorphism Development Markers Involved in Early Sexual Maturation from Transcriptomes of Rainbow Trout (*Onchorhynchus mykis*)** Dr. Sajad Nazari
16:15 – 16:30	**Sterilization of Sterlet (Acipenser ruthenus) by heat shock and microinjection of Dead-End(DND) gene knockdown agent(** Mahsa Borhani

16:30 – 16:45	**Transcription of Bcl-2 as an Anti-apoptotic Gene in Two Species of Coral Reefs, Sensitive and Tolerant to Thermal Stress** Pegah Javid

Room 2 *- August 24*

Biotech Commercialization *by: Dr. Amir Meimand* ***Panelist:***	
14:00 – 14:30	**Venture Building and Open Innovation Impact on Biotechnology Business Development and Investment** Mohammadreza Hemmati Mojarrab
Bioethics & Bioethics Law *by: Dr. Mahmoud Hekmatnia* ***Panelist:***	
14:30 – 15:00	**Biotech & Religion** Ayatollah Alidost
14:30 – 15:00	**The Review of Iranian Law and Parliamentary Plan** Dr. Mahdi Moalla
15:00 – 15:30	**Fundamental Rules in Economic Law** Dr. Mahmoud Hekmatnia

Room 3 *- August 24*

Tissue Culture *by: Dr. Pejman Azadi* ***Panelists:*** *Dr. Ahmad Moini, Dr. Mehran Enayati Shariatpanahi, Dr. Kourosh Vahdati, Dr. Abbas Saidi*	
14:00 – 14:25	**Application of Reverse Breeding and Haploidy in Production of F1 hybrids** Dr. Enayat Shariat Panahi

14:30 – 14:50	**Transformation of DRO1 and CKX4 genes in Order to Modify Rice Root Architecture and Improved Drought Tolerance in Rice** Zahra Ghorbanzadeh
14:55 – 15:10	**The Effect of Different Concentrations of Polyethylene Glycol and Sorbitol on the Production of Potato (Solanum tuberosum) Microtubules In vitro** Banafsheh Jamshidi
15:15 – 15:30	**Efficient in vitro plant Regeneration via Indirect Somatic Embryogenesis from Petal Cultures of *Pomegranate cv.* Ganesh in Double Phase Media** Mehrnaz Falaki Khalilsaraie
15:35 – 15:50	**Graphene Nanoparticles: A Biodegradable and Efficient nano-additive for Increasing of Asexual Embryogenesis of Date Palm** Sadaf Abedi

Room 4 *- August 24*

Pharmaceutical Biotechnology *by: Dr. Eskandar Omidinia* ***Panelist:***	
14:00 – 14:15	**Designing of High-Resolution Melting Technique with Bioinformatics Analysis of Different Gene Regions for Separating Different Types of *Toxoplasma gondii*** Reza Fotouhi-Ardakani
14:15 – 14:30	**Application of gene Therapy with Viral Vectors in Infectious and Noninfectious Diseases** Sepideh Saeb
14:30 – 14:45	**Fusion of LTB (L) Bio-adjuvant to Newcastle Virus Hemagglutinin (HN): A new Chimeric Vaccine Candidate** Mehregan Rahmani
14:45 – 15:00	**Designing of sgRNA and donor DNA for Disruption of PUF5 gene in Leishmania major through CRISPR/Cas9 system** Elaheh Davarpanah

15:00 – 15:15	**In-silico Design by Semi-rational Method to Improve Soybean Peroxidase Enzyme Functionality** Alireza Zargarani
15:15 – 15:30	**Fabrication of Hepatic cell-sheet using Decellularized Extracellular Matrix and Thermoresponsive Polymer** Maryam Asadi
15:30 – 15:45	**Design, Fabrication and Characterization of 3D Composite scaffolds of Amniotic Membrane/Hyaluronic Acid/Nano-hydroxy Apatite for application in bone/cartilage interface** Mahboubeh kabiri

Room 5 *- August 24*

Microbial Biotechnology *by: Dr. Fatemeh Tabandeh*
Panelists*: Dr. Iran Alemzadeh, Dr. Naeimeh Enayatizamir, Dr. Mohammad Pazoki*

14:00 – 14:10	**Evaluation of the Activity of the New Enzyme Benzoyl formate Pseudomonas aeruginosa Decarboxylase Expressed in E.coli** Shahab Areze
14:10 – 14:20	**Co-production of Cellulase-xylanase Enzymes by Different Species of Trichoderma fungi using Corn Bran Waste of High Fructose Corn Syrup Factories** Hamed Askari
14:20 – 14:30	**Semi-industrial Optimization of L-Asparaginase Production from Candida utilis** MohammadReza Lesani
14:30 – 14:40	**Development of an Inexpensive Culture Medium for Over-production of Bacterial Cellulose-based on the Response surface Statistical Design Method** Maryam Nasr Esfahani
14:40 – 14:50	**Question and Answer**

Panelists: *Dr. Fatemeh Yazdian, Dr. Mohammad Reza Zolfaghari, Dr. Gholamreza Ahmadian*	
14:50 – 15:10	**Simulation of Microbial Process using Molecular Dynamics** Dr. Fatemeh Yazdian
15:10 – 15:20	**Synthesis and Characterization of GAAMCs Nanobiocomposite Hydrogel: Antibacterial Activity** Fatemeh Karchoubi
15:20 – 15:30	**Application of Cold Atmospheric Plasma for expression improvement of Green Fluorescent Protein in Recombinant Yeast** Zeinab Kabarkouhi
15:30 – 15:40	**Production of Biodegradable Polylactic acid Film Containing Lippia Citriodora Nanoemulsion to Investigate the Biological Properties** Mahsa Hojatoleslami
15:40 – 15:50	**Antifungal Activity of the Essential Oils of Thymus eriocalyx and Thymus daenensis Species on Two Phytopathogenic fungi** Mehdi Mardi
15:50 – 16:00	**Question and Answer**

Keynote Speakers Abstracts

12th National and 4th International Biotechnology
Congress of the Islamic Republic of Iran

دوازدهمین همایش ملی و چهارمین همایش بین المللی
بیوتکنولوژی جمهوری اسلامی ایران

Dr. Mohammad Pourkheirandish
(University of Melbourne - Australia)

Grain Dispersal Evolution in Cereals

Cereal inflorescences are developmentally diverse. Inflorescence branching is dependent on the developmental fate of the axillary apical meristem. The final architecture of the inflorescence is the product of the number of meristems and their arrangement. It is believed that the panicle is a primitive form of inflorescence, from which the spike evolved. Wheat and barley have spike inflorescences and are ranked world's first and fourth most economically important crops. Grains from wild cereals break off at the inflorescence and are scattered on the ground as the plant senesces and dries. During this process, which makes harvesting difficult or impossible, the inflorescence stem, or rachis, becomes brittle and breaks; the phenotype is referred to "brittle rachis". In contrast, mature grain from domesticated cereals is easily harvested because they remain attached. The progenitors of wheat and barley have the brittle rachis as their grain dispersal mechanism. Loss of the natural mode of grain dispersal was perhaps the most important single event in the process of wheat and barley domestication. The evolution of the dispersal unit in wheat and barley is distinct from others such as rice and maize. Here we discuss how grain dispersal characteristic evolved in cereals, where and when ancient farmers selected the non-shattering types.

Dr. M.A. Malboobi
(National Institute for Genetic Engineering and Biotechnology - Iran)

Two Decades Of Research For Introducing Transgenic Sugar Beet Plants Resistant To Rhizomania Disease Based On Gene Silencing Against Beet Necrotic Yellow Vein Virus

Rhizomania is the most damaging disease of sugar beet worldwide which is caused by Beet necrotic yellow vain virus (BNYVV). Using intron-hairpin RNA (ihpRNA) expressing constructs to activate RNA silencing mechanism against cp21 sequences encoding BNYVV coat protein or its 5'-UTR, with different lengths and orientations. Both on-spot and stable transformation methods, effective resistance to rhizomania significantly correlated with the transgene presence. Among the examined constructs, S3 and S6 constructs, carrying 720-bp full-length CP21 and a 120-bp 5'-UTR sequence of it, respectively, generating hairpin RNA structures with small intronic loops were the best choice for producing high frequencies of resistant events. Several events for each constructs were produced and subjected to several rounds of green-house and contained field trials using either clonally propagated or seed grown events. Resistance to rhizomania were bio-assayed by DAS-ELISA, RT-PCR and/or real-time PCR while collecting phenotyping and genotyping data. Examination of all data concluded to selection of two events for which many risk assessment experiments including were arranged. The outcomes of molecular, genome, proteome and metabolome, crop trait indicies, bioassays, toxicology and allergenicity tests as well as phenotypic indices were all in favor of "substantial equivalence" to the parental sugar beet plant. The inheritance of transgenes and resistance were confirmed over generations in stably transformed plants. The compiled data demonstrate that the RNA silencing mechanism via BNYVV CP-ihpRNA is a promising approach for rhizomania disease management.

Dr. M. Iqbal Choudhary
(University of Karachi - Pakistan)

Biocatalytic Based Synthesis Of Chemical Space-An Approach For Cost – Effective Drug Discovery

Biocatalysis or biotransformation is an important field of biotechnology, which utilizes biological systems, such as microorganisms, cells, and pure enzymes to catalyze the synthetic process. Biocatalysis offers a remarkable arsenal of highly chemo-, regio- and stereo-selective methods for chemical conversions, which are often difficult to achieve even from state-of-the-art synthetic procedures. Thus requires environment friendly reaction conditions. In last two decades, this methodology has become an indispensable tool for asymmetric synthesis, not only at the academic level but also at the industrial scale. There is a need to fully exploit the potential of biotransformation in creating new and novel chemical space for the discovery of drug leads against prevalent diseases.

During this presentation, recent developments in biotransformation technologies and the potential of microbes and plant and animal cell cultures to create new molecular entities from the existing compounds will be presented, along with the results of our work in this field. This includes the use whole microbial and plant cell suspension cultures for the structural transformations of various classes of bioactive compounds, including anti-cancer, anti-inflammatory, oral contraceptive, etc. The main objective of the on-going research study is to discover new and effective lead molecules from existing one, for improved therapeutic activity against various drug targets

Dr. Mohammad Rashidian
(Harvard Medical School - United States)

Imaging Of Immune Responses

Immunotherapy has revolutionized cancer treatment. Notwithstanding the encouraging results, responses remain heterogeneous among patients and some face serious side effects. Predicting and monitoring the response remains a great unmet need. Developing methods to achieve these goals without multiple biopsies or other invasive methods is critical. The success of immunotherapy is a direct result of changes it brings to the tumor immune landscape. Therefore, noninvasive monitoring of immune responses can be used to tackle this issue. We have recently developed robust immuno-PET imaging methods to monitor the dynamics of specific subsets of immune cells in real-time. We will discuss these new advances and their potential to evaluate an ongoing response to cancer treatments.

Dr. Mahdi Taghadosi
(Kermanshah University of Medical Sciences - Kermanshah - Iran)

The Biotechnology in the Era of COVID-19 Pandemic

Coronavirus Disease 2019 (COVID-19), caused by the novel virus SARS-CoV-2, is often more severe in older adults. Besides age, other underlying conditions such as obesity, diabetes, high blood pressure, and malignancies, which are also associated with aging, have been considered risk factors for COVID-19 mortality. A rapidly expanding body of evidence has brought up various scenarios for these observations and hyperinflammatory reactions associated with COVID-19 pathogenesis. Advanced glycation end products (AGEs) generated upon glycation of proteins, DNA, or lipids play a crucial role in the pathogenesis of age-related diseases and all of the above-mentioned COVID-19 risk factors. Interestingly, the receptor for AGEs (RAGE) is mainly expressed by type 2 epithelial cells in the alveolar sac, which has a critical role in SARS-CoV-2-associated hyper inflammation and lung injury. Here we discuss our hypothesis that AGEs, through their interaction with RAGE amongst other molecules, modulates COVID-19 pathogenesis and related comorbidities, especially in the elderly.

Dr. Kayhan Azadmanesh
(Pasteur Institute of Iran-Iran)

Developing And Rolling Out Diagnostic Tests For Emerging Infectious Diseases Laboratories Network: The Iranian Laboratory Network For COVID-19

Detecting the emerging diseases has always been a huge challenge for health systems, when there is not enough information about the causing agent to develop proper diagnostics. Developing a practical diagnostic method and rolling it out to a country are even bigger challenges. COVID-19 was a typical example of how difficult the situation could be. To tackle this problem in a country level, different disciplines like biotechnology, virology, epidemiology… worked hand in hand. This is a brief report of what was done in Iran.

In the dawn of the pandemic and based on the genome sequences published by Chinese and then other scientists, PCR and Real Time PCR methods were developed. Then we had to roll out the established protocols and tests to a large number of labs all around the country. Standardization of so many labs and assuring of quality of tests were then a big obstacle. So, a training system followed by an External Quality Assessment Program was established. Sine the number of needed molecular tests for CoVID-19 was unprecedented, the next unparalleled challenge was providing consumables (for example extraction kits, PCR kits…) to the labs. To fulfil this demand local large scale production of those kits were established and an evaluation system for their authorization was created.

Developing non nucleic acid-based tests (NAT) was another universal approach for this disease. Soon after the beginning of the pandemic it became obvious that the antibody detection-based tests have no role in diagnosis of the viral infection. However, antigen detection kits, despite their lower sensitivity, are very helpful. Faster NATs, like LAMP or RAA, were also developed but did not become popular.

Emerging new variants of the virus was another challenge for the labs. These variants had different transmission efficiencies, and at least in a few cases, made some molecular tests unreliable. Besides, it became a threat for vaccine development. So, we had to look for a monitoring system utilizing large scale sequencing and NGS, as

well as new molecular tests to detect those variants.

Establishing the National Laboratory Network for COVID-19 from February 2020 till now, is an exceptional experience to face future large scale outbreaks.

Dr. Hossein Baharvand
(Royan Institute - Iran)

Towards The Generation Of A GMP Compliant Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial Cells For The Treatment Of Age-Related Macular Degeneration: The First Pre-Clinical Study For Safety And Efficacy In Iran

Age-related macular degeneration (AMD) is the primary cause of blindness in adults over 60 years of age, and clinical trials are currently assessing the therapeutic potential of retinal pigmented epithelial (RPE) cells to treat this disease. Human embryonic stem cells (hESC)-derived RPE cells have the potential to provide the unlimited and reliable source for treatment of AMD. To serve this capacity, these cells must undergo a xeno-free and good manufacturing practice (GMP) grade process. Here, our experience in the development of a protocol for generation of RPE cells from hESC under GMP compliant condition will be described. These RPE cells produced a confluent pigmented monolayer with related gene expression, polarized, and functional RPE cells. We also verify that stability of hPSC-RPE cells banking. The administration of REP cells into the subretinal space of a mammalian model, the Royal College of Surgeons (RCS) rat, demonstrated survival as a monolayer and maintained visual function for 24 weeks. There were no adverse effects attributable to the grafted cells and no cell overgrowth or tumor formation, thus paving the way for a future retinal degeneration clinical trial.

Dr. Fereidoun Mahboudi
(Pasteur Institute of Iran - Iran)

The Role Of Medical Biotechnology In Health Promotion In Iran

Recombinant proteins such as Insulin, Erythropoietin, Human Growth Hormone, Interferon and blood coagulation factors created a revelation in treatment of genetically disorders patients. In addition, monoclonal antibodies have saved the life a huge number of people who suffering from cancer and autoimmune diseases. More than 170 biopharmaceutical products have entered the market since 1980. The total revenue in world market is more than 200 billion USD. Iran with 24 biopharmaceutical products as called as biosimilar is one of the key players in the world. The total revenue based on the world market is around 1.8 billion USD for local manufactures. The price in local market is between 30 to 50% of the regional price. The price of biopharmaceutical products are high in terms of the income for people who are living in most of the third world countries. Therefore, it is not affordable for most of the people. The local production of these products has tremendous effects on the health conditions in Iran. We produce high numbers of the monoclonal antibodies for cancer treatment. The affordability of the price and accessibility of the biosimilars in Iranian market is big advantages for promotion of health in any country. We are proud that we are among those countries that have these two parameters. Unfortunately in last 5 five year there is a ban on manufacturing monoclonal antibodies and recombinant proteins for market authorization by government. If the situation goes like these Iran will be facing a big health problems and people should buy the medicine with more than 2 to 3 times more expensive from neighboring countries. Iran has potentiality to produce more than 18 more biosimilars which have been supported by Biotechnology council department under the Vice president for Science and technology.

Dr. Sirous Zeinali
(Pasteur Institute of Iran - Iran)

National Programs For Prevention Of Genetic Disorders In Iran, 20 Plus Years Of Practice

Prevention is the most cost effective procedure that some countries choose to reduce cost of hospitalization and treatment. This is more true when it comes to inherited genetic diseases. There are several time course that this can be implemented. Pre-marriage, pre-pregnancy, prenatal or neonatal diagnosis are among most practiced policies being carried out. One of the widely of such programs is prevention of hemoglobinopathies such as alpha- or beta-thalassemia or Sickle Cell Disease (SCD).

Iran with a population of about 80 million, is in the hemoglobinopathies belt which starts from East Asia and ends up in Mediterranean and Africa. In 1997 the National Program for Prevention of Thalassemia started. In this program, every couple to be, upon registering their marriage are asked to go to a designated Health Center as part of National Primary Health Care Center, equipped with the necessary manpower and laboratory know how to carryout CBC and hemoglobin variant level. In the program, the male partner is testes first and if his test indicates that he is or might be a carrier of beta-thalassemia, then the female partner is tested. If both are carrier or suspicious of being carriers, then they are referred to one of several designated and accredited medical genetic laboratory. In there, they are tested by molecular methods to see if they are true carriers or not and if so, what are their mutations and linked RFLP markers. In either case they are given a written letter to be taken to the Health Center and from there to the Marriage Registry Center. This program required the most comprehensive management and programing. Several achievements have been obtained which the most prominent of them is sharp decline of beta-thalassemia new cases in Iran since 1997. Implementation of this program has provided the Ministry of Health the knowhow and managerial experience to implement few more programs. The Genetic Office at the Deputy for Health at the Ministry of Health has masterminded the whole process and program.

The Genetic Office initiated implementation of several other programs several years later. One is diagnosis of PKU in neonates at the first 10 days of their birth. The primary part is obtaining blood spots from newborns and then performing tests to see if the child is actually affected. If so, then the families are trained and free nutrition and other medicine are given. This program in the past 4-5 years had evolved to include about 20 other inborn errors of metabolism. The program has aimed to increase it to 40+ by utilization of NGS. This program has required involvements from clinicians, medical geneticists, nutritionist and medical laboratories; therefore Genetics Office has created several networks to better manage the whole programs.

The other program which has been in practice for more than a decade is prenatal screening of pregnant women for chromosomal aneuploidies most prominently Down Syndrome. The program initially was aimed at being rather compulsory but recently the Iranian Parliament passed a law to make it voluntary.

By implementing such programs, we have see sharp increase in the expansion of centers providing these services, increase in medical and laboratory expertise, production of necessary equipment, reagents and consumables. As a whole we have seen advancement of medical genetics, biotechnology, etc. as a result of these implementations, similar to what we have seen for Covid-19.

Dr. Changiz Eslahchi
(Shahid Beheshti University - Iran)

Predicting Anticancer Drug Response Using Machine Learning Approaches

Precision medicine can be used to classify the patients into subgroups that vary in response to a medical treatment. Tailoring efficient treatments based on their personalized characteristics can improve the quality of therapies, avoid extra expense and diminish undesirable side effects. One of the prominent challenges in precision medicine is to select the most appropriate treatment strategy for each patient based on the personalized information. Recognizing whether a patient is sensitive or resistant to a drug is of high importance. Since the providing huge data about patients› sensitivity against drugs is not easy and probable, the sensitivity of cell lines against drugs are extensively studied. The availability of massive data about drugs and cell lines facilitates the possibility of proposing efficient computational models for predicting anticancer drug response in cancer cell lines. In this study, we propose ADRML, a model for Anticancer Drug Response Prediction using a machine learning approach to integrate the cell line information with the drug information systematically in order to make accurate predictions about drug therapeutic. The proposed model decomposes the drug response information into the low-rank feature space and uses these features to predict the drug response for unknown cell line-drug pairs. The evaluation of ADRML performance on various types of cell lines and drug information, in addition to the comparisons with previously proposed methods, shows that ADRML provides accurate and robust predictions. Further investigation verify that predicted drug responses reveals drug pathways activity.

Dr. John FRASER
(Retired from Florida State University - United States)

Technology Transfer', 'Knowledge Exchange' and 'Commercialization'. A History and Lessons Learned.

This 20-minute presentation will discuss what Technology Transfer, Knowledge Exchange and Commercialization is and why it is important. A History in different parts of the world will be presented as well as Lessons Learned to be successful.

Dr. Farhad Ghavam
(Chief Scientific Officer (CSO) at Eurofins Bio Diagnostics Inc - United States)

Developing an Amplicon Based Genotyping by Sequencing Panel in Sunflower

The advent of next-generation sequencing technologies and the ongoing reduction of sequencing costs have made genotyping by sequencing (GBS) a common practice for genotyping in plant breeding. Among different methods of GBS, amplicon based GBS is an accurate and economical method for genotyping large number of samples in an economic way.

The first sunflower 10K SNP array was developed thorough a collaboration between Eurofins BioDiagnostics and the National Sunflower Association (NSA). Since the development of the SNP array in 2012, more than 500 public lines were genotyped using the NSA array and a number of scientific manuscripts has been published using the 10K SNP illumina bead array. Using this public data, the best markers with optimal performance and high minor allele frequency (MAF) were selected to develop an AgriSeqTM GBS panel. The AgriSeq Panel containing 700 of these markers well distributed on the genome will be discussed in this presentation, which can help QTL mapping, marker assisted backcrossing, and genomics selection projects in sunflower.

Dr. Kianoush Khosravi Darani
(Shahid Beheshti University of Iran)

Applications Of Mycoprotein In Immunity (Food And Humans)

The role of mycoproteins in food safety is well known and has long been considered as an alternative source of plant and animal proteins by researchers. A newer point in this article is the role of mycoprotein in food safety and even human safety. The use of mycoprotein as a prebiotic, and the ability to bind to certain toxins in food have been hypothesized and few reports have been published. Another important issue is the ability to produce antimicrobial peptides. Human beings will soon face an increase in the number of antibiotic-resistant bacteria and an increase in therapeutic failure in the treatment of infection with antibiotic-resistant microbes, which in turn will lead to a prolonged period of disease. At the same time, there is an increase in the possibility of occurrence of pandemic infections, the length of hospitalizing of patients, the demand for more expensive drugs as well as complications for treatment and the risk of mortality. Fungi, on the other hand, produce many antimicrobial peptides as a new generation of antibiotics and are a good option for introducing new biomolecules with antimicrobial properties. Many of these antimicrobial peptides have antitumor activity and, while causing death in many cancer cells, prevent them from growing by lesser-known mechanisms. Introduction of substances with important and multiple medicinal effects of fungi has turned these organisms into an important source for isolation and identification of new pharmacological molecules. In this article, we will review the main roles of mycoproteins in safety and describe the mechanism of its action of antimicrobial peptides and the antimicrobial spectrum of each peptide.

Dr. Sathyanarayana N. Gummad
((Chief) Department of Biotechnology - India)

Process Development For Caffeine Degradation In Effluents & Food Products

Caffeine is a purine alkaloid which is a major constituent of coffee, tea and other beverages. Caffeine acts as a central nervous system stimulant but it also has negative withdrawal effects. Decaffeinated beverages are being used to overcome its negative effects. In addition, effluents from coffee and tea processing plants have high concentrations of caffeine at ranging between 1 g/l, which will affect the microbial community in soil and water. Hence from both food and environmental point of view, caffeine degradation is necessary. Conventional decaffeination processes are expensive and uses toxic solvents. Hence development of a process involving an enzymatic (specific) degradation of caffeine to non-toxic compound is necessary. Identification of enzymes specific to caffeine degradation will solve the problem of chemical extraction of caffeine in food products and as well as treating the wastes containing caffeine. *Pseudomonas* sp. was isolated from coffee plantation area capable of utilizing the caffeine as sole carbon and nitrogen source. The rate of caffeine degradation was enhanced in the presence of sucrose. The isolate was characterized as *Pseudomonas putida* based on 16S rRNA analysis. The effect of different nutrients, physical parameters affecting caffeine degradation was studied and optimized using statistical experimental design. Kinetics of caffeine degradation by whole cells and immobilized cells were performed. Localization studies revealed that caffeine degrading enzymes are located in cytoplasm and they are inducible in nature. Purification and biochemical characterization of the enzyme was performed. Optimization of conditions for maximum production of caffeine degrading enzymes is studied in bioreactors. Induced cells were used as biocatalyst to degrade caffeine in commercial tea samples and effluent samples.

Dr. Jamshid Amiri Moghaddam
(E.V.Hans-Knöll - Institut - Germany)

Marine Bacterium As Potential Protective Agents For Oyster Aquaculture

Marine bacteria have come into the focus for natural product discovery as a consequence of the emergence of antibiotic resistance. This was additionally boosted by the limitations encountered in drug developments from traditional producers of drug leads from terrestrial environments. The marine bacterium Labrenzia sp. 011 of the family Rhodobacteraceae was isolated from the coastal sediment of Kronsgaard, Germany. This strain produces two cyclopropane-containing medium-chain fatty acids, namely cis-4-(2-hexylcyclopropyl)-butanoic acid and cis-2-(2-hexylcyclopropyl)-acetic acid, which showed activity against a range of microorganisms. It is of special interest, that these compounds strongly inhibit Pseudoroseovarius crassostreae DSM 16950 (genus Roseovarius), the causative agent of Roseovarius oyster disease (ROD). The latter is a bacterial-induced infection and causes major problems in oyster aquaculture. Bacteria of the genus Labrenzia have been proposed as protective agents against ROD.

The genome analysis of bacteria of the genus Labrenzia was expected to provide information to understand the mollusk-protective role of Labrenzia spp.. Therefore, the genome of Labrenzia sp. 011 was sequenced and assembled into 65 contigs. It has a size of 5.1 Mbp and a G+C content of 61.6%. Comparative genome analysis defined Labrenzia sp. 011 as a distinct new species within the genus Labrenzia, whereby 44% of the genome was contributed to the Labrenzia core genome. The genomic analysis revealed several conserved cyclopropane fatty acid synthases (CFAS) genes in bacteria of the genus Labrenzia, putatively responsible for methylation and cyclopropanation of long-chain fatty acids. In addition, a gene cluster encoding for two distinct CFAS genes is proposed as the biosynthetic origin of the isolated cyclopropane fatty acids from Labreniza sp. 011. In conclusion, the here investigated marine bacterium, Labrenzia sp. 011, harbors a high unique genetic and metabolic diversity, rendering it as a promising protective agent for oyster aquaculture.

Dr. Melekşen Akın
(Horticulture at Iğdır University - Turkey)

Classificationification And Regression Trees For Tissue Culture Data Analysis

Decision trees are non-parametric techniques that can project complex non-linear associations between the variables and high-order interaction effects without requiring the restrictive distributional assumptions of the conventional linear models. Besides showing the effect-size relationship of the variables, these algorithms are considered as supervised machine learning and can be used in prediction of a target based on various inputs. These methods recursively split the data into subsets based on several independent variables and generate easy to interpret elegant trees. Decision trees are appropriate to analyze numeric and categorical data, thus can be applied both for classification and regression problems. Classification and Regression Trees (CART) is one of the most well-known decision tree algorithms. The CART method generates a classification tree when the response is qualitative, and a regression tree in case the target is quantitative. Model evaluation metrics for classification and regression models differ. Accuracy, No Information Rate, Sensitivity and Specificity are useful metrics in classification problems, whereas Root Mean Squared Error is the commonly used statistic for regression problems. This study aims to provide a gentle introduction to the implementation principles of classification and regression trees through the “rpart” and “caret” packages in R software and to motivate future applications of these methods on multi-dimensional in vitro data.

Keyword: CART, classification, recursive partitioning, regression, tissue culture

Dr. Mohammad J. Taherzadeh
(University of Borås - Sweden)

Resource Recovery From Wastes Using Biotechnology

Anaerobic digestion (AD) is well known technology that is used for internationally for biogas production from e.g. manure, sludge or food wastes. However, low value and difficult transportation of methane, shift the focus of research to use AD for production of hydrogen and/or volatile fatty acids (VFAs). Both H2 and VFAs are intermediate products of anaerobic digestion (AD). While hydrogen is separated from the digested as gas, VFAs need special separation such as membranes and even purification for some applications. VFAs are a platform for production of a variety of chemicals and materials, such as bioplastics, carbon source for denitrification, food and feed. This presentation covers the production and separation of VFAs and also discuss some applications that are developing at our university in Sweden.

Dr. Ashley J. Stevens
(Past President of University Technology Managers - United States)

The Challenges Of Creating A Tech Transfer Ecosystem In An Emerging Economy

Technology transfer has such a challenging business model that it can only be successfully practiced as a not-for-profit activity, even in well-developed academic research ecosystems. Reasons include: academic inventions are early stage and unvalidated technically and commercially; only 25% of inventions submitted by academic inventors are ever licensed and only 3% result in a marketed product; it takes a median of four years to find a licensee to invest in developing a product from the innovation; and then the patents expire. If this were not daunting enough, there are additional challenges in establishing tech transfer in an emerging economy.

This session will discuss the business model of tech transfer, the challenges in an emerging ecosystem such as Iran and will show that there is a pot of gold at the end of the rainbow, but that it's not located where you might expect.

Dr. Naira Baghdasaryan
(UNESCO/UNITWIN Network- Armenia)

Non-Ionizing Radiation-Induced Stimulation Of Bull Sperm Motility

Ving cells, starting from prokaryotes to eukaryotes, have a quantum-mechanical sensitivity to signals and an ability to respond to them. However, the nature of such a universal sensor(s) and its role in controlling the living state of organisms is not fully evaluated. There are many hypotheses in this regard, but nobody has given a reliable explanation to these phenomena. Among them the most popular explanation is the so-called “Water hypothesis” suggesting that water, which has a quantum-sensitivity and is a dominant component of living organisms that serves as a medium for a majority of metabolic reactions, is a target, through which the quantum-mechanical cross-talk of the living organisms with the environmental medium is realized. It is well-documented that non-ionizing factors such as Infrasound Frequency (IS), Electromagnetic fields (EMF) and Mechanical Vibration (MV) can cause changes in physicochemical properties of water and this effect has frequency-dependent nature. The biological effect of non-ionizing factors-treated PS on cells and organisms was also shown by our Center. It was documented that EMF- and MV-treated PS can provoke a specific cell response at “window” frequencies. This pathway is considered as an indirect influence of these factors on cells and organisms.

However, the experiments performed on E.coli with the use of direct and indirect treatments by EMF and MV reveal that there are some differences in the nature of the cell response after the treatment of the medium with and without the microbial strain addition. This fact allows us to hypothesize that not only the indirect impact but also the direct stimulation of the cell can be the pathway of cell response. To prove this hypothesis, we performed a series of experiments on bull sperm. The high sensitivity of sperm velocity and viability to physicochemical properties of water bathing medium (rheotaxise and chemotaxise) makes it a very convenient experimental model for studying the cellular mechanism of biological effect of EMF and MV. The experiments have shown that the sensitivity of sperm motility and viability

to MV has frequency-dependent nature. This effect can be recorded in experiments performed on cryopreserved sperms, while fresh sperm treatment has no statistical changes on sperm characteristics. To reveal the cellular and molecular mechanism of MV direct impact on sperm, the intracellular levels of cAMP, cGMP and Ca were investigated. The results have shown that it has more pronounced activation effect on the sperm velocity (38±4%, *** $P<0.001$), which is accompanied by the decrease of intracellular level of cGMP (26±8% *: $p<0.05$), elevation of intracellular level of cAMP (43.5±7%, *: $p<0.05$) and activation of 45Ca-uptake by sperm (285±25%, *: $p<0.05$).

Thus, the obtained results allow us to conclude that:

The biological effect of non-ionizing factors can be realized not only through the changes of physicochemical properties of cell bathing medium, but also through the direct stimulation of specific mechanosensory of cell,

The results support the hypothesis that the stimulation of 2Hz-sensitive mechanical-sensors serves as an initial signal for switching the cyclic nucleotides dependent Ca-uptake pathway on, which is responsible for stimulation of sperm motility.

Dr. K. R. S. Sambasiva Rao
(Acharya Nagarjuna University - Guntur - India)

Cold active enzymes and their potential industrial applications

More than three quarters of Earth's surface is occupied by cold environment, including the ocean depths, polar and alpine regions. These cold ecosystems have been habituated and successfully colonized by a class of extremophilic microorganisms called psychrophiles. To cope up with the environmental conditions, the psychrophiles have evolved themselves by producing specific enzymes. These psychrophilic enzymes display unique features, high specificity and catalytic efficiency at low temperatures associated with high thermo sensitivity and a range of structural features that correlates with the enzymes cold adaptation. Because of these unique properties, they have several potential applications. Cold active proteases, lipases, amylases, and celluloses are very much used in detergent industry. In food industry, β-galactosidase is used for removal of lactose from milk and milk products, pectinases helps in the juice extraction, enzymes such as amylase, proteases and xylanases helps in the retention of the aromas and moisture levels backed food stuffs and cold active proteases are used for tenderization of meat. Psychrophilic enzymes were also been used in bioremediation of polluted soils and waste waters during the winter in temperate countries, when the degradative capacity of the endogenous microflora is impaired by low temperatures. The main advantage of these enzymes was attributed to be, high activity of enzymes at low and moderate temperatures and offers potential economic benefits, for example, through substantial energy savings in large-scale processes that would not require the expensive heating of reactors and also can be used during transport and storage of goods at low temperatures and they have several other advantages over thermophiles and mesophiles. However, it is leaving a challenge of maintaining similar growth conditions in large scale production of these enzymes. The present study has been emphasized at isolation and production of some selected cold adaptive enzymes such as β-galactosidase and pectinase from marine psychrophiles. The cold active enzymes represent an extremely versatile

group of enzymes that are capable of catalyzing a variety of important reactions, thereby presenting a fascinating field for future research.

Abstracts

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Pharmaceutical Biotechnology

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Production of nanobody-based anti-PSMA CAR T cells by lentiviral vectors

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Abstract

For many years, cancers have been treated using traditional therapies, such as surgery, radiation therapy, and chemotherapy. Although these approaches have contributed to improved outcomes but they also have limitations and severe side effects. Recently, targeted cancer therapies, have been developed and applied as standard therapies for many cancers. One approach to cancer immunotherapy entails genetically engineering patient's T cells to express chimeric antigen receptors (CARs) that recognize and attack tumor cells.

In this study, we aim to design and prepare CAR T cells against Prostate Specific Membrane Antigen (PSMA) based on a PSMA nanobody by lentivirus transduction.

At first, a second generation CAR construct was subcloned into pCDH, a lentivial expression vector. The HEK293 cells were cotransfected with pCDH, pMD2G and pSPAX, to produce a GFP expressing lentiviral vector. Then, Jurkat cells were transduced with lentivirus and CAR expression was evaluated by fluorescent microscope and flow cytometry.

The microscopic images and flow cytometry results confirmed the successful expression of CAR construct in the transduced HEK293 and Jurkat cells.

Our results showed that CAR expressing lentivirus-based vectors can transduce efficiently Jurkat cells and may be used as valuable tools for production of CAR T cells.

Keyword: Chimeric Antigen Receptor, Nanobody, Prostate Cancer, PSMA, lentiviral vector

Making antibacterial filter using polyacrylonitrile, polyvinyl alcohol-gelatin and copper ions based on electrospinning method

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Abstract

Nowadays, linking Nano science with biology can offer new approaches to dealing with microbes and infectious diseases. Bacterial contamination and related resistance is one of the concerns facing human society today. Nano science uses various techniques, materials and elements to try to solve such problems. Electro spinning process is one of the best methods for making Nano fibers, which we also used in this study using poly acrylonitrile and polyvinyl alcohol-gelatin polymers. Electro spinning is a method that can be used on a large scale. During electro spinning, UV radiation was used to improve the quality of the fibers and sterilize them. After making the Nano fiber, it was impregnated with three solutions of copper sulfate, salt and copper sulfate and salt, and each Nano fiber was imaged by SEM electron microscopy. The Nano fibers were then placed in culture medium of Escherichia coli 25922 and Staphylococcus Aureus 25923 and the plates were incubated. The next day, by examining the plates, we observed a halo of non-growth around the Nano fibers impregnated with copper sulfate solution and found that these Nano fibers have antibacterial properties and prevent the growth of bacteria. As a result, antibacterial filters and Nano fibers can be made using electros pinning and UV irradiation and copper ions.

Keyword: Nano fiber, electro spinning, copper sulfate, bacteria, UV irradiation

Generation and Characterization of a Camelid Diabody Against Placental Growth Factor for Targeting Angiogenesis

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Abstract

Antibodies are potential molecules that be used as therapeutic agents in several diseases. Applying engineered antibodies like nanobodies rather than traditional antibodies are more advantageous. So, inhibition of angiogenic factors like Placental Growth Factor by using nanobodies could be beneficial in suppression of the angiogenesis progression. In this study, we developed a recombinant anti-PLGF diabody based on affinity enhancement mutant form of anti-PLGF nanobody to suppress the angiogenesis progression. The diabody was cloned and expressed into a bacterial system then the purity was authorized using western blot assay and the affinity was assessed using ELISA. Proliferation, 3D capillary tube formation, and migration assays were employed as functional assays. The data was analyzed using SPSS and $P<.05$ was considered statistically significant. The EC50 was estimated for endothelial cell proliferation and capillary tube formation about 100 ng/ml and 65 ng/ml, respectively. Migration of the diabody-treated MCF-7 cells was inhibited about 69% rather than control. Inhibition of PLGF with monoclonal antibody have showed that it is significant in angiogenesis suppression but due to intrinsic properties of nanobodies, it is suggested to use of them. The small size is caused to remove through liver or kidney system rapidly, so it is important to use bivalent for extending of the half-life. Our findings indicate that inhibition of PLGF by means of the diabody can prevent growth and proliferation of endothelial cells and tumor cells.

Keyword: Diabody, VHH, Anti-Angiogenesis, Placental Growth Factor.

Evaluation of cytarabine and mercaptopurine drug complex on Ln-cRNA URHC expression in acute lymphoblastic leukemia

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Abstract

T-cell acute lymphoblastic leukemia (T-ALL) represents approximately 12% to 15% of all newly diagnosed ALL cases in pediatric patients. Mercaptopurine and Cytarabine are Chemotherapy drug used for the treatment of ALL. The aim of this study was to evaluate the simultaneous effect of a complex of two chemotherapeutic drugs, cytarabine and mercaptopurine, at certain concentrations and at 24, 48, 72h on changes in URHC expression in the Jurkat E6.1 cell line. After cell passage, Jurkat E6.1 cell line was treated with a complex of cytarabine with a concentration of 1µM and mercaptopurine with a concentration of 5µM in groups of 24-48-72h. RNA was extracted and cDNA synthesis was performed. URHC expression were examined by RealTime PCR. GAPDH gene was selected as a reference gene. Finally, the results were analyzed using Rest software. Changes expression of URHC treated with 1µM cytarabine and 5µM mercaptopurine at 24, 48 and 72h showed a significant decrease in expression in the first 24h after URHC treatment (p-value <0.001) and the rate was equal to 0.233. Expression decrease in URHC at 48h was insignificant, expression in URHC expression at 72h had a statistically significant decrease in expression (p-value <0.001) and the rate of this decrease was 0.133. Therefore, the highest decrease in gene expression, which indicated the highest effect of drug complex in significantly reducing the expression of URHC, was observed in a rate of 0.133 at 72h.

Keyword: Jurkat E6.1, T-ALL, lncRNA URHC

Fusion of LTB (L) bio-adjuvant to Newcastle Virus Hemagglutinin (HN): A new Chimeric Vaccine Candidate

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Abstract

Newcastle disease (ND) is one of the most important viral diseases among birds. Despite using live attenuated vaccines, the virus continues to infect both domestic and wild birds and has the potential to create huge losses for the poultry industry. Live vaccines have several benefits and some limitations, including the probability of viral spreading and reversing back to virulence, but subunit vaccines are safer alternatives. However, subunit vaccines may be less immunogenic than live attenuated vaccines. Hence, bio adjuvants, for example, *ltb* from (*ETEC*) can be added to boost the immunogenicity. Accordingly, a chimeric construct consisting of *ltb* and two repeats of conserved and epitopic parts of *hn* (hemagglutinin neuraminidase) of Newcastle virus was designed and cloned into the pTG-۱۹T cloning and subsequently pET-۲۸a expression vectors. The presence of the gene *lhn2* was validated using restriction enzymes digestion, colony-PCR, and finally Sanger sequencing. The recombinant chimeric protein was successfully expressed in *E.coli* host and purified. This study provided successful data of cloning, subcloning, and production of recombinant LHN2 protein as a new vaccine candidate against the Newcastle virus.

Keyword: Newcastle virus, Hemagglutinin Neuraminidase, Recombinant vaccine, Chimeric protein

Doxorubicin-loaded Chitosan- Montmorillonite- Carbon quantum dots Hydrogel Nanocomposite for the Treatment of Breast Cancer

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Abstract

Cancer has remained one of the most deadly diseases. Although chemotherapy is the only option in recent years, adverse side effects, development of multiple drug resistance, and poor physiochemical properties can compromise its many advantages in cancer treatment. Accordingly, natural compounds due to their less toxicity are considered as a promising approach to cancer treatment. Doxorubicin (DOX), isolated from Streptomyces strains, has demonstrated applications in cancer treatment. However, short in vivo circulation time, low solubility, and poor permeability are among major restrictions for the use of doxorubicin as an anti-cancer drug. Purpose of the present study is to improve DOX loading and prolong the release time in addition to reducing side effect for the drug. For this purpose, we loaded doxorubicin into a hydrogel nanocarrier of chitosan (CS)-montmorillonite (MMT)-carbon quantum dots. The incorporation of CQDs into the CS-MMT hydrogel nanocarrier improved the loading and entrapment efficiency up to 49% and 91%, respectively. The fabricated nanocarriers' morphology was observed using Field Emission Scanning Electron Microscope (FEEM). The incorporation of all components in the fabricated drug delivery system was approved using the FTIR spectrum. Through the drug release study, we showed that the release of DOX from fabricated nanocarriers was pH-dependent. Furthermore, zeta potential value was +31.5, confirming the nanocarriers' good stability. Finally, by comparing the cytotoxicity of doxorubicin-loaded CS-MMT-CQDs with control group, doxorubicin as a free drug, and CS-MMT-CQDs nanocarrier on the MCF-7 cell line, we identified that CS-MMT-CQDs-DOX caused significant cytotoxicity. These findings provide evidence suggesting that the DOX-loaded nanocarrier is a promising drug delivery system with the potential to enhance doxorubicin loading as well as improve cytotoxicity and sustained-release

of DOX.

Keyword: Doxorubicin, Hydrogel nanocarrier, Enhanced drug loading, Sustained release, pH-sensitive nanocarrier

Modification of PVA nanofiber by GO- Cu containing curcumin; emphasis on antibacterial activity and wound healing acceleration

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Abstract

In recent years, applying various wound dressings with antibacterial activities to expedite tissue repair stages has gained remarkable attention. The intertwined three-dimensional structure of nanofibers provides unique spaces for carrying drugs and repair agents during the wound healing process. The purpose of this work is the optimization and fabrication GO-Cu-Cur nanocomposite and PVA/GO-Cu-Cur as a wound dresser in order to accelerate the wound repairing process. The PVA/GO-Cu-Cur was synthesized by the electrospinning method, and by the FTIR was characterized. SEM analysis indicates that the diameter of the nanofibers is 328nm. Mechanical characteristic of PVA/GO-Cu-Cur was investigated by the tensile resistance, and it reveals that the GO-Cu-Cur nanohybrid cause to improvement of mechanical characteristic. The MIC and optical density (OD) results show that these materials have a great ability to inhibition of gram-negative and gram-positive bacteria growth. The toxicity, survival, and cell proliferation of the fibroblast cells and cell immigration and wound repairing by the MTT and, the scratch test were investigated respectively, and results show that the GO-Cu-Cur nanohybrid does not have a toxic effect. And also, the nanohybrid concentration of 0.05 mg/mL can lead to wound repairing percentage of 35% after 24h. The approach in this study may provide an alternative to make an antibacterial wound dressing to achieve an effective drug based bandage.

Keyword: polyvinyl alcohol, graphene oxide, Curcumin, cu nanoparticles

pH sensitive Agarose/chitosan nano-carrier loaded with 5-Fluorouracil and Curcumin against MCF-7 cell lines

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Abstract

Controlled drug delivery systems are promising engineering technologies used in the treatment of many cancer tumors. This technology is for the targeted delivery and controlled release of therapeutic agents and has received great attention because of its advantages over chemotherapy methods. This study aims to prepare a pH sensitive nanocomposite of chitosan/agarose with exclusive properties in the field of cancer treatment as a carrier against breast cancer MCF-7 cells. 5-Fluorouracil, Curcumin, and a compound of both of them were loaded into the nano-carrier. To confirm the efficiency of this carrier several analyses such as XRD, FESEM, FTIR, DLS, and zeta potential were accomplished. In addition, the biocompatibility of nano-carrier and the effect of 5-Fluorouracil and Curcumin on cancer cells were evaluated with MTT assay and flow cytometry analysis which demonstrated that chitosan/agarose@Curcumin/5-FU represents 58% less viable cells for the MCF-7 breast cell lines than chitosan/agarose@5-FU. Eventually, this study demonstrated that chitosan/agarose@Curcumin/5-FU is an adequate candidate for the treatment of breast cancer cells.

Keyword: Drug delivery system, Agarose, Chitosan, Curcumin, 5-Fluorouracil

Investigation of Immunological Axis of IL23-IL17 Serum Levels in Patients with Polycystic Ovarian Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most important endocrine disorders in women with low levels of chronic inflammation. The aim of this study was to evaluate the serum levels of IL-17, IL-23 and demographic factors in patients with polycystic ovary syndrome for the first time. In a case-control study, 60 patients with PCOS and 60 healthy individuals referred to Imam Ali Clinic in Shiraz and 5cc of venous blood received of all participants. The Serum levels of IL-17 and IL-23 cytokines levels were measured by ELISA method. Data were analyzed using SPSS19 software. Median concentrations of IL-17 and IL-23 in patients (0.9 and 5.8, respectively) were significantly higher than controls (3.50 and 3.00, respectively) (P = 0.000) and (P =0.003). The results of this study revealed the central role of TH17 cells in the inflammation related to increase of IL-17 and IL-23 level in non-obese women with PCOS.

Keyword: Polycystic ovary syndrome, Interleukin 17, Interleukin 23

Design, cloning, expression and purification of synthetic fusion protein (F) from Newcastle virus with LTB bioadjuvant in prokaryotic expression system

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Abstract

Newcastle disease virus (NDV) is a dangerous virus that infects a wide range of birds that causes serious damage to the poultry industry. Binding of the virus to the host cell membrane is mediated by two surface glycoproteins, hemagglutinin-neuraminidase (HN) and fusion (F), which are considered as an important target for the immune response in poultry. The main purpose of this study was to synthesis chimeric *lf* gene and to express a recombinant protein in the Escherichia coli host. For this purpose, the SOEing PCR was performed and the F gene fused to ltb moiety. Cloning and subcloning techniques were used to insert *lf* gene into the expression and subsequently to *E.coli* cell. The recombinant protein was successfully expressed and purified. The immunogenicity in animal model is under progress.

Keyword: Newcastle disease virus, hemagglutinine-neuraminidase, SOEing PCR

Preparation and evaluation of nano metal-organic framework coated with chitosan and conjugated with Folic acid containing methotrexate for targeted chemotherapy of folic acid receptor positive cancer cells

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Abstract

Cancer is still one of the most challenging diseases. Over the past two decades, new drug delivery systems have been developed that have alleviated the problems associated with chemotherapy. These systems include nanoparticles containing organic and inorganic compounds. Nanoparticles are used in a variety of applications, such as drug delivery to cancer tumor cells, invading cancer cells, and increasing the sensitivity of cancer cells to imaging and observing them more closely. Nanoparticles have good potential for cancer prevention, diagnosis and treatment. In the field of health, one of the important challenges is the efficient delivery of drugs in the body using non-toxic nanoparticles. Most of the available carriers show weak drug loading or rapid drug release (usually less than 5% by weight of the transported drug versus carriers). In this context, porous solids, with the ability to adjust their structure and porosity for better interaction Drugs and high loads are well-suited as nanoparticles for drug delivery and imaging. Metal and Organic Frameworks (MOFs) are a unique class of composite porous solids based on metals and organic bonds. In compare with traditional porous materials, they have a larger surface area, adjustable pore shape size and larger pore diameter, adjustable composition and functional pore surface, which give them unique advantages and properties for applications in adsorption and release therapeutic drugs are possible. In the present study, a Nano porous metal organic framework (MOF) based on zinc [Zn2(bdc)2(dabco)] coated with a monodisperse layer of chitosan was synthesized as a pH-responsive and target-selective system for delivery of methotrexate (MTX) that was conjugated with folic acid is affect in the treatment of folic acid receptor positive cancer cells such as colon cancer. Porous metal organic framework [Zn2(bdc)2(dabco)] was synthesized

under solvothermal conditions. We were evaluated the drug release at 4 ° C, RT, and 37 ° C. MOF nanoparticles of zinc acetate were first synthesized in dimension of less than 200 nm. Characterization of samples was studied by DLS, Fournier transform infrared (FT-IR), X-ray powder diffraction (XRD), Transmission Electron Microscope (TEM) and Scanning Electron Microscopy (SEM). Cytotoxicity was assessed by MTT assay and also apoptosis and cell cycle inhibition were evaluated by flow cytometry test. In addition, gene expression of autophagy and apoptosis was examined by Real Time PCR. The targeted nanoparticle was displayed 78% of drug loading efficiency in 3 days. In addition, cell toxicity, apoptosis, cell cycle inhabitation was assessed by cellular and molecular methods. In vitro cytotoxicity assessment and IC50 values for the human colon cancer cell (HCT116) was determined by MTT assay which indicated high and significant mortality of the targeted nanoparticles (MOF-CS-FA-MTX) in the HCT116 cell lines in comparison with the HGF as a normal cell. The cell death type was confirmed by apoptosis and cell cycle control tests. The molecular analyzes of the treated cells show high expression of BAX, ATG5, BCL2 apoptosis genes and Beclin1, MTORC1 autophagy genes and GAPDH as a control gene. Then, we examined the results of each of them on gel electrophoresis. The results obtained in this study revealed that this specific nanoparticle had suitable morphological properties and MOF-Chitosan Nano systems containing methotrexate and targeted with folic acid Targeted on the cancer cell line with folic acid receptor activated the autophagy molecular pathway. Therefore, our studies in the project show that this synthesized Nano complex can be a promising factor in the treatment of cancer.

Keyword: MOF, Methotrexate, Folic acid, colon cancer, Chitosan, drug delivery

Preparation and evaluation of nano metal-organic framework coated with chitosan and conjugated with Folic acid containing methotrexate for targeted chemotherapy of folic acid receptor positive cancer cells

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Abstract

Cancer is still one of the most challenging diseases. Over the past two decades, new drug delivery systems have been developed that have alleviated the problems associated with chemotherapy. These systems include nanoparticles containing organic and inorganic compounds. Nanoparticles are used in a variety of applications, such as drug delivery to cancer tumor cells, invading cancer cells, and increasing the sensitivity of cancer cells to imaging and observing them more closely. Nanoparticles have good potential for cancer prevention, diagnosis and treatment. In the field of health, one of the important challenges is the efficient delivery of drugs in the body using non-toxic nanoparticles. Most of the available carriers show weak drug loading or rapid drug release (usually less than 5% by weight of the transported drug versus carriers). In this context, porous solids, with the ability to adjust their structure and porosity for better interaction Drugs and high loads are well-suited as nanoparticles for drug delivery and imaging. Metal and Organic Frameworks (MOFs) are a unique class of composite porous solids based on metals and organic bonds. In compare with traditional porous materials, they have a larger surface area, adjustable pore shape size and larger pore diameter, adjustable composition and functional pore surface, which give them unique advantages and properties for applications in adsorption and release therapeutic drugs are possible. In the present study, a Nano porous metal organic framework (MOF) based on zinc [Zn2(bdc)2(dabco)] coated with a monodisperse layer of chitosan was synthesized as a pH-responsive and target-selective system for delivery of methotrexate (MTX) that was conjugated with folic acid is affect in the treatment of folic acid receptor positive cancer cells such as colon can-

cer. Porous metal organic framework [Zn2(bdc)2(dabco)] was synthesized under solvothermal conditions. We were evaluated the drug release at 4°C, RT, and 37°C. MOF nanoparticles of zinc acetate were first synthesized in dimension of less than 200nm. Characterization of samples was studied by DLS, Fournier transform infrared (FT-IR), X-ray powder diffraction (XRD), Transmission Electron Microscope (TEM) and Scanning Electron Microscopy (SEM). Cytotoxicity was assessed by MTT assay and also apoptosis and cell cycle inhibition were evaluated by flow cytometry test. In addition, gene expression of autophagy and apoptosis was examined by Real Time PCR. The targeted nanoparticle was displayed 78% of drug loading efficiency in 3 days. In addition, cell toxicity, apoptosis, cell cycle inhabitation was assessed by cellular and molecular methods. In vitro cytotoxicity assessment and IC50 values for the human colon cancer cell (HCT116) was determined by MTT assay which indicated high and significant mortality of the targeted nanoparticles (MOF-CS-FA-MTX) in the HCT116 cell lines in comparison with the HGF as a normal cell. The cell death type was confirmed by apoptosis and cell cycle control tests. The molecular analyzes of the treated cells show high expression of BAX, ATG5, BCL2 apoptosis genes and Beclin1, MTORC1 autophagy genes and GAPDH as a control gene. Then, we examined the results of each of them on gel electrophoresis. The results obtained in this study revealed that this specific nanoparticle had suitable morphological properties and MOF-Chitosan nano systems containing methotrexate and targeted with folic acid Targeted on the cancer cell line with folic acid receptor activated the autophagy molecular pathway. Therefore, our studies in the project show that this synthesized nano complex can be a promising factor in the treatment of cancer.

Keyword: MOF, Methotrexate, Folic acid, colon cancer, Chitosan, drug delivery

Effect of Mixed Lymphocyte Culture (MLC) on the proliferation of allo-immune lymphocytes

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Abstract

Mixed Lymphocytes Culture (MLC), is a method which study the interaction of cell-cell between subpopulations of lymphocytes and the production of compounds caused by these interactions. Activation of T-helper lymphocytes in presence of MLC leads to B-lymphocyte proliferation. Cyclosporine A is an immune system inhibitor agent which inhibit cytotoxic T lymphocytes produced after stimulation of B-lymphocytes against foreign antigens. In alloimmunization, stimulated B lymphocyte can produce antibodies against exposed foreign red blood cell antigen. As B lymphocytes has low lifespan, using adjuvants like cytokines can promote their proliferation and antibody production capacity. The aim of this study was to produce MLC and investigating its effect on proliferation of alloimmunized lymphocytes in vitro. Positive and negative RhD lymphocytes was exposed to each other in vitro. Supernatant was isolated and filtered as MLC. Produced MLC and cyclosporine added to alloimmunized lymphocytes in 4 different patterns and proliferation of cells measured by microscopic observation and trypan blue dye exclusion assay. The most cell proliferation was seen in group received MLC and cyclosporine simultaneously. The lowest proliferation reported in a group received no MLC, nor cyclosporine. According to the results, further investigation on MLC as an effective agent on proliferation of stimulated B lymphocytes which can lead to subsequent production of antibody in these cells is recommended

Keyword: MLC, Alloimmunization, Lymphocytes proliferation

Nanoencapsulation of Radachlorine to increase the efficiency of photodynamic therapy

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Abstract

Photodynamic therapy is a new clinical and non-invasive treatment for cancers and some other diseases. This treatment uses a combination of three non-toxic factors, *i.e.* a photosensitizer, molecular oxygen and light to kill cancer cells. In this study, we report for the first time that Radachlorine loaded poly-(D, L lactic-co-glycolic acid) (PLGA) nanoparticles (Radachlorine-PLGA NPs) leads to enhanced photodynamic therapy efficiency when compared with Radochlorine-mediated photodynamic therapy alone. Radachlorine-PLGA NPs were prepared using water/oil/water double emulsion solvent evaporation method and then characterized. The photocytotoxicity was evaluated on MDA-MB٢٣١- cells. Cells were incubated for ٤ hours with Radachlorine alone or Radachlorine-PLGA NPs and then exposed to light (٦٦٢ nm) for ٢ min, and light dose of ١٠ J cm^{-2}. After 24 h of incubation, the cellular viability was measured using the MTT assay. Our results showed that Radachlorine-PLGA NPs increase phototoxicity and lead to 80% cell death. The IC_{50} for Radachlorine-PLGA NPs was lower than the IC_{50} value for the free Radachlorine on MDA-MB-231 cells. The IC_{50} value was 0.68 mg/ml for Radachlorine and 0.44 mg/ml for Radachlorin-PLGA NPs. The results obtained in this study suggest that the encapsulation of Radachlorine in PLGA nanoparticles may be a promising strategy to improve the efficacy of conventional photodynamic therapy.

Keyword: Photodynamic therapy, Radachlorine, Nanoencapsulation, Cancer

Design & expression of a new artilysin against antibiotic resistant *A. baumannii*

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Abstract

Currently, antibiotic resistance of pathogenic bacteria is a critical problem needs to specific consideration of scientists. Gram-negative *Acinetobacter baumannii* stands on top of the WHO list of antibiotic resistant bacteria and leads to 58 % of death in patients with this infection. Bacteriophage endolysins hydrolyze peptidoglycan layer of bacterial membrane is suitable candidate among different substitutes are suggested instead of current antibiotics. There are several reports show significant antibacterial effect of these enzymes. In addition, there aren't probability of bacterial resistance to phage endolysins and this is important benefit of these antibacterial enzymes. In this research, we designed a recombinant peptidoglycan hydrolase fusion gene and then expressed it in *E. coli* host. We used a modified positive charged antibacterial peptide, BMAP-27B cathelicidin and an *A. baumannii* specific phage endolysin, Ply F307, linked with rigid linker in artilysin gene construct. Cathelicidines are small positive peptides naturally produced by immune system of human and some animals against infective bacteria. For artilysin fusion gene design, nucleotide sequence of BMAP-27B was located on 5' end as one partner and Ply F307 endolysin gene combined to it after linker sequence on 3' site as the other one. The designed fusion gene was cloned in pET-28a expression vector after it synthesized and replicated in *E. coli* DH5α. For this purpose, competent *E. coli* DH5α was transformed with synthesized gene harboring plasmid at first. Competent cell preparation was done through $CaCl_2$-$MgCl_2$ chemical approach and transformed cells were cultured at 37°C and agitating rate of 250 rpm for 20h. Then, designed fusion gene was isolated from parent plasmid with Nde1 and Xho1 double digesting and extracted from low melting agarose gel. The gene was inserted to Nde1.Xho1 double digested pET-28a under overnight ligase treatment. *E. coli* DH5α transformed with recombinant plasmid was incubated overnight in 37°C. For screening of positive transformed clones, each colony was cultured in liquid media and plasmids were extracted from them. Recombinant plasmids harboring the designed fusion gene was introduced

in *E. coli* BL21 (DE3). For expressing of designed protein recombinant hosts were induced with 1mM IPTG as inducer in 37°C. Cloning of fusion gene was confirmed with Nde1.Xho1 double digestion in 37°C for 2h and sequencing. Expression results verified with western blotting was don fallowing protein bond observation in SDS-PAGE.

Keyword: Artilysin, *Acinetobacter baumannii*, Antibiotic resistant, Endolysin, Recombinant

Cloning & expression of a new chimeric endolysin including cell wall binding domain KZ and PlyF307 endolysin against antibiotic resistant *A. baumannii*

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Abstract

Following WHO announcement about resistance of dangerous pathogenic bacteria to usual antibiotics, using of recombinant phage endolysins was interested for combating with these infective agents. One of antibiotic resistant bacteria is *A. baumannii*, a non-fermentative Gram-negative coccobacillus, leads to high rate of deaths caused by hospitals infections. Although the recombinant endolysins show the significant effect on Gram- positive bacteria, in Gram- negative bacteria the outer membrane causes to desirable result wouldn't see. Therefore, scientists use different ways to rise their functionality on Gram-negative bacteria. This research is looking for to fusing peptidoglycan binding domain of *ØKZ* phage endolysin, KZ144, to PlyF307 endolysin and its cloning and expression in *E. coli*. For this purpose, nucleotide sequence of CWB-KZ was ligated to PlyF307 sequence through a rigid linker. Sequence arrangement of fusion gene was CWB-KZ, linker and PlyF307 from 5' to 3' respectively as shown in schematic figure. CWB-KZ is cell wall binding domain of KZ144 enzyme from *ØKZ* bacteriophage that infects *Pseudomonas aeruginosa*. PlyF307 also is a small globular protein made by *A. baumannii* specific phage has proper effect on different species of it when produced in recombinant form. Synthesized fusion gene harboring plasmid was amplified in *E. coli* DH5α. Then, amplified gene was introduced in pET-28a plasmid and expressed in *E. coli* JM109 (DE3). For this purpose, parent plasmid bearing synthesized fusion gene at first was amplified in competent *E. coli* DH5α for 20 hours at 37°C with 250 rpm agitation rates. Making of competent cells was done by chemical method of $CaCl_2$-$MgCl_2$. Then, Nde1 and Xho1 endonucleases were used for cleaving plasmid contains synthesized gene and releasing of it. In this time, released gene was cut and purified from low melting agarose gel. Purified gene was ligated to Nde1.Xho1 double digested pET- 28a

under DNA ligase reaction for 16h. Following to transformation of *E. coli* DH5α with ligation product, the transformed cells were incubated overnight at 37°C in LB agar contained kanamycin as selective marker. The colonies were cultured in LB broth and plasmids were extracted subsequently. Plasmids harboring designed gene were introduced in competent *E. coli* JM109 (DE3) as host and the fusion gene was induced by 1mM IPTG for 5h at 30°C. Cloning of new chimeric endolysin was confirmed by 2h Nde1.Xho1 double digestion in 37°C at first and nucleotide sequencing followed it. Protein expression also evaluated through SDS-PAGE and then verified by western blotting. In this step, it needs to optimize expression process of chimeric protein and study about its antibacterial function after purification.

Keyword: Cell wall binding domain -KZ, *Acinetobacter baumannii*, Antibiotic resistant, Endolysin, Recombinant

Fabrication and evaluation of novel antibacterial wound dressing containing curcumin and surfactin

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Abstract

The skin is the largest organ in the body and protect internal organs from external injuries, so any perforations in this barrier leave the body susceptible to infections. Therefore, fabrication of an antibacterial wound dressing is a promising strategy for the advancement of wound care. Due to the expanding concern regarding antibiotic resistant bacteria, polymeric nanofibers loaded with nature-derived compounds have shown a remarkable potential for wound dressing. The present study utilized a double-nozzle electrospinning technique to yield an antibacterial wound dressing composed of curcumin (Cur) and surfactin (Sur)-loaded poly(ε-caprolactone) (PCL)-gelatin (Gel). In order to prepare polymeric solutions for electrospinning, a weighed amount of PCL was dissolved into chloroform-methanol (3:1, v/v) mixture to prepare a 15 % w/v solution and stirred for 6 h. Then, Sur was added to the PCL solution. In a parallel manner, an appropriate amount of Gel was dissolved into acetic acid (90% (v/v)) to prepare a 15 % w/v solution and stirred for 6h. Then, Cur was added to the Gel solution. The prepared dressings were characterized by scanning electron microscopy (SEM), water contact angle measurement, tensile testing, antibacterial test, and Cur release study. The cytotoxicity and cell attachment on the prepared dressings were assessed *in vitro*. The SEM micrographs proved that with the addition of Sur into the PCL solution, the mean fiber diameter decreased

from 657 ± 203 nm to 530 ± 115 nm (P<0.05). The possible mechanism for this phenomenon can be found in the chemistry of Sur which is an anionic surfactant and it may have decreased the surface tension of polymeric solution. It should be noted that the effect of Cur on the Gel's fiber diameter was not significant (P>0.05). The SEM images showed that the PCL/Sur-Gel/Cur fibers are smooth with an average diameter of 387 ± 127 nm. The PCL fibers exhibited a high water contact angle of 143.68 ± 0.58° which confirms the hydrophobic nature of PCL. Unlike PCL, Gel fibers exhibited a low contact angle of 23.25 ± 2.56° proving the hydrophilic nature of Gel. The hydrophilicity of PCL was found to improve significantly when both Gel and the drug molecules were added (P<0.05). The contact angle of PCL/Sur-Gel/Cur scaffold was observed to decrease to 20.36 ± 1.35° which can be resulted from the hydrophilic chemical groups of Gel, proton donor oxygen in Cur, and decrease the surface tension by Sur. Interestingly, it was found that Cur and Sur worked as a plasticizer and improved the elongation at break of samples. The results showed that the addition of Cur and Sur caused a decrease in the elastic modulus from 33.61± 4.44 MPa to 18.612.45 ± MPa and an increase in the tensile strength and elongation at break from 1.150.17 ± MPa to 2.460.07 ± MPa and 28.664.13 ± % to 865.12 ± %, respectively. The dressings encompassing Sur exhibited an excellent antibacterial activity against *Staphylococcus aureus* as commonly found bacteria in wounds infection after 24 h (>99%). Release studies showed that, the existence of PCL fibers among the Cur-loaded fibers reduced the burst initial release of Cur (50 % after 8 h) and exhibited a sustained Cur release up to a period of 2 weeks and released about 90% of Cur in this time point. Moreover, prepared dressings exhibited desirable cell viability and attachment for human adipose-derived stem cells without any cytotoxic effect. Taking together, the PCL/Sur-Gel/Cur scaffold can be a suitable candidate for wound dressing applications due to the optimum properties such as suitable mechanical properties, good antibacterial activity, and high biocompatibility.

Keyword: Electrospinning, Polycaprolactone, Surfactin, Curcumin, Wound dressing

Investigating cytotoxic effects of benzothiazolopyrimidine derivatives on cancer cell lines *in vitro*

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Abstract

Cancer is known as the second cause of death worldwide. Breast and colorectal cancer account for first and third most common cancers, respectively. Chemotherapy is considered as one of the standard treatments for cancer. Drug resistance and series side effect are two main challenges of chemotherapy. To overcome these problems, novel anticancer agents are being developed. Antiproliferative properties of benzothiazolopyrimidine as an attractive drug scaffold have been investigated in many studies. The aim of present study was to evaluate the cytotoxic effects of two synthetic benzothiazolopyrimidine derivatives on cancer cell lines *in vitro*. To determine IC_{50} values of benzothiazolopyrimidine derivatives, MCF-7 and HCT 116 cancerous cells were treated with different concentrations of compounds for different time periods and cell viability was assessed using MTT assay, followed by data analysis with GraphPad Prism 8.3.0 software. The results demonstrated that benzothiazolopyrimidine compounds had significant toxic effects on HCT 116 colorectal cancer cells at p-value <0.05. Our results indicated that benzothiazolopyrimidine with bromide group at a concentration of 50 to 100 µM inhibited HCT 116 cell growth at 48 and 72 hours. These results suggest that benzothiazolopyrimidine derivatives have antiproliferative effects on colorectal cancer cells.

Keyword: Cancer, Cytotoxicity, MTT assay, Benzothiazolopyrimidine

Production and integration of glioblastoma cell spheroid in microfluidic chip

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Abstract

Spheroids are an attractive example of 3D cell culture for cell-related testing. Now a days, spheroids are used as the most popular 3D cell culture model in various fields such as research, discovery and specification of drugs, effective dose determination, study of processes such as migration and metastasis. Various techniques have been introduced to produce the spheroid model, in which microfluidic chips are considered as an acceptable method of self-confidence in terms of efficiency, repetition and control of animation, important components in the formation of the spheroid, and the creation of similar physiological conditions. Microfluidic chips cells only make the conditions necessary to create spheroid in the desired number, measurement and composition, the possibility of cellular studies such as the integration of cancerous masses in the chip itself. In this study, a well-shaped chip was designed and fabricated to produce spheroid in large numbers and similar measurements. The geometric structure of the chip is such that it is possible that after the spheroid has formed, they are taken out of the wells and the important components in their integration are in the distances between the chips. One of the benefits of integrating spheroids from different cell lines is that they create heterogeneous conditions that better mimic the body physiological conditions and produce acceptable reliable results in drug tests

Keyword: Spheroid, Microfluidic, Spheroid integration

Designing of sgRNA and donor DNA for disruption of PUF5 gene in *Leishmania major* through CRISPR/Cas9 system

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Abstract

Leishmaniasis is a common infectious-parasitic disease in humans and animals caused by more than 20 species of *Leishmania* parasites belonging to the Trypanosomatid family. The parasite is transmitted through sandfly bites to vertebrate hosts, including humans. One of strategies in designing an effective vaccine against infectious diseases is the preparation and use of genetically attenuated microorganisms. One of the candidate proteins for genetic attenuation of the parasite is PUF5 which is one of the RNA-binding proteins (Pumilio RNA-binding protein) and is involved in post-transcriptional modifications in stability or instability of primary transcript. The main goal of this study is investigate the role of PUF5 in *Leishmania major* by gene deletion using CRISPR system. For this purpose, in the first step, sgRNA primers and donor DNA sequences are designed. In this project, five different software including: LeishGEdit, EuPaGDP, CCTop, CRISPOR and CHOPCHOP were used and sgRNA primers with the highest efficiency score for both 5' and 3' ends of gene were selected. Next, donor DNA sequences were designed to substitution Puf5 gene with an antibiotic marker. These two primers, after amplification in the presence of a vector carrying the antibiotic resistance gene as a template, are placed in both sides of that to cause cross-over recombination and resulting the replacement of the marker gene with the PUF5 gene.

Keyword: Gene deletion, *Leishmania major*, PUF5, sgRNA, CRISPR/Cas9.

Expression and intein mediated purification of P28-IL24 and P28-M4 fusion proteins for targeted cancer therapy

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Abstract

Targeted cancer therapy by means of cell-penetrating peptides that have the ability to deliver cytotoxic agents only to cancer cells and have no effect on normal cells is a good way to solve the problems caused by chemotherapy. Azurine is a CPP secreted by Pseudomonas aeruginosa and is a member of the cupredoxin family of copper-containing proteins that can specifically penetrate cancer cells and have cytostatic and cytotoxic effects. P28 is an Azurin-derived peptide that has specific transportability to some cancer cell lines. We previously constructed fusion proteins P28-IL24 and p28-M4 as targeted agents against breast and uterine cancer cells since IL-24 and M4 have pro-apoptotic properties. However, the expression yield for the fusion proteins was very low, which could be due to the toxic effect of P28 on host bacterial cells. In the current study, in order to increase the yield, intein mediated protein purification was performed. Hence, the amino terminus of P28 can be coated by intein to prevent toxicity toward the host cell. The P28-IL24 and P28-M4 nucleotide sequences were subcloned into the *NcoI* and *XhoI* sites of the pTWIN1 plasmid, using standard molecular biotechnology techniques. The expression of recombinant proteins was increased compared to our previous study. The best condition for total protein production was determined to be 0.8 mM IPTG, incubation at 37 °C, and for 6h. Finally, increased ratio of soluble protein fraction was obtained at 0.1mM IPTG, and 24h of incubation at 4 °C.

Keyword: pTWIN, Cell-penetrating peptide, Interleukin 24, M4, P28

Synthesis of keratin nanoparticles from poultry waste

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Abstract

Keratin is a natural biopolymer that has attracted much attention in recent decades due to its disulfide cross-links, hydrophobic interactions associated with negative electric charge, and the presence of hydrogen bonds in its strong mechanical structure. As the main waste produced from poultry farms and slaughterhouses, they are produced in large quantities daily. They have a high keratin protein content and are considered one of the most abundant sources of this protein. Over the past century, this protein has led to the introduction of nanotechnology and the production of keratin-based biomaterials. In the present study, keratin nanoparticles were synthesized from chicken feathers. The structure and morphological properties of keratin and keratin nanoparticles were investigated using FESEM, FTIR, XRD, and Biuret test characterizations. X-ray diffraction pattern showed a crystalline peak at 37.5 and 20°C, indicating keratin and keratin nanoparticles, respectively. The crystal size of keratin nanoparticles was 75.6 nm. The presence of amides I, II, and III was also confirmed in the FTIR analysis. This study aimed to synthesize keratin nanoparticles from chicken waste, obtained by using glutaraldehyde as a cross-linking agent. Keratin nanoparticles have physical and chemical properties. They are unique and can provide new directions of application in medical and other fields.

Keyword: keratin Nanoparticles, Glutaraldehyde, Poultry Waste, Data Analysis

Fabrication of hepatic cell-sheet using decellularized extracellular matrix and thermoresponsive polymer

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Abstract

Liver tissue engineering through cell sheet technology could provide new options for the treatment of patients with liver failure. Decellularized extracellular matrix (ECM) along with the potential of temperature-responsive polymer (pNIPAAm) as an intelligent surface could develop the hepatocyte cell sheets (HLCs). In this study three in vitro microenvironments models were generated that including I: pNIPAAm hydrogel (pN hydrogel), II: decellularized ECM incorporated into pNIPAAm hydrogel (dECM + pN hydrogel), and III: decellularized ECM scaffold (dECM scaffold). Then adipose tissue-derived mesenchymal stem cells (AT-MSCs) were differentiated on hepatocyte-like cells and their structure and function were investigated. dECM scaffold was obtained after decellularization of rat liver, and its efficiency was analyzed. pN hydrogel and dECM + pN hydrogel (1:3 and 2:3 ratios) were made, and scaffold structure was analyzed. After coating each well of culturing plates with these constructs, AT-MSCs were cultured and differentiated into HLCs. After recellularization, differentiation patterns, and hepatogenic marker expression were studied at different time points via biochemical tests and qRT-PCR. Multipotency of AT-MSCs was confirmed via positive results for osteogenesis and adipogenesis. The dense and intact cell sheets were produced in dECM + pN hydrogel, in comparison with pN hydrogel and dECM scaffold. Also, the expression level of hepatocyte markers including, alpha-fetoprotein, cytokeratin 18, cytochrome P450-2E1, and phosphoenolpyruvate carboxykinase showed a statistically significant difference in dECM + pN hydrogel.

Keyword: cell sheet, pNIPAAm hydrogel, extracellular matrix, decellularized, liver

A review of strategies to improve expression and efficiency of CAR-T cells

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Abstract

Cancer therapy has evolved over time, and in recent decades, has shifted from traditional approaches such as surgery, chemotherapy, and radiotherapy that are not very effective to immunotherapy. One of the newest and most promising cancer immunotherapy strategies in CAR technology, or chimeric antigen receptors, through which T lymphocytes will be able to specifically recognize tumor antigens and eradicate the tumor in an MHC-independent manner. Chimeric antigen receptors (CARs) have achieved significant success in treating some hematological malignancies such as lymphoma and leukemia. Typically, several factors likely contributed to the efficacy of a CAR-T cells therapy that needs to be considered while designing a CAR-containing expression cassette and the vector used to transfer the CAR construct to T lymphocytes. In this review, we aim to indicate some of these factors and explain their role in increasing CAR-T expression and therapeutic efficacies.

Keyword: Chimeric antigen receptor (CAR), Expression cassette, RRE, cPPT, WPRE

Evaluating the in vitro anti-tumor activity of Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells

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Abstract

Chimeric antigen receptors (CARs) are innovative receptors designed to bring together the superiority of BCRs and TCRs. So far, CARs which target CD19 showed tremendous therapeutic effect and led to complete remission in patients with chemorefractory or relapsed B-cell malignancies however solid tumors are still a major of concerns. Among the different problem lacking tumor specific antigen is of great importance. Mesothelin is a glycoprotein which is highly expressed on tumor cells but rarely express on normal tissue; So, we have chosen this antigen as target and tried to produce CAR T cell using the IVT prepared mRNA. This CAR T cells are safer to the labile nature of mRNA. To these ends, we have extracted a fully-human anti MSLN CAR construct sequence from a patent with the US number 9272002 B2. This construct harbors a fully human single chain variable fragment (ScFv) against mesothelin fused with a CD8a hinge, and intracellular T cells signaling domains of 41BB and CD3z. We cloned this construct in a suitable vector for IVT and electroporated the generated mRNA to T cells and could successfully overexpress the CAR MSLN construct in human primary T cells. we could prove that this CAR construct was functional in terms of proliferation, cytotoxicity and cytokine secretion.

Keyword: CAR T cell therapy, IVT, mRNA, TGFβRII.

Interference of mmu-miR-96 in insulin signaling pathway in mRPE cell line

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Abstract

Diabetic retinopathy (DR) is one of the most common cause of blindness which is associated with the progression of diabetes. One of the key biochemical pathways in diabetic retinopathy is impaired insulin signaling. The miR-183 cluster, which includes miR-183, miR-96 and miR-182, is highly expressed in the retina and plays an important role in retinal development through targeting the cognate genes expression. Pri-mmu-miR-96 codding gene was taken from NCBI. The cleavage sites of KpnI and XhoI enzymes were inserted in upstream and downstream of the sequence, respectively. The gene was ordered and the synthesized gene was sub-cloned to pAAV2-MCS-EGFP vector by using the related enzymes. PRKCE, Akt2, FOXO4, SGK genes involved in the insulin signaling pathway, predicted by TargetScan database, were selected as miR-96 targets, for further studies. The developed construct was extracted at maxi prep scale. mRPE cell line was transfected by the vector through calcium phosphate method and RNA extraction was performed 48 hours later. Using Real-Time PCR, expression of PRKCE, Akt2, FOXO4, SGK genes was measured. Recombinant vector named "AAV2-MCS-EGFP-mmu-miR-96" was constructed and accuracy of the cloning was confirmed by restriction enzyme digestion. Stem loop Real-Time PCR confirmed that, the cells overexpressed the desired gene. Quantitative expression of PRKCE, Akt2, FOXO4, SGK genes by Real-Time PCR showed that, the expression was changed 0.29, 0.3, 2.8 and 0.77 times, respectively, compared to the control. This information suggests that miRNA-96 interferes with insulin signaling and inhibitors such as antagomir-96 are promising in reducing symptoms in DR.

Keyword: mmu-miR-96, mRPE cell line، Akt2، FOXO4, Insulin signaling.

An example of application of minigenes in expression of the peptides involved in the regulation of cellular signaling pathways

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Abstract

Currently, the use of small peptides (up to 20 amino acids) in activating or inhibiting different cellular signaling pathways is very common in biological research. However, synthesis of such peptides with acceptable purity is expensive and also there is an uncertainty in the stabilization of these peptides in the cells. An alternative way to overcome these limitations, is construction of minigene expression vectors expressing the appropriate peptides in the cell. It is expected that the peptide will be made as long as the minigene construct exists in the cell. If possible, stable transfection of these constructs can produce cell lines which make the peptides for a longer period of time. We have successfully used such a minigene construct to produce a short peptide in colon cancer cells to study the interaction between Gaq (a family of alpha subunits of trimeric G-proteins) and b-Catenin signaling pathways. We briefly present the results in this paper and in this conference.

Keyword: minigene constructs; short peptides; signaling pathways

Calculation of penetration diffusion coefficient of tetracycline in Poly (vinyl alchol) based polymer hydrogels

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Abstract

Wound dressings are the biomaterials that promote wound healing by providing a suitable micro-environment. Among the wound dressings, special attention has been given to hydrogel wound dressings due to their unique properties which can meet the essential requirements of ideal wound dressings. The results of biomedical properties indicated that hydrogel films are non-thrombogenic, non-hemolytic, antioxidant, and mucoadhesive, and are permeable to oxygen and moisture while impermeable to micro-organisms. In this study, tetracycline hydrochloride was released from biodegradable polymer PVA wound dressings and the penetration diffusion coefficient of tetracycline in PVA hydrogels was calculated experimentally using the diaphragm cell technique. According to the objectives of the project in the experimental section, after preparing PVA polymer hydrogels by the freezing-thawing method, the drug release and diffusion coefficient of hydrogels were investigated by ultraviolet (UV) spectroscopy. The diaphragm cell consists of two tanks separated by a membrane of the hydrogel. Tank number one contains PBS and the drug and tank number two contains PBS. As can be expected, the concentration of the drug in Tank 1 decreases over time, while there is a corresponding increase in the concentration of tetracycline in Tank 2. At any time, the concentration values in the two tanks can be used to calculate the diffusion coefficient.

Keyword: Wound dressings, hydrogel, Poly (vinyl alchol), Diffusion coefficient

Physicochemical characterization of MiRGD peptide nanocarrier for delivering anti-angiogenic sFLT01 gene to retinal pigment epithelial cells

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Abstract

Nonviral vectors with low toxicity and immunogenicity have been developed to advance the delivery of active materials. Recombinant MiRGD peptide carrier is composed of several biological motifs to overcome barriers in gene transduction pathway. We aimed to study physicochemical properties of MiRGD peptide carrier to efficiently transfer sFLT01, anti-angiogenesis gene, into retinal pigment epithelial cell (RPE). Recombinant peptide was purified by Ni-NTA affinity chromatography. Purity of the carrier was determined by SDS-PAGE. sFLT01 gene was cloned and transformed into the desired bacteria. Nanoparticles were produced by MiRGD nanocarrier and sFLT01 plasmid at different N/P Ratios. DNA binding of MiRGD carrier was examined by gel retardation assay. The stability of MiRGD/pDNA nanoparticles in the presence of serum was investigated. Size and zeta potential measurements of nanoparticles were performed using DLS. Activity of HIV gp41 motif of MiRGD peptide was determined by hemolysis assay. DNA binding motif of 16 mer histone H1 of MiRGD peptide effectively condensed pDNA and neutralized its negative charges, through increasing N: P ratio. Nanoparticle was stable in the presence of serum and effectively protected pDNA from degradation by the serum nucleases. Size of nanoparticle decreased and the surface charge of the MiRGD/pDNA nanoparticles remained positive at higher N: P ratios. HIV gp41 motif of peptide showed hemolytic activity in both acidic environment (pH 5.4) and either physiological condition (pH 7.4) which showed endosome disruptive activity of peptide. As a result,

MiRGD peptide was promising as gene carrier in further experiments in RPE cells.

Keyword: Drug delivery, Peptide nanocarrier, sFLT01 gene, nonviral vectors, RPE

In-silico design by semi-rational method to improve Soybean Peroxidase enzyme functionality

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Abstract

Peroxidases are oxidoreductases found in almost every living organism playing an important role in physiological processes. They are of the key antioxidant enzymes controlling reactive oxygen species (ROS) by catalyzing the oxidation of electron donor substrates also exploiting the reduction of hydrogen peroxide to water, making them useful biotechnological tools with the potential for a range of applications, such as medical kit development, immunoassay tests, and bioremediation. these enzymes have been obtained from a natural source, a process that is laborious and affected by different uncontrollable conditions and results in low yields. To overcome this hurdle, efforts have been made to heterologously express them in different expression systems to construct a more commercial way for its production but this process has been plagued by inclusion body formation and limited availability of heme and iron within a bacterial cell, etc. Our candidate enzyme Soybean Peroxidase (SBP) exhibits significantly high thermal and conformational stability and high enzyme activity both in the organic and inorganic substrates. But even when successful recombinant enzymes showed reduced function and activity so in this study, we took a step back optimizing this enzyme before recombinant production using previous studies on HRP-C and SBP and utilizing computational tools and in silico design we were able to significantly improve the affinity of the enzyme for the favorable ligand (ABTS) by inducing these mutations N197D, L237K, N240E, R231K, S234V, L237Q, Q238E, N262R, and improve the enzyme shown in the molecular dynamics analysis.

Keyword: In-silico design, Soybean Peroxidase enzyme, Enzyme Improvement, Nanome, GROMACS

Expression and intein mediated purification of P22-DT-386BR2 fusion protein for targeted cancer therapy

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Abstract

Due to the adverse side effects of current cancer chemotherapeutics development of targeted anti-cancer medications is under intensive focus. We previously constructed fusion proteins p28-DT (as control) and p22-DT386BR2 as targeted agents against breast and uterine cancer cells since DT-386BR2 have pro-apoptotic properties. However, the expression yield for the fusion proteins was very low, which could be due to the toxic effect of p28 and p22 on host bacterial cells. In the current study, in order to increase the yield intein mediated protein purification was performed. Hence, the amino terminus of p28 and p22 can be coated by intein to prevent toxicity toward the host cell. The p28-DT and p22-DTBR2 nucleotide sequences were subcloned into the *Nco*I and *Xho*I sites of the pTWIN1 plasmid, using standard molecular biotechnology techniques. The expression of recombinant proteins was increased compared to our previous study. The best condition for total protein production was determined to be 0.8mM IPTG, incubation at 37°C, and for 6h. Finally, increased ratio of soluble protein fraction was obtained at, 0.1mM IPTG, and 24h of incubation at 4°C. Purification of soluble protein was performed by IMPACT-TWIN Purification Kit at 24h of incubation at 4 °C.

Keyword: pTWIN, Cell-penetrating peptide, Diphtheria toxin linked to BR2, DT-386BR2, DT

Construction and Evaluation biocompatible magnetic nanofiber with controlled release of anticancer drug

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Abstract

Breast cancer is one of the most common cancers in human societies. In traditional methods of cancer treatments, like chemotherapy, the drug is injected intravenously and systemically absorbed and metabolized which results in affecting both healthy and cancerous cells. In modern approaches, different nanoparticles are used as smart carriers for controlled and directed drug delivery. Among them, iron oxide nanoparticles have been investigated and used vigorously owing to their magnetic properties used in directed and focused drug delivery and their potential in hyperthermia treatments by applying an external magnetic field. In the present study, a biodegradable and biocompatible nanofiber was constructed using PLLA, loaded with iron oxide nanoparticles and the widely used anticancer drug doxorubicin. The morphology and chemical structure of the fibers were investigated with SEM and FT-IR. The diameter of nanofiber is about 304.7. The presence of iron oxide nanoparticles in the nanofibers was proved by EDX method. Examination of drug release pattern showed a slow release of doxorubicin of 74% over 21 days. The hydrophilicity, mechanical and magnetic properties of nanofiber were also investigated. Also, MTT assay was invoked to assess any probable scaffold cytotoxicity on cancer cells. Collectively our results showed that our biocompatible drug carrier platform with paramagnetic and hyperthermia properties has high potential for controlled and slow drug release with potential application to be used as a drug release prosthesis specially in tumor resected tissues or prevent tumor relapse.

Keyword: paramagnetic, controlled release, hyperthermia, PLLA, cancer

Design, Fabrication and Characterization of 3D Composite scaffolds of Amniotic Membrane/Hyaluronic Acid/Nano-hydroxy Apatite for application in bone/cartilage interface

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Abstract

Today, treatment of injuries to cartilage and bone tissues has become a main issue in health organizations. Tissue engineering can vastly help osteochondral regeneration via introducing osteochondral grafts that can facilitate bone and cartilage fusion at their interfaces. In the present study, to mimic structure and ECM of osteochondral tissue, we fabricated 3D spongy scaffolds of Amniotic membrane and Hyaluronic acid via freeze-drying. By crosslinking the scaffolds with NHS/EDC, their degradation rate reduced vastly. In order to strengthen scaffolds and improve their mechanical properties hydroxy apatite was introduced in to the composite scaffolds. In order to assess mechanical properties of the two studied groups (Amniotic membrane/ Hyalorunic acid and Amniotic membrane/ Hyalorunic acid /Hydroxy Apatite), we measured compressive strength of the spongy scaffolds in their wet state. Porosity, morphology and cell/scaffold interaction were examined via SEM and alcohol absorption tests. Biodegradability, biocompatibility (via MTT assay) and cell infiltration assays (via DAPI staining) were all performed. Data showed appropriate porosity and pore size for cell infiltration. No cytotoxicity associated with scaffolds or their degradative by-products on fibroblasts was observed. Collectively, our results showed that the fabricated 3D spongy scaffolds were suitable for bone, cartilage and osteochondral tissue engineering regarding their mechanical, morphological and biological properties.

Keyword: Osteochondral tissue engineering, Amniotic Membrane, Hyaluronic Acid, Hydroxy apatite, Scaffold

Preparation, evaluation and in vivo assessment of nanofibers containing herbal extracts with antibacterial agents to prevent and control bacterial infection with *Staphylococcus aureus*

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Abstract

Bacterial contamination is one of the problems which has made wound care and control difficult to control and restrict. The Chitosan/Poly ethylene oxide electrospun nanofibers containing *Calendula officinalis* extract were prepared and the morphology of the nanofibers was examined with scanning electron microscopy (SEM). The bonds in nanofibers were investigated by Fourier Transform Infrared spectroscopy (FT-IR). Other properties such as water uptake were also investigated. Antibacterial test was performed to evaluate the antibacterial effect of nanofibers and the MTT test was also performed to examine the biocompatibility. The results showed that the prepared fibers are homogeneous and without beads, and FT-IR results confirmed the presence of the extract in nanofibers. Water uptake of the nanofibers were increased and nanofibers showed a significant antibacterial effect against Staphylococcus aureus. According to the obtained results, it seems that the nanofibers containing *Calendula officinalis* extract can serve as a suitable candidate for wound dressing to treat and control wound infection and facilitate wound healing.

Keyword: Wound infection, herbal extract, nanofiber, electrospinning.

Comparison of sensitivity and specificity between IHC and FISH tests for Her2 amplification in paraffin embedded breast cancer blocks

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Abstract

Breast cancer is the leading type of malignancy-related death in women worldwide. It is well documented that 20%-30% of breast cancers show her2 amplification or overexpression. Determination of HER2 amplification is performed mainly by IHC and FISH techniques. The gold standard FISH required advanced technique, expertise, and more costs than the IHC method. This study aimed to determine the sensitivity, specificity, and accuracy rate of IHC compared to the gold standard FISH technique. The IHC results from Paraffin-embedded breast carcinoma tissues belonged to 44 women with breast cancers were compared with the FISH method. The FISH results represented 16% false positive and 9% false negative in comparison to IHC. The sensitivity of IHC for identifying true HER2+ was equal to 66.6%, and the specificity of IHC in determining true negatives was equal to 78.1%. Hence, the FISH method is more accurate even for the negative IHC results. The confidence level of IHC positive results was 53.3%, for negative results were equal to 86.2%. These results have shown that the accuracy of the IHC method is far beyond standard compared to the FISH method.

Keyword: FISH, IHC, HER2+, Breast cancer

Isolation and characterization of exosomes separated from animal samples induced by myocardial infarction

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Abstract

Cardiovascular disease is the most cause of death in all countries. Myocardial infarction tests are usually meaningful and measurable after a stroke.in recent years the replacement of new methods for timely detection with proper prognosis and the use of exosomes as membrane _ like particles capable of carrying various compounds has been considered. The use of exosome has fewer risks and more benefits compared to cell therapy. In this study 20 male Wister rats were given myocardial infarction by Isoprenaline injection . After blood sampling and isolation of serum from blood, induction of myocardial infarction was confirmed by blood tests using standard enzyme tests. Exosomes were then isolated using extraction kits. exosome size and morphology were examined by scanning Electron microscope and size with dynamic light scattering (DLS). After using the injection method of myocardial infarction drug which is less invasive compared to surgical method. The exosome was separated using a 4-degree centrifuge at 3000 rpm. Scanning electron microscope images confirmed the structure and size measurement by dynamic light scattering analysis also showed a single bell_ shaped size distribution with a peak of 80 nm. Our approach for isolation exosome derived from a blood serum sample containing myocardial infarction and confirmation of the nature and morphology of exosomes is an important step for future studies in understanding the function of exosomes and their relationship with cardiovascular disease.

Keyword: Cardiovascular disease, Myocardial infarction, Isolation exosome.

Synergistic effect of glycolipid biosurfactant with ciprofloxacin against methicillin-resistant *staphylococcus aureus*

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Abstract

There is a method currently used to address antibiotic resistance in which biosurfactants, known as antimicrobials, are combined with antibiotics to enhance the final efficacy of antibiotics. Using biosurfactants and antibiotics together is a relatively recent concept, and corresponding data on the possible synergistic impact is limited. The current study has focused on glycolipid biosurfactant synthesis from *Shewanella* Sp. strain 12b and evaluating the synergistic efficacy mixed with ciprofloxacin against methicillin-resistant *Staphylococcus aureus*. After biosurfactant production, it was extracted using ethyl acetate: methanol (3: 1 v/v) solvent. Well diffusion, minimum inhibitory concentration, and minimum bactericidal concentration assays were conducted to assess the biosurfactant antimicrobial and synergistic efficacy. *Staphylococcus aureus* indicated resistance to ciprofloxacin (15.63 µg/ml), with no inhibition zone, but in the presence of the antibiotic with the biosurfactant, the inhibition zone was reported to be 16.33 mm. The biosurfactant functioned synergistically with ciprofloxacin, and the findings showed that the biosurfactant has a high potential to overcome the pathogen's resistance. Accordingly, several effective medicines might be produced against resistant bacteria in the future.

Keyword: Antibiotic effect, biosurfactant, drug resistant, *Staphylococcus aureus*, synergistic effect

Anti-diabetic properties of novel curcumin-polyhydroxy derivatives: Synthesis, molecular docking, and their inhibitory effects on α-glucosidase activity

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Abstract

One of the most prevalent metabolic disorders in humans is diabetes. α-Glucosidase (α-Glu) is an important enzyme in the human intestine that hydrolyze carbohydrates, and inhibition of its activity can lower blood sugar levels to effectively prevent hyperglycemia-induced tissue damage. The present study aimed at synthesizing several new curcumin derivatives and further evaluation of these compounds for possible antioxidant and anti-diabetic properties along with inhibitory effect against α-Glu, as a therapeutic target for attenuation of postprandial hyperglycemia. In search of potent α-Glu inhibitors, we have synthesized curcumin-polyhydroxy derivatives, characterized by FTIR and MS then screened for antioxidant and α-Glu inhibitory activity. The antioxidant potential of the derivatives was evaluated by DPPH and FRAP methods. Also, the stability of curcumin and synthesized derivatives was investigated in the simulated intestinal medium with pH = 7.5 and the gastric environment with pH = 1.2. Finally, the binding interaction details of compounds to α-Glu was determined using molecular docking. Curcumin derivatives were found to exert significant inhibition activity on α-Glu with an IC_{50} value of L1, L2, L3, and L4 were 24.35, 16.59, 14.44, and 23.32 μM respectively. Analysis of antioxidant ac-

tivity and stability data showed that, in the simulated conditions of the stomach and intestines, the synthesized derivatives have considerable stability. Although, almost all synthesized derivatives have less free radical scavenging potential than curcumin. Molecular docking indicated that curcumin derivatives mainly interacted with amino acid residues located in the active site of α-Glu. These antioxidant inhibitors may be potential anti-diabetic drugs, not only to reduce glycemic index but also to limit the activity of the major reactive oxygen species (ROS) producing pathways.

Keyword: Curcumin-based derivatives, Anti-diabetic, α-Glucosidase, Molecular docking

Mechanism of interaction between curcumin and porcine pancreatic α-amylase revealed by fluorescence quenching and molecular docking

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Abstract

Curcumin, as the substantial constituent of the turmeric plant (Curcuma longa), plays a significant role in the prevention of various diseases, including diabetes. It possesses ideal structure features as an enzyme inhibitor, including a flexible backbone, hydrophobic nature, and several available hydrogen bond (H-bond) donors and acceptors. The objective of this work was to understand the mechanism responsible for interactions between the curcumin and porcine pancreatic a-amylase using a combination of fluorescence quenching and molecular docking study. The fluorescence quenching studies at various concentrations of the curcumin were performed to investigate the mechanism of interaction between curcumin and porcine pancreatic α-amylase. The fluorescence spectra at three different temperatures (296, 303, and 310 K) were recorded in the range of 300–500 nm with an excitation wavelength at 280 nm. Molecular docking study was performed on AutoDock Vina program to speculate the possible binding site(s) between curcumin and α-amylase. The results showed that the intrinsic fluorescence of α-amylase was quenched by the interaction with curcumin through a dynamic quenching mechanism, and the curcumin-α-am-

ylase complex was spontaneously formed mainly driven by the hydrogen bonds and van der Waals forces. The binding forces involved in curcumin-α-amylase interaction were also explored using both experimental as well as theoretical docking approaches. Molecular docking indicated that curcumin with binding affinity energies of -8.5 (kcal/mol) mainly interacted with amino acid residues located in active site of α-amylase by hydrogen bonds and Van der Waals forces. This study indicated that curcumin could bind to α-amylase and that it occupied the active catalytic site of α-amylase, resulting in the inhibitory effect due to steric hindrance, but more preclinical and clinical studies should be carried out in the future. Our study provided a basis for the application of curcumin as a functional ingredient in food system and suggested a kind of natural plant as the treatment of type 2 diabetes mellitus.

Keyword: α-Amylase, Starch digestion, Fluorescence quenching, Molecular docking

Application of gene therapy with viral vectors in infectious and noninfectious diseases

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Abstract

Virus-based vectors, also called viral vectors are being studied in the field of gene therapy because of their innate biological and structural properties. Their ability to protect and specific delivery of genetic elements into the cells, promising results in the pre-clinical studies, going through clinical trials and approval of some products as a therapeutic option in some cancers introduce viral vectors as a powerful tool in the field of gene therapy. In this review, first an introduction about viral vectors is presented and then some studies about application of viral vectors in various fields of human diseases such as prevention and treatment are briefly discussed.

Keyword: viral vector, gene therapy

On-column refolding and purification of Interferon alpha-2b

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Abstract

Interferon alpha-2b is a type of interferons used to treat viral infections and cancers. This protein usually produced using recombinant DNA technology in *Escherichia coli*. The bottleneck of the production of recombinant protein with a form of inclusion body is refolding and purification. On column refolding is the efficient method that purifies and refold the protein at the same time. After propagating *Escherichia coli* with a high cell density cell culture method, the solubilization was performed with solubilization buffer. In this study, the dilution method was performed in optimal refolding buffer, which contains 0.64mM urea, 5.57mM cysteine, 1.8mM cysteine, 0.4M arginine, 5% glycerol, 50mM sodium chloride, 1mM EDTA, 50mM Tris buffer, pH 8 at three concentrations of protein. After dilution refolding, on-column refolding was performed with anion exchange chromatography at pH and urea gradient with three protein concentrations. The IFN-α2b activity and purity was tested with bioassay and size exclusion HPLC. The solubilization agent used in this study led to the proper solubilization of protein to prepare protein for refolding process. The best refolding of tested concentration of protein in dilution refolding method was 50 µg/mL, which had 55% bioactivity and 70% purity. The on-column refolding method used in this investigation showed that the best concentration of IFN-α2b resulted from 1 mg/mL of protein, which had 90% bioactivity and 92% purity.

Keyword: Interferon alpha-2b, Inclusion body, Dilution refolding, On-column refolding

Designing of High-Resolution Melting technique with bioinformatics analysis of different gene regions for separating different types of *Toxoplasma gondii*

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Abstract

Determination of *Toxoplasma gondii* genotypes plays an important role in the treatment management and epidemiology of toxoplasmosis. Enough information from different gene regions of the parasite is important to select the appropriate region to design the High-Resolution Melting (HRM) reaction. This study was designed and optimized with the approach of isolating different types of *Toxoplasma gondii* using bioinformatics predictions and HRM method.

Overall, 50 samples of muscle tissue of livestock with three standard strains RH (type I), PRU (type II) and VEG (type III) were prepared and analyzed. The B1, ROP5, ROP8 and ROP18 genes were selected to isolate different types. For each gene, several sequences from GenBank were obtained from different types. Bioinformatics analyzes were employed to predict the temperature resolution of DNA between different types. Then, specific new primers were designed and synthesized for developing HRM.

By optimizing HRM, specific primers designed for the B1, ROP5 and ROP8 genes in accordance with bioinformatics predictions were able to isolate only a type (types I, II, and II, respectively) with an acceptable temperature difference of other types. The ROP18 gene was also able to isolate each three types I, II and III according to the predictions with average temperatures of 85, 84.6 and 86.4 ° C. As a result, according to the obtained results, the results of bioinformatics analyzes were in good agreement with the results of DNA temperature melting behavior.

Keyword: *Toxoplasma gondii*, Bioinformatics predictions, High-Resolution Melting

Evaluation of Lectin Activity of Recombinant Pebulin Protein, A Type 2 Ribosome-Inactivating Protein Isolated From Dwarf elder (Sambucus ebulus L.) by Hemagglutination Assay

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Abstract

Ribosome-inactivating proteins (RIPs) were first studied as proteins that are widely present in plants. RIPs are also found in algae, fungal and bacteria that catalytically and irreversibly inhibit protein synthesis. *Sambucus ebulus L*. (Dwarf elder) is one of the medicinal and valuable species of the North of Iran which because of having a complex mixture of diverse types of RIPs and related lectins, It is a suitable plant model for studying these proteins. In this study, recombinant pebulin using the pG-Tf2 chaperone plasmid was expressed in *E. coli*. Finally, the recombinant protein was purified by Ni-NTA affinity purification and Lectin activity of the purified protein was examined by hemagglutination assay as a simple method to evaluate the lectin activity. The results of hemagglutination assay showed that purified pebulin is biologically active and indicated strong hemagglutination of erythrocytes in all human blood groups (ABO). The hemagglutination analysis revealed that pebulin has the lectin activity and can bind to sugar chains on the cell surface and enter into the cell. Compared with other type 2 RIPs, pebulin at higher concentrations induced the agglutination of human erythrocytes, indicating its less toxicity on the cells. Dissimilarities in hemagglutination potential, proposing presence of differences in their sugar specificities and cell recognition.

Keyword: Ribosome-inactivating protein, Escherichia coli, Recombinant protein, Ligand modeling, Hemagglutination activity

A Preservative with Bleaching and Emulsifying effects

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Abstract

In cosmetic products, additives are substances that aren't consumed as main ingredients, actually they are added to these products in the processes of preparation, packaging or storage in order to make them safer, improve their appearance, help to present a stable, attractive and easier to apply product, without being stricken by environmental conditions. The protection and quality of toiletries or medicine products are important elements in regards to the health of the consumers. Adding preservatives to the formulations helps the cosmetic manufacturer achieve the first objective of products i.e. meeting the requirements of the users while being safe under normal conditions of use. Despite advances in manufacturing conditions (raw materials with exhaustive bacteriological controls and manufacturing in sterile areas) and the containers used (single-dose ampoules, opaque and hermetic bottles which are used for precise amount), there is still possibility of colonizing cosmetics without preservative in their composition. Principally, adding extra compounds as emulsifier and blanching agent causes more noxious compounds in end products which should be avoided. Hence, it is desirable that some components of the formulation fulfill this function. In our study, combination of sodium thiosulfate and Citric acid contains

emulsifier and bleaching substances. When this mixture was added to *Azadirachta indica* (Neem) gum or *Acacia Senegal* (Gum Arabic), beside the above-mentioned properties, turned these gums into a thickener and stabilizer agent. This formulation can prevent the spreading of microorganisms. It will be showed from the findings and results that this formula can be used as a preservative agent in the cosmetic and pharmaceutical industry with significant emulsifying and bleach potential. The rare side effect of this additive is mild skin hypersensitivity reaction only in sensitive individuals. The purpose of the study was to develop a powerful preservative based on synthetic and natural ingredients, with bleaching and emulsifying effects.

Keyword: preservative; bleaching; emulsifying; sodium thiosulfate; citric acid; neem gum

hTERT-molecular targeted therapy of ovarian cancer cells via folate-functionalized PLGA nanoparticles co-loaded with MNPs/siRNA/ wortmannin

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Abstract

Effective telomerase-molecular targeted cancer therapy might be a promising approach for the efficient treatment of ovarian cancer. Therefore, folate-functionalized PLGA nanoparticles (NPs) were co-loaded with hTERT siRNA, Wortmannin (Wtmn), as a potent PI3K inhibitor and magnetic nanoparticle (MNPs) as a theranostic agent to gain a multifunctional NPs for targeted drug delivery as well as molecular targeted therapy. ^{1}HNMR, FTIR, DLS, FE-SEM and TEM were applied to characterize the synthesized NPs. *In vitro* discharge pattern for siRNA and Wtmn from the dual drug-loaded NPs showed an early fast release followed by a constant release up to 200 h. According to the MRI analysis, by increasing the concentration of Fe_3O_4 in NPs, the weaker T2 signal intensity was enhanced, and a considerable contrast was detected in the MRI images. MTT assay and median-effect analysis showed that the Wtmn/siRNA-loaded MNPs-PLGA-F2 NPs display the most synergistic cytotoxicity on the SKOV-3 ovarian cancer cells. Moreover, the Wtmn/siRNA-loaded MNPs-PLGA-FA NPs could significantly reduce the expression of hTERT, AKT, and p-AKT than the single drug-encapsulated NPs ($P<0.05$). Taken together, the findings showed that the multifunctional NPs relying on combinatorial therapy might have considerable potential for effective telomerase-molecular targeted therapy of ovarian cancer.

Keyword: hTERT, siRNA, Wortmannin, Magnetic nanoparticles, PLGA, Molecular targeted therapy, Ovarian cancer.

Physicochemical experience of PEGylated LipoNiosomal nanocarriers containing pomegranate peel extract

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Abstract

Natural products generally have effective ingredients with minimal side effects. The use of nanocarriers containing herbal compounds instead of conventional chemotherapy drugs can increase its effectiveness while reducing the side effects of treatment. In this study, Lipo-Niosomal containing pomegranate peel extract was synthesized and its physicochemical properties were investigated. Lipo-Niosomes containing the extract were synthesized by thin layer dehydration using cholesterol, DSPE-mPEG (2000), span 60, dipalmitol phosphatidylglycerol and then pomegranate peel extract was loaded in the Lipo-Niosomes. Physicochemical properties were evaluated using FTIR, AFM and zeta sizer. Release was calculated at 37 ° C. The synthesized Lip-Niosomes have an encapsulation efficiency of 45% and the average particle size was 91.28 nm and the surface charge was -26.2 mV. FTIR and AFM results showed no evidence of chemical reaction between the extract and the nanoparticle. Its particle has spherical morphology.

Keyword: Lipo-Niosomal, Nanocarriers, Chemotherapy

The biocompatibility of hemostatic agent

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Abstract

Hemorrhage is the leading cause of death from trauma in conflicts and accidents that on time stop bleeding, leads to survival of injured person. Hemostatic bandage is applied topically as an adjunct to manual compression and is indicated for the local management and control of surface bleeding from vascular access sites. This hemostatic Bandage contains a hemostatic agent, Kaolins with special formulation that functions to stop bleeding. Kaolins is effective to absorb water from blood that causes blood concentration and clot formation. It is considered as a fast option to control bleeding in emergencies. On the other hand, the biocompatibility evaluation is important international requirements in usage of medical devices. In the present study, in vitro biocompatibility of hemostatic bandage in cell cytotoxicity is evaluated for the purpose of stopping the bleeding and wound healing by international standards. The results in comparison to commercial product, indicate no cytotoxicity of the hemostat bandage.

Keyword: Biocompatibility, Hemorrhage, Kaolins

Evaluation of auto-oxidation of glutaraldehyde polymerized hemoglobin

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Abstract

The need for a blood substitute to treat or prevent hypoxia due to blood loss during surgery or illness has always been felt in medicine. Therefore, the use of hemoglobin solutions, which carry oxygen in the blood, is a suitable alternative to blood. An efficient and effective blood substitute is able to deliver oxygen to tissues without the need for precise blood type adaptation. HBOC It is a common name for all hemoglobin-based oxygen transporter products, and one of the most popular product is the polymerized with glutaraldehyde. The aim of this study was to investigate the amount of oxidized hemoglobin during the polymerization of hemoglobin by the addition of glutaraldehyde. For this study, hemoglobin was extracted from bovine blood using filtration and ion exchange chromatography. Blood cells and stroma were removed and pure hemoglobin was obtained at the concentration of 0.028×10^{-3} M and confirmed by SDS-PAGE electrophoresis. After deoxygenation, polymerization was performed by glutaraldehyde and the reaction was stopped with $NaBH_4$. Cross-linked hemoglobin was observed by SDS-PAGE electrophoresis. Absorption at 400, 540, 610 and 630 nm was monitored to investigate auto-oxidation during polymerization. The results have shown that, the rate of auto-oxidation and Met-hemoglobin production did not increase significantly. It can be said that hemoglobin polymerization with glutaraldehyde is suitable for the formation of oxygen-carrying blood substitutes with good oxygen delivery properties.

Keyword: Auto-oxidation, Glutaraldehyde, HBOC, Hemoglobin, Polymerization

A novel mother of vinegar-inspired regenerative dressing accelerates wound healing in diabetic rat model

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Abstract

Diabetic ulceration is one of serious global health issues in patients with diabetes mellitus leading to foot amputation, psychiatric morbidity and mortality. In spite of advances in wound dressing, treatment options for diabetic ulcers are too limited mainly due to the complexity of related factors and poor immune response. The development of the natural, cheap, safe, and effectual regenerative dressing would help providing a better care and improving the wound closure and healing rate, leading to a permanent solution for regeneration of damaged tissue. We herewith characterized and evaluated healing efficacy of a novel bio inspired gelatinous extracellular cellulosic scaffold, mother of vinegar from ***acetic acid*** bacteria in type I streptozotocin-induced diabetic rat model. Obtained nanostructured bacterial cellulose biofilm result in accelerated improvements in healing rate of diabetic wounds in rat model with increased collagen regeneration and epithelialization. This multifunctional membrane displayed hemostatic, and promoted cell migration and collagen synthesis with a potent antimicrobial and antibacterial property that can alter the wound bed bioburden. Altogether, it can be considered as a feasible and safe candidate for enhance diabetic ulcers treatment reduce the risk of infection in partial- and full-thickness wounds, as well as over percutaneous line sites.

Keyword: Diabetic ulcers, mother of vinegar, Rat model, wound healing

FSH follicle stimulating hormone and its pharmaceutical compounds

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Abstract

Follicle-stimulating hormone (FSH) is produced by the pituitary gland in the brain. In women, FSH helps control the menstrual cycle and egg production by the ovaries. Follicle-stimulating hormone is one of the essential hormones for the development of puberty and ovarian function in women and men. Preparations containing this hormone have been used to assist in pregnancy using in-vivo and in-vitro techniques. The amount of FSH varies throughout a woman's menstrual cycle and is highest before the egg is released (ovulation). Its main use is in the treatment of female infertility, either in cases where ovulation does not occur spontaneously or to cause overstimulation of the ovaries to cause the growth of multiple follicles in reproduction with medical help (e.g., fertilization Laboratory and Embryo Transfer (Folitropin is a recombinant follicle-stimulating hormone. There are two follitropins available (follitropin alpha and follitropin beta), which are slightly different in carbohydrate structure in formulations.

Keyword: Hormone, FSH, Infertility, Ovary, Biotechnology

MicroRNAs: Novel biomarkers for male infertility treatment

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Abstract

MicroRNAs (miRNAs) are small non-coding RNAs, which can regulate the expression of hundreds of genes at the post-transcriptional level. MicroRNA applications have been extensively studied over the years, from potential biomarkers of cancer to targeted therapies for a variety of diseases. In this article, in addition to the mentioned applications, the role of microRNAs in male infertility is discussed. Male infertility is a clinical disorder with a significant number of cases being idiopathic. Former studies have shown that the abnormal expression of microRNAs can be associated with some reproductive disorders in men. Later, these studies were reviewed the latest findings published in this field to evaluate the relationship between up-/down-regulation of various microRNAs and their role in male infertility. MicroRNAs were discovered to be numerous and stable in the seminal liquid, which led to an easy identification utilizing regular RNA detection methods. Future investigations should focus on discovering future treatments against male infertility by targeting specific microRNAs, and also on developing modern and improved contraceptive methods.

Keyword: microRNAs, biomarker, male infertility, gene expression

Evaluation of recombinant antimicrobial effect of peptide TP4 on some food-borne bacterial strains

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Abstract

Food poisoning is one of the diseases transmitted through contaminated food and beverages. It causes by the presence of infectious microorganisms, including some bacteria in food, and one of the most common symptoms of this disease can be Diarrhea and vomiting in the patient. The use of antimicrobial peptides has been considered for various reasons such as increased bacterial resistance to common antibiotics. The aim of this study was to evaluate the minimum inhibitory and bactericidal concentration of recombinant antimicrobial peptide (TP4) Tilapia Piscidin 4 against four important bacteria that are the cause of food poisoning in humans. In this study, the antibacterial activity of this peptide was evaluated on Bacillus cereus, Yersinia enterocolitica, Escherichia coli O157: H7 and Staphylococcus aureus by plate microtiter method to determine MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration). The MIC of TP4 peptide against gram-positive and gram-negative strains was 4 and 2µg/ml, respectively and the MBC for Y.entrocolitica and B.cereus was 4 and against S.aureus and E.coli was 8µg/ml. As a result, TP4 has an appropriate antimicrobial effect on foodborne bacterial strains.

Keyword: Food poisoning, Antimicrobial activity, Antimicrobial peptide, TP4, Food-borne bacterial strains

A review on expression of neural cells marker

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Abstract

Neural stem cells (NSCs) are pluripotent cells that maintain the proliferation and production of progenitor cells and can become differentiated cells in response to specific stimuli. Differentiated cells include neurons, astrocytes, and oligodendrocytes that can be derived from NSC cells in-vivo and in-vitro conditions. These cells are found during embryonic development as well as in the brain, spinal cord, and peripheral nerves of adults in all mammalian species. The presence of these cells in specific areas (hippocampus) of the brain leads them to proliferate continuously. Continuous neurogenesis requires dynamic adjustments throughout life and is controlled by physiological, pathological, and pharmacological factors. With the knowledge of peripheral neurons derived from peripheral neurons, it can increase the treatment of central nervous system (CNS) and peripheral diseases. In this article, we first identify neuronal stem cells in the fetal brain of adult mammals and neurogenic areas, and then describe the determinants of cell proliferation, differentiation, and cell function.

Keyword: Neural stem cell, Neuron, Diiferentiated cell, Cytology

A review study on role of Klf4, C-Myc, Oct4 and Sox2 achievment induced pluripotent stem cell by reprogramming

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Abstract

General characteristics of self-renewing and proliferating stem cells, which according to their ability and potential to differentiate into four groups of all-powerful stem cells, enable you to read the theory of weapons stem cells in the umbilical cord or embryonic stem cells, several Ability The theory of hematopoietic stem cells and monovalent, such as spermatogonia sol, are divided. Ready-made cells, only developing into two placental layers, have a special distinction in the three germinal layers of oderm, oderm and oderm, so such cells are able to create a complete and living organism. This important feature has become the use of the benefits of these cells in fundamental studies and research and therapeutic fields. However, research and experiments on cell line cells, especially embryonic cells, with certain problems, such as new ones applied to the human embryo, may also keep the transplant cells from being robots. Hence, scientists and researchers have come up with a solution to the immunological and moral problems of embryonic stem cells.

Keyword: Induced pluripotent stem cell, Pluripotency, Diferentiated cell, Stem cell

New perspectives on plant metabolites for photodynamic treatment of cancer

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Abstract

Photodynamic therapy is a promising therapeutic approach to the management and elimination of cancer. Plants in nature are one of the potential sources for obtaining light-sensitive compounds that are less toxic than synthetic compounds. Although much work has been done on photodynamic therapy in recent decades, relatively little attention has been paid to the study of medicinal plant extracts in recent years. The aim of this study was to identify common light-sensitive reactive groups in nature, which are mainly derived from plants. In addition, biotechnology strategies in the cultivation of plant laboratory systems for the production of plants sensitive to natural light on a large scale are discussed. These compounds are capable of photosensitivity by adequately botanically describing known and unknown plants used in photodynamic therapy to kill tumor cells in the field of herbal medicines against cancer. The success of light-sensitive plants in photodynamic therapy encourages researchers to work with botanists to identify and study natural reactive compounds from different plant species, so they use these plants as options for synthesizing light-sensitive plant metabolites. They have minimal side effects that are less toxic and more selective in various cancer treatments using photodynamic therapy. In addition, new biotechnology-based techniques, such as targeted genome-editing techniques, provide significant opportunities for the production of natural products in plants, mainly when associated with recent advances in bioreactor scalability and design.

Keyword: Photodynamic therapy, Cancer, Photosensitizers, Biotechnology

Cloning and expression of protein biomarker; a new strategy for rapid cancer diagnosis

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Abstract

Human Pyruvate kinase is an important isoenzyme to control and regulating the cellular metabolic budget which providing energy by transferring a phosphoryl group from phosphor-Enol pyruvate to pyruvate. The M_2 isoenzyme of this protein is the most active form which appears in neoplastic tissues. By the cancer development, there are conformational changes in this protein which turn it from tetrameric to dimeric form; so, the enzymatic function will be change. On the other hand, releasing the dimeric form of M_2PK in cancer patient plasma and body fluid makes it a good sensitive biomarker for cancer diagnosis in the early stages. The purpose of this study is to cloning and expression of the enzyme active site to reaching more information about this protein and enhance the cancer diagnosis process by designing more rapid and sensitive constructions against this biomarker. To achieve this goal, total RNA from colon cancer patient tumor was extracted and cDNA was synthesized. Then the polymerase chain reaction method was accomplished to amplify the target sequence. The acquired sequence was digested by restriction enzymes and cloned into the PET32a expression vector. The obtained structure was transferred into the BL21 E. coli bacteria strain as an expression host. After the colony emerging, the presence of the targeted sequence was confirmed by the Sanger Sequencing method and the protein expression validated by the ELISA assay. In the following way, Future studies around the Modeling and functional analysis of obtained polypeptide might be useful.

Keyword: Pyruvate kinase type M_2, Neoplasm, Cloning, Active Site

Cloning and expression of anti-CD19 scfv in a prokaryotic host for use in CAR – T cell research

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Abstract

According to estimated data from the world health organization (WHO), cancer is the first or second cause of death for young adults and ages above 70 years. Among the cancer different types, hematopoietic cancers (HCs) are responsible for 8 to 10 percent of cancer deaths. They all originate from the cells in bone marrow and lymphatic system. The most common (HCs) are: leukemia, myeloma and lymphoma. CD19 antigen by having unique properties is being used as a target antigen for treatment of malignancies like lymphoma and leukemia. This antigen is widely expressed on B lymphocytes, is rarely lost during neoplastic transformation and it is not expressed on normal tissue and other stem cells outside the B lineage cells. This transmembrane protein can be the target molecule , for the antibody which is produced against it. Immunotherapy is the mechanism in which immune system cells are modified to help improve the immune system functionality against malignancies and cancers especially. CAR-T cell therapy is the novel types of immunotherapy and is one the most effective tools in treatment of hematopoietic cancers like different types of lymphoma and leukemia malignancies like acute lymphoblastic leukemia (ALLs). this is the technology of targeting malignant cells with special targeted cell receptors. The common produced antibodies are showing some insufficiency according to their large size and high weight. So in these cases single chain fragments of variable (scFv) is preferred cause of its small size or other unique and suitable features. In this research we expect to achieve the anti-CD19 scfv by cloning the sequence in bacterial plasmid and to express the antibody for research application. At the end of the research we expect the proper cloning and expression of scFv antibody by the prokaryotic cell. It is concluded from this research that *E.coli* strains are one of the best tools in cloning and expression procedure for recombinant and other humanized or mouse antibodies. These strains are easy to manipulate and not too

expensive. The use of affinity tags like HIS-tag seems to be effective for the proper expression of these proteins and it is an effective agent for the purification processes like IMAC-chromatography.

Keyword: Cloning, Protein expression, scFv antibody

Mechanisms of microRNAs and mechanisms regulating epigenetic miRNA relationships in human cancer and its potential

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Abstract

MicroRNA s (miRNAs) are small non-coding RNAs with the capability of modulating gene expression at the post-transcriptional level either by inhibiting messenger RNA (mRNA) translation or by promoting mRNA degradation. The outcome of a myriad of physiological processes and pathologies, including cancer, cardiovascular and metabolic diseases, relies highly on miRNAs. However, deciphering the precise roles of specific miRNAs in these pathophysiological contexts is challenging due to the high levels of complexity of their actions. Indeed, regulation of mRNA expression by miRNAs is frequently cell/organ specific; highly dependent on the stress and metabolic status of the organism; and often poorly correlated with miRNA expression levels. Such biological features of miRNAs suggest that various regulatory mechanisms control not only their expression, but also their activity and/or bioavailability. Several mechanisms have been described to modulate miRNA action, including genetic polymorphisms, methylation of miRNA promoters, asymmetric miRNA strand selection, interactions with RNA-binding proteins (RBPs) or other coding/non-coding RNAs. Moreover, nucleotide modifications (A-to-I or C-to-U) within the miRNA sequences at different stages of their maturation are also critical for their functionality.

Keyword: miRNA, methylation of promoters miRNA, cancer, cell

Efficient *in vitro* plant regeneration via indirect somatic embryogenesis from petal cultures of Pomegranate cv. Ganesh in double phase media

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Abstract

Reliable and reproducible protocol for plant regeneration via somatic embryogenesis from segments of petal, excised from unopened flower buds of mature pomegranate (Punica granatum L.) cv. Ganesh has been developed using double phase Murashige and Skoog's medium containing three fourth strength of salts. Embryogenic callus was induced on media supplemented with individual and combined plant growth regulators such as Indole-3-acetic acid, Zeatin, Thidiazuron and natural additive as coconut water in both phases. The liquid phase was discarded during five weeks of culture for the development, maturation and conversion of somatic embryos into plantlets on semisolid phase. The frequency of plant regeneration was more on media supplemented with 0.5 mg l^{-1} each of Zeatin and IAA along with 15% coconut water within 12 weeks of culture period. The genetic fidelity testing of regenerated plants and artificial seed production from embryogenic callus is under experimentation.

Keyword: In Vitro regeneration, somatic embryogenesis, petal segments, Punica granatum L.

Study of esophageal cancer-producing suppressor genes

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Abstract

One of the most important factors in silencing cancer-producing genes can be considered as nefarious methylation of suppressor genes that cause the extinction of these genes and by slowing down their expression, pave the way for cancer. In this study, we investigated the methylation of three suppressive genes of p14, p15 and p16 in patients with squamous esophageal tissue cancer (ESCC) and its relationship with demographic and pathological characteristics of patients. For this purpose, 44 non-familiar ESCC patients, 44 tumor tissue samples and 19 healthy samples (adjacent to tumor) were prepared and extracted by DNA and RNA. Then, with the specific primer of methylated and non-methylated states of these genes, the chain reaction of methylation-specific polymerase (MSPCR) was performed. The expression of these genes was also investigated by RT-PCR. The results were 53%, 9% and 27% for p14, p15 and P16 genes, respectively, and no methylation was observed in any of the normal samples (adjacent to the tumor).

Keyword: Methylation, Esophageal cancer, Suppressor, PCR

Immunogenic efficacy of protein-based vaccine from a chimeric gene consisting OmpW, TcpA and CtxB, of *Vibrio cholerae*

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Abstract

Vibrio cholera is one of the major causes of mortality in children under 5 years and travelers particularly in developing countries. Therefore, it is very important to improve preventive measurements and treatment strategies. The three most important pathogenic factors in Vibrio cholerae that are able to stimulate the immune system are: a) B subunit of CtxB Cholera enterotoxin, responsible for toxin binding to eukaryotic cells, b) TcpA, an essential factor for bacterial colonization, and c) OmpW, the highly conserved extracellular protein, as a stabilizing agent against environmental shock. The recombinant chimer proteins that include several immunogens can make the humans more persistent with high efficiency against the pathogenicity of bacteria. In the present study, we evaluated the protective efficacy of produced specific immunoglobulin G antibody (IgG) against chimeric recombinant proteins ompW, tcpA and ctxB (otc) in mice Recombinant chimeric protein (otc) was expressed in E. coli BL21 (DE3) after the addition of IPTG (1 mM) induction and purified by an Ni-NTA affinity chromatography column, and analyzed by SDS-PAGE and was confirmed through Western blot. BalbC mice were immunized subcutaneously with 20 µg of purified recombinant protein three times with two-weeks intervals. The activity and specificity of produced IgG antibody was examined by indirect ELISA using recombinant proteins and whole cells of bacteria as a target. The protective effect of produced of IgG against CT toxin was also investigated on Y-1cell line. After determining the bacterial lethal dose, the viability of neonatal mice from immunized mothers were challenged. The expression of the recombinant protein was confirmed by the detection of the protein by Western blot using anti-His-tag antibodies. The produced IgG showed specific binding activity to the chimeric protein and whole cells of bacteria in indirect ELISA, and a significant difference was observed between test and control IgG groups. The IgG antibody titer increased

with the initial immunization and reached its peak following the third immunization. Our data indicated that the antibody titers was increased more than 3 folds in immunized mice compared to control group. OTC IgG antibody at a concentration of 5% (v/v) was able to reduce the effects of CT cytotoxicity on Y-1 cells.Animal challenge showed 100% survival rate against one LD dose of bacteria for pups from immunized mothers whereas pups of unimmunized mothers were totally died. The results of this study indicate that anti-chimeric IgG antibody can be applied in immunotherapy for vibrio cholerae infection. Therefore, this recombinant construct can be as a Protein-based vaccine candidate.

Keyword: Protein-based vaccine, Protectively, Diarrhea Enterotoxin, Vibrio cholera

Study of immunogenicity effects of recombinant Newcastle virus LHN2 protein epitopes loaded into Albumin nanoparticles

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Abstract

Nanosystems are able to play a role in vaccine technology with the possibility of targeted delivery and controlled release for cellular safety. Albumin protein is a natural nanocarrier that has compatible, biodegradable and non-toxic properties. Newcastle disease is a highly contagious viral disease that affects most bird species and causes irreparable economic damage to the poultry industry. Newcastle virus attacks host cells by binding to its two surface glycoproteins, HN and F, and causes disease. The main purpose of this study is to load the recombinant protein into albumin nanoparticles as carriers and adjuvants and to evaluate the immunogenicity in the animal model. For this purpose, the synthesized LHN2 gene and recombinant protein were expressed and purified and loaded into albumin nanoparticles. Immunization is underway in the animal model.

Keyword: Albumin, Newcastle virus, Nanocarrier

Evaluation of Cytotoxicity of Recombinant Pebulin Protein, A Type 2 Ribosome-Inactivating Protein Isolated From Dwarf elder (Sambucus ebulus L.) On Breast Cancer Cell Line (MCF7)

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Abstract

Ribosome-inactivating proteins (RIPs) are toxins with N-glycosidase activity on the rRNA of mammalian, fungal, plant, algae and bacterial ribosomes that irreversibly inhibits protein synthesis. *Sambucus ebulus L.* (Dwarf elder) is one of the medicinal and valuable species of the North of Iran which because of having a complex mixture of diverse types of RIPs and related lectins, it is a suitable plant model for studying these proteins. In this study, in order to increase the expression of recombinant protein in soluble form, co-expression of the target protein with pG-Tf2 chaperone plasmid and reduction of growth temperature after induction were used to increase protein solubility and function. Finally, the recombinant protein was purified by Ni-NTA affinity purification and the anti-cancer activities of the purified protein were examined. The results of antitumor activity assay also showed that this protein had a dose- and time-dependent lethal effects on MCF7 cancer cell line and decreased survival percent by increasing pebulin concentration. Based on the findings of cytotoxicity of recombinant pebulin protein, it can be considered as a potential antitumor material, especially for the development of RIP-based immunotoxins.

Keyword: Ribosome-inactivating protein, Anti-cancer activity, Breast cancer cell line, Recombinant protein, Escherichia coli

MB-67

Phycocyanin-loaded 3D alginate hydrogel promote skin wound healing with effective regeneration of hair follicles after complete loss of epidermis and dermis in a rat model

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Abstract

Despite advances in skin regeneration technologies, the majority of currently available wound dressings in the market are unable to attain scarless skin rejuvenation with complete recovery of addendums such as hair follicles. Management of full-thickness skin lacerations mainly in head area even after clinical treatment remains a major challenge, mostly due to incompetent outcomes of skin treatments. We previously reported supportive effects of phycocyanin on reorganization of the dermis with significantly increased epidermal and vascular markers in rat model. Herewith, we have assessed utilization of a phycocyanin-loaded 3D alginate hydrogel as a new type of wound dressing for repairing full-thickness skin injury with enhanced hair follicles regeneration in a rat model. Data presented here confirmed anti-inflammatory, antioxidant and antimicrobial effects of phycocyanin as well as stimulation of angiogenesis during healing process. We found that expressions of TGF-β1 prevented scar tissue formation, and induced mesenchymal stem cells migration into injury sites for regeneration of skin appendages. We found that after 14 days, necrotic tissue, inflammation and collagen deposition in the hydrogel group loaded with phycocyanin 3D decreased compared to the other groups, but the most important observation was the formation of hair follicles at the wound site. Further

evaluation of epidermal regeneration, epidermal dermis junction, leukocyte infiltration and collagen deposition, in addition to Real-Time RT-PCR and tissue staining, confirmed that treatment of alginate hydrogel with Phycocyanin significantly improved the healing process by reperfusion and hair follicle regeneration. The data presented here show that phycocyanin has effectively improved scarless skin regeneration with complete healing process of skin addendums such as sebaceous glands and hair follicles and can therefore be used as an effective therapeutic strategy for the topical treatment of skin wound healing.

Keyword: Phycocyanin, Hair regeneration, wound healing, skin repair

Optimization of graphene foam for better detection of H2O2

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Abstract

One of the most important issues that human beings are currently facing is the rapid diagnosis of diseases and various biological elements such as oxygenated water. According to the researches, the electrochemical analysis method has attracted the attention of many researchers in this field due to its low detection range and fast response time. In this research, the conductivity of graphene and graphene-silver hybrid and its resistance were measured with an ohmmeter. A new biosensor made of the mentioned materials has the ability to detect oxygenated water. Due to their simplicity, low cost, and high electrical conductivity, these electrodes are made with desirable electrochemical properties. Composite electrode foams have a high limit of detection and sensitivity of 0.11µM and 0.8µA /µM Ag NPs 3DG composites are fabricated directly as a test electrode for the electrochemical detection of hydrogen peroxide (H2O2). Various equipments such as scanning electron microscopy, X-ray diffraction and Raman spectroscopy are used to describe the morphology and structure of the composite. Electrochemical experiments show that AgNPs-3DG-based sensors have fast amperometric sensing, low detection limit, wide linear response amplitude and complete selection for non-enzymatic H2O2 detection, indicating the synergistic effect of high electrocatalytic activity of Ag NPs and conductivity. In this research, in order to optimize the graphene-based electrode, the parameters that have improved the structure of graphene foam were identified and decorated with Ag particles according to the best design interval and in order to improve the conductivity and increase the detection limit.

Keyword: Hummers method, Graphene foam, Graphene-silver hybrid, Composite strength, Optimization.

Green synthesis of silver nanoparticles with Spirulina Cyanobacteria extracts with Folic acid targeted chitosan polymer coating containing Imatinib for targeted colon cancer therapy

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Abstract

Introduction: In the recent years, ongoing research has focused on development of nano-scale objects as efficient anti-cancer therapies. Biosynthesis of nanoparticles is reviewed in detail in this study. Comparison of different synthesis methods, namely physical,chemical and green methods giving emphasis to biological synthesis is documented heret[1]. NPs show totally novel or enhanced properties taking into account particular characteristics, i.e., size (1–100 nm), shape, and structure. Silver nanoparticles are important because of their exceptional chemical, physical, and biological properties, and hence applications. In the last decade, numerous efforts were made to develop green methods of synthesis to avoid the hazardous byproducts. It also describes the comparison of efficient synthesis methods via green routes over physical and chemical methods, which provide strong evidence for the selection of suitable method for the synthesis of Ag-NPs Among the various nanoparticles, silver nanoparticles have gained much attention due to their unique anti-cancer properties[2]. However, concerns about the synthesis of these materials such as use of precursor chemicals and toxic solvents, and generation of toxic byproducts have led to a new alternative approach, green synthesis[3]. This eco-friendly technique incorporates use of Spirulina extract as reducing and capping agents. Moreover, by using folic acid targeted chitosan as a biocompatible polymer, the silver nanoparticles and Imatinib effectively and actively targeted colon cancer cell line[4].

Methods: The physical and chemical characterization of nanoparticles was evaluated by FT-IR, TEM and DLS. The viability of cells was assessed by MTT and the expression of autophagy and apoptotic genes considered molecularly by Real Time PCR. Moreover, the apoptotic cells and cell cycle arresting was measured by Flow Cytometry.

Results and Discussion: According to DLS results and analysis of TEM, nanoparticles have spherical shapes and size of 100±50 nm. The MTT assay indicated significant decrease in viability of cells treated by folic acid targeted chitosan-Ag-Imatinib nanoparticles in comparison with non-targeted nanoparticles. The apoptotic and autophagy genes overexpression was 8-fold (caspase9), 3-fold (BAX), 7-fold (ATG5), 2-folds (BECLIN1), and 3-folds (mTORC1) genes in cancer cells. More than 50% of cell cycle arrest and 45.05% of apoptosis were obtained for cancer cells after treatment with nano-complex. Hence, the synthesized nano-complex could be promising for cancer therapy. The auction results from this study showed that the lethal effect of silver nanoparticles on cells depends on protection and time. Also, the expression of caspase9 gene by real-time PCR testing at the mRNA level showed that silver nanoparticles significantly reduce the expression of this gene. The use of these nanoparticles can be considered in inhibiting the metastasis of the larger Sultan River

Keywords: Targeted delivery, Colon cancer, Chitosan, Folic acid, Silver nanoparticle, Green synthesis

Apoptotic and antiproliferative effect of rutin nanoencapsulated in human estrogen receptor positive breast cancer cells MCF7

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Abstract

Rutin (Rut) has received considerable attention in recent years for its anticancer potential activities. However, short half-life and weak bioavailability of Rut limited its use as a chemotherapeutic agent. The present study is intended to evaluate the efficiency of PLGA-PEG as a nano-carrier for Rutto increase anticancer effects on MCF7 brest carcinoma cells. Rut-loaded PLGA-PEG nanoparticles (NPs) were characterized through Dynamic Light Scattering (DLS), Fourier-transform infrared spectroscopy (FTIR) and field emission scanning electron microscopy (FE-SEM). Anti-proliferative and apoptotic effects of nanoformulated Rut were evaluated using MTT and flow-cytometric assays, respectively. Also, real-time polymerase chain reaction (Real-Time PCR) was used to determine the gene expression levels of apoptotic genes,. Evaluation of cytotoxicity showed that Rut-NPs had more cytotoxicity than free Rut in a time-and dose-dependent manner. The nuclei fragmentation and the percentage of apoptotic cells induced by Rut-NPs were significantly higher than free Rut. Also, it was found that Rut-NPs triggered more cell cycle arrest at sub-G1 checkpoint than free Rut. Compared to Rut treated cells, the mRNA expression levels of hTERT was significantly altered in Rut-NPs treated cells. In conclusion, it is supposed that nano-encapsulation of Rut into polymeric PLGA-PEG NPs may be a convenient drug delivery system to enhance its anticancer effects for ovarian cancer therapy.

Keywords:Rutin ; polymeric nanoparticles; apoptotic effect; breast cancer

Plant derived decellularized scaffold for enhanced 3D osteogenic differentiation of human stem cells

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Abstract

Decellularized plant-based scaffolds has been recently absorbed high attention to be used in human tissue culture due to their beneficial properties such as low cost, biocompatibility, biodegradability, and ease of use without any problem to the environment. Despite critical advances in the manufacture of bioengineered platforms for bone tissue engineering, delivery of supplements in complex designed human tissues stays a challenge and bone tissue remains a challenging arena to obtain a satisfying functional and structural restoration after damage. Surprisingly, the plant-based platforms are biocompatible, since they held onto insignificant safe reaction upon implantation into a host, making them a practical choice for transplantation researches of close autologous tissues with efficient physically induction of osteogenesis. We evaluated osteogenic ability of various decellularized plant derived scaffolds on mesenchymal stem cells (MSCs). Various structural characterizations of decellularized scaffolds by Atomic Force and Scanning electron microscopes, Infrared spectroscopy and Brunauer-Emmett-Teller, Tensile, and Contact angle tests confirmed their proper 3D structure for bone formation with interconnected pores, and moderate surface roughness. Efficiency of osteogenic differentiation were confirmed by alkaline phosphatase and calcium deposition assay in MSCs differentiated on the decellularized plant scaffolds compare to those cells differentiated on tissue

culture polystyrene Petri dish as a 2D control. Altogether, we conclude that decellularized plant derived scaffolds are able to support stem cell proliferation and efficient osteogenic differentiation in 3D structure and they can be considered as a promising potential for use in bone tissue engineering.

Keywords: natural cellulose based scaffolds, tissue engineering , bone regenerative, green scaffold technology, MSCs

Targeted Delivery of Chemotherapeutics to Breast Cancer Model Using S3 Peptide against Na $^{+}$/K^{+} ATPase

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Abstract

In this study, Doxorubicin (DOX) was encapsulated in biocompatible and biodegradable poly (lactic acid-glycolic acid) (PLGA) nanoparticles. The nanoparticles were targeted against the alpha subunit of Na $^{+}$/K^{+} ATPase using a 13-amino acid peptide (S3). The nanoparticles were been evaluated for the encapsulation efficacy, morphological properties, *in vitro* release of DOX, and cytotoxicity. *In vitro* tests showed that the NPs can release their cargo in a pH-dependent manner; besides, the targeted NPs (S3-PLGA-DOX) had a higher cytotoxicity effect on 4T1 human cancerous cells than non-targeted ones (PLGA-DOX). Flow-cytometry experiments on 4T1 and HeLa cell lines confirmed an increased cellular uptake of S3-PLGA-DOX NPs compared to PLGA-DOX and free DOX. Blocking the receptors by pre-treatment of HeLa cells using free peptides reduced the cellular uptake of targeted NPs, indicating the role of the peptide-ligand interaction for the endocytosis process. These results shows the potential of Na $^{+}$/K^{+} ATPase as a target molecule for selective delivery of chemotherapeutics to breast cancer.

Study of the regulatory role of HSV-1 miR-H4 on protocadherin 19 gene

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Abstract

Over the last decades, microRNAs (miRNAs) have emerged as important molecules associated with the regulation of gene expression in humans and other organisms, expanding the strategies available to diagnose and handle several diseases. One of the important identities of Herpes Simplex Virus Type-1 is its ability to induce latency in neurons for all of the host life time and occasionally recurrent infections. In latency the expression of lytic phase genes is disrupted, whereas the Late Associated Transcript (LAT) expression to smaller transcripts and dependent introns accumulated in cell nucleus. It is increasingly clear that miRNAs-encoded by viruses can affect the viral life cycle and host physiology. this study was designed to show the effect of miR-H4, on protocadherin 19 gene. The bio-information tools including (Human target, RNA22, MR-microT , human Atlas) were used to selection of target genes. in vitro studying was performed by cloning miRNA in PCDH plasmid and transforming in E.coli recombinant strain DH5α . the extracted plasmids were transfected in HEK293. The relative quantitative expression assay of gene and microRNA was performed by Real Time.

In this study we showed that HSV-1 miRNAs could regulate the transcription factors of protocadherin 19 gene in cell PCDH19 is UP-regulated in sample group (in comparison to control group) by a mean factor of 3.317 (S.E. range is 3.202 - 3.438).

Keywords: LAT miRNAs , HSV-1 , protocadherin 19 .

Biotechnology and Coronavirus

Corona Virus/Covid-19

COVID-19: Synthesizing and Optimization of niosomes containing the anti-corona Virus drug Kaletra (lopinavir) to reduce adverse effects of drug

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Abstract

COVID-19, the cause of Acute Respiratory Syndrome(SARS-CoV-2), causing a major threat to public health. Kaletra (Lopinavir) is an antiviral drug used to treat corona virus disease which has many side effects on normal cells. Niosomes are drug carriers that could enhance stability, improve function and reduce side effects of drugs. The aim of this study is synthesis and optimization a niosomal formulation to reduce the side effects of Kaletra (Lopinavir) on normal lung cells.

Methods: In this study, after synthesizing of niosomal formulations containing Kaletra with different molar concentrations of lipids and cholesterol by thin film method and characterized physico-chemical features of them with spectrophotometry method and DLS, the optimal formulation was selected and its toxicity was assayed on normal lung cells (MRC-5) in comparison with the free form of drug.

Results: The results of this study showed that the optimal niosomal system containing Kaletra with an encapsulation rate of 86±2.6%, size 172 nm, zeta potential -13±2.4 mV, dispersion index of 0.26±0.01 and maximum release of drug 70%, decrease normal lung cells (MRC-5) mortality than the free form of the drug in same concentration of drug. Encapsulation of Kaletra anti-coronary drug in niosome could reduce its side effects on lung cells. Due to the great effects of this drug in the treatment of coronary heart disease, this method can be a new window in using this drug to fight the coronary pandemic.

Keyword: Covid-19, Kaletra, Niosome, Drug Delivery Carriers

COVID-19 and Renin Angiotensin Aldosterone System: Pathogenesis and Therapy

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to ACE2 component of the renin angiotensin aldosterone system (RAAS) and infects the human cells. There is a crosstalk between the RAAS and kallikrein/kinin pathway and association of both pathways with hyaluronic acid degradation and neprilysin activity. In the present review we looked at the components of the RAAS and its association with kallikrein/kinin pathway, hyaluronic acid degradation and neprilysin activity and their role and alterations in SARS-CoV-2 infection. Also, we discussed about various COVID-19 therapies based on recombinant ACE2, ACE inhibitors, angiotensin receptor blockers, renin inhibitors, antihyperglycemic drugs, Janus activated kinase inhibitors and the antioxidant of resveratrol in relation to the modulation of the RAAS components. Further, clinical trials in this field have been explained.

Keyword: COVID-19, SARS-CoV-2, ACE2, RAAS inhibitors, antihyperglycemic drugs, Janus kinase inhibitor

In silico virtual screening recommends Suvorexant as potent medicine to inhibit coronavirus 3CL^{pro} protease

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Abstract

the ongoing COVID-19 pandemic affected the entire world since late 2019. After more than a challenging year, no established treatment has been employed to contain the virus. search for potential medicines to inhibit coronavirus via blocking the activity of 3CLpro protease. This protease plays an essential role in the production of viral particles, so inhibiting it can offer a notable solution in combating the infection. all the FDA approved drugs were docked against the active site pocket of the 3CLpro protease in PyRx software. Then, the physicochemical properties of ligands with the greatest binding affinity were studied to select the best candidate drugs.

Of more than 1600 FDA approved drugs, 18 ligands had the best binding affinity greater than -8 kcal/mol with the target protein. The most potent drug is Suvorexant (Belsomra) with a binding affinity of -8.5 kcal/mol. This ligand can establish deep links comprising four hydrogen bonds, three carbon-hydrogen bonds and seven Van der Waals interactions with the amino acids of the active site pocket of the 3CLpro enzyme. This identified FDA approved drug can be utilized after the successful completion of additional *in vivo* examinations to control and adequately improve patients with COVID-19 infection.

Keyword: 3CL^{pro}, COVID-19, Drug design, Suvorexant,Virtual screening

Molecular cloning and sequences analysis of the Receptor-Binding Domain and protease cleavage site of SARS-CoV-2

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Abstract

Globally, the coronavirus diseases 2019 (COVID-19) outbreak as a serious health threat has been spreading at an unimaginable rate. The SARS-CoV-2 spike (S) glycoprotein plays a vital role in binds and fusion to the angiotensin-converting enzyme 2 (ACE2) as a receptor on the host cell. The aim of our study is RNA extraction, cDNA synthesis, and then through specific primers, we isolate two genes including the Receptor-Binding Domain (RBD) and S1/S2 and S2' protease cleavage site of SARS-CoV-2 via polymerase chain reaction (PCR). In the next step, after double digestion, we ligated the sequences into the pET28a (+) vector and transformed them into E.coli strain(DH5a) as a cloning host. The clones were then evaluated for recombination using three common methods including colony PCR, double digestion of final constructs, and sequencing of recombinant plasmids. As a hypothesis molecular cloning of these two genes, which play a key role in the pathogenicity of SARS-CoV-2 can initiate the production of these two proteins as promising candidates as vaccines (active immunotherapy) due to inducing neutralizing antibodies and also via production of an immunoaffinity column based on these two proteins we can isolation neutralizing antibodies against these two proteins from the convalescent serum that can be used as a promising method in the treatment of COVID-19 patients (passive immunotherapy) against SARS-CoV-2.

Keyword: COVID-19, polymerase chain reaction (PCR), Molecular cloning

Neutralization of corona virus using 3-Layer surgical mask coated with an ionic solution

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Abstract

The world is dealing with coronavirus pandemic. There are many ongoing studies developing vaccines and few of them have reached the market. Vaccination progress and loose spread of the virus encouraged us to come up with a solution that can completely destroy the virus and can be sprayed to the surface of the 3-Layer surgical mask. The ionic crystals in this solution are activated with our breathing system and counter the surface of any air born virus including the corona virus. Based on the ionic crystals content, the performance of the enhanced mask can be guaranteed from 3 up to 7 days. This solution is environmental-friendly, allows us to use a smaller number of masks per week and more importantly assures a safe breathing environment. Moreover, the enhanced 3-Layer surgical mask can be safely disposed without any virus content. The stability and consistency of the enhanced mask for minimum period of 3 days has been confirmed using 200 µml of the solution containing sodium and potassium crystals.

Keyword: Covid-19, pandemic, melt-blown, sodium

Effect of hsa-miR-4735 on *nsp2* gene in SARS-CoV-2 virus

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Abstract

Recent introduced mutations in SARS-CoV-2 improve viral compatibility with humans. In addition to amino acid changes, mutations can interfere with the sequences targeted by host miRNAs, which in turn can affect the translation of viral RNA, and thus, can help the virus to escape the host miRNA defenses. All of the human microRNA's sequences have got from miRbase database (http://www.mirbase.org) and then binding positions on the complete SARS-CoV-2 genome isolated from Wuhan were analyzed. The complementary sequence between miRNA and target gene based on P-value was determined by the RNA22 bioinformatics database (http://cm.jefferson.edu/rna22/Interactive/). Maximum folding energy for heteroduplex (Kcal/mol) <-12 has been regarded as a cut-off for binding energy between miRNA and mRNA duplexes and Also, only microRNAs that were bound to the virus genome by canonical binding were investigated. Based on the obtained data, hsa-miR-4735 targets *nsp2* gene that is the subset of ORF1ab genes in SARS-CoV-2 isolated from Wuhan with -12.2 Kcal/mol folding energy and 6mer target binding type. *nsp2*, is an RNA-binding protein that accumulates in cytoplasmic inclusions (viroplasms). *nsp2* is involved in coronavirus (CoVs) genome replication. Therefore, these data determined that, hsa-miR-4735 may affect the virus genome replication by targeting the *nsp2* gene.

Keyword: SARS-CoV-2, miRNA, miRNA-4735, nsp2

Applications of CRISPER technique in Covid-19

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Abstract

The Covid 19 pandemic, which has affected most countries, was first observed in Wuhan, China in 2019 and quickly spread to other countries. The rate of transmission of this disease is very high and it affects the respiratory system more than other parts of the body and it has symptoms such as flu which is called acute respiratory syndrome. Due to the high prevalence rate and high mortality rate, diagnostic methods such as RT-PCR to detect it due to insufficient accuracy and limitations, have moved to more advanced and accurate methods such as Crisper technique. CRISPER technique has been tested in diagnosis and treatment in this disease, which has been approved by the FDA in the diagnostic section, but it is not approved in treatment sections. remains in the experimental stages. Attention to the Crisper technique is increasing due to its high accuracy, although in addition to the advantages, it also has disadvantages, including the delivery system, which is the most important limitation of this technique.

Keyword: Covid-19, CRISPER, RT-PCR, cas12/13

Reconsidering Plant Secondary Metabolites in Attenuation of Covid-19 Pandemic

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Abstract

The global public health-threating Covid-19 disease, caused by severe acute respiratory syndrome (SARS-CoV-2), is a current pandemic with large mortality worldwide. There are many trials underway to discover therapy through testing existing drugs as well as new vaccines. But the severe side effects are still a concern, and vaccination has not been widely available in any country to date. Therefore, many plant-based natural products could provide a starting point for testing against the virus. Numerous natural products such as Magnoflorine, tinosponone, cirsimaritin, chrysoeriol, vasicinone, quercetin, luteolin, epigallocatechingallate, curcumin, apigenin, chrysophanol, Hesperidin, emodin, chrysin, nimbin, withaferin A, piperine, mangiferin, thebaine, berberine, and andrographolide, chebulagic acid, silybin, withaferin A, cordioside, catechin, quercetin, protopine, allocryptopine, crocin, digitoxigenin, theasinensin D, lepidine E, Ursolic acid, carvacrol, oleanolic acid, cyanidin 3-glucoside, baicalin, glabridin, cetylglucopetunidin, isoxanthohumol, carnosol, and etc. From different plant families can interference with SARS-CoV-2 via various mechanisms of actions and strengthen the immune system. Bearing this in mind, studies highlight the importance of natural products against Covid-19 pandemic.

Keyword: Covid-19, Immune system, Natural products, SARS-CoV-2.

Microfluidic System for Detecting COVID-19 Virus using Nano Molecularly Imprinted Polymers

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Abstract

Rapid detection of viral contamination remains a pressing issue in various fields related to human health. One of the most important usage of viral contamination detections is used in measuring the presence of Corona Virus and its variants in human samples. The majority of currently available assays for SARS-COV-2 (COVID-19) like PCR are expensive, time-consuming, and labor-intensive. So we necessarily need a novel rapid assay. In the past some assays were developed for detecting some compound like morphine or bacteria or some viruses like Tobacco Mosaic Virus. In this article we theoretically suggest a novel combination of microfluidics containing integrated NanoMIP with Biosensors is developed using the Spike Protein of Corona virus.

NanoMIPs specific to the spike protein of SARS-COV2- (COVID-19) are synthesized using proprietary methodology, whereby a peptide specific to a region of the whole spike glycoprotein molecule are immobilized on a solid phase, monomers and cross linker are added, controlled polymerization is initiated and, ultimately, nanoMIPs with high affinity for the spike protein are eluted The affinity of the nanoMIPs for the spike glycoprotein is assessed using SPR (surface plasmon resonance) nanoMIPs are then conjugated to CPNs ™ and evaluated using dot blot for spike glycoprotein detection Real virus particle detection was confirmed. Then the specific Biosensors are coated by these NonoMIPs and then biosensors are installed in the designed kit in (figure 1). The flow of corona viruses sample in the microfluidic canals causes the conjunction between nanoMIP and spike protein of Covid 19. Consequently, biosensors will detect these reactions and then they report them to the

computer device which is connected to this kits.

In this study, a NMIP film are formed on the surface of sensing electrodes to enhance the selectivity of sensing. An electrochemical method is then used for detection of Covid 19 Spike Proteins.

NanoMIPs specific to the spike protein of SARS-COV-2(COVID-19) were synthesized using proprietary methodology, whereby a peptide specific to a region of the whole spike glycoprotein molecule was immobilised on a solid phase, monomers and cross-linker were added, controlled polymerization was initiated and, ultimately, NanoMIPs with high affinity for the spike protein are eluted. The affinity of the Nano MIPs for the spike glycoprotein was assessed using SPR (surface plasmon resonance). NanoMIPs were the conjugated to CPNs™ and evaluated using dotblot for spike glycoprotein detection. Real virus particle detection will be confirmed. Then A MIP–morphine electrode is integrated into the microfluidic chip.

Fig. 1 shows a designed diagram of the microfluidic MIP–morphine sensing system accurately. The sample injection can be automated by using micro pumps .Each biosensor consists of 3 main part: 1. A NanoMIP film which is formed on the surface of biosensor 2. A Fluorescent sensitive layer is installed under the first layer, this layer can detect the reaction between NanoMIP and the spike protein of Covid 19 completely. 3. In the last layer an electric chipset was designed to report the condition of fluorescent layer, accordingly if any viruses particle is present in sample the layer will reports its presence to the central computer which is connected to the main microfluidic system. A microfluidic device that combines the microchannel with the micropumps and the microvalves. The dimensions of the chip are 4.5 cm × 11.5 cm.

Results:

1.detection limit of under 0.3 ng of spike glycoprotein using a simple set up. It demonstrates that detection of 100 individual CPN ™ particles is possible with optimized optics and image processing)

2.MIP Diagnostics proprietary NanoMIPs specific for SARS-COV-2 (COVID-19 spike protein have been shown to have high affinity for the spike glycoprotein and conjugation with Stream Bio's CPNs TM produces a conjugate for detection of COVID-19 with a very long shelf life under ambient conditions The combined reagent provides assay developers with a new, significantly more robust option for producing assays that could lead to a new generation of point of care and decentralized testing for COVID-19. Also this novel method will be able to develop in industrial field. We can completely suggest the details of this method to who can invest on.

Keyword: SARS-CoV-2, spike , NanoMIP, microfluidics

Investigating the interaction of antimicrobial peptides, specifically probiotic bacteriocins, on spike proteins of SARS-CoV2 virus

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Abstract

Acute Respiratory Syndrome-2 spread from Wuhan, China in late 2019, causing illness and death among many people around the world, and was finally significantly controlled in 2021 with general vaccination. The causative agent of this disease, which is the SARS CoV-2 virus, belongs to the beta-coronavirus family and uses its surface spike proteins to enter and amplify inside the target cell. This protein consists of two subunits, S1 and S2, each subunit and region of which has a different function. In this study, we used bioinformatics software to find antimicrobial peptides secreted by bacteria (especially probiotic bacteria (bacteriocins)) and other organisms to inhibit spike protein.

In this study, 300 peptides were docked with heptapeptide repeats HR1 and HR2 from subunit S2, and among them, seventeen peptides showed high affinity. Based on peptide safety studies, only 2 peptides, CAP18 protein (1LYP) and PlnK peptide (2KEG) out of seventeen peptides, had no side effects, of which peptide, PlnK, is of probiotic origin. Despite the success of 2JPK probiotic peptide called lactococcin-G in obtaining the highest molecular docking results, this peptide is an allergen and also produces interleukin-4.

Keyword: SARS-CoV-2, Bacteriocin, Lactococcin-G, CAP18 protein, PlnK peptide

Evaluation of clinical symptoms and rapid diagnostic test and PCR of covid-19 in HIV patients

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Abstract

From the end of 2019 until today, a new virus from the corona family of viruses is threatening human society and other animals. Concerns about the new coronavirus are due to the fact that for the third time in less than two decades, the world is facing a deadly coronavirus epidemic. That has emerged and mutated as a virus transmitted between humans and animals, or otherwise adapted to allow pathogenesis in humans. The outbreak began in China, where the effects of the disease have been widespread. Since then, the disease has spread to many other countries. Prior to 2002, coronaviruses were not thought to be an acute problem, but with the spread of the new coronavirus, researchers have found that coronaviruses can cause more severe illnesses than the common cold. Covid virus 19 begins in some people with mild symptoms that may progress to shortness of breath and severe seizures. The most common side effects are fever and cough, as well as muscle aches and fatigue. Patients with moderate to severe disease suffer from shortness of breath. In a small percentage of patients, bloody sputum has been reported. In some patients with the new coronavirus, chest pain as well as upper respiratory tract symptoms (eg, runny nose, sneezing, and sore throat) have been reported. Headaches and gastrointestinal symptoms (eg, nausea, vomiting, diarrhea) are uncommon but can occur. There are two types of coronavirus diagnostic tests, PCR and Rapid. PCR is the gold standard for definitively diagnosing the new coronavirus, which actually detects RNA, or virus-specific genetic material, and can detect the virus within a few days of infection. This test can be detected in people who do not even have any symptoms. Rapid test is another diagnostic way to get a quick answer about the presence or absence of coronavirus. Rapid testing is valuable in patients with Covid-19 symptoms, and its

use in asymptomatic individuals is very limited. However, the results of rapid corona testing may not always be correct.

Due to the fact that people with underlying diseases such as heart disease, diabetes, tumors and immunodeficiency such as AIDS, the symptoms of Covid 19 are more severe and people are more at risk. Therefore, it is necessary to diagnose these patients quickly and correctly so that patients can start quarantine as soon as possible.

In this study, 50 HIV-positive individuals covered by the Behavioral Diseases Counseling Center, each with more than two suspected symptoms of Covid 19, were randomly selected and clinically evaluated, followed by Covid19 and Rapid PCR tests. Covide 19 test was performed for these patients and the results of these two diagnostic tests were compared.

Conclusion: The most common symptoms among people are fever and then cough. In people with Covid-19 symptoms, PCR and Rapid diagnostic tests were 92% consistent, and in asymptomatic individuals, 60% were inconsistent, and despite a negative Rapid test, PCR was positive. Currently, the most standard test for detecting Quid is 19 molecular tests or PCR. There is no false positive in the molecular test, but there is a false negative. This means that if we test 10 people with definite coronavirus infection, 7 people may be definitely diagnosed and 3 people may be negative despite being positive. This means a false negative. As a result, the most standard corona test is the molecular test. The US Infectious Diseases Control Center's guidelines state that there are two things that can be done on the first day when a patient has very mild symptoms of Covid 19 disease and a rapid diagnostic test is negative. Negative, to confirm, perform a molecular test. Second, if the rapid diagnosis test is negative, the same test should be done the next day. Because the volume of the virus in the body on the first day may be 30%, but this amount may reach 70% on the second day. Because the severity of the disease varies from asymptomatic or mild to severe, a significant proportion of patients with obvious evidence of clinical infection have severe disease. The overall mortality rate among the diagnosed cases is about 2% and our knowledge of this disease is incomplete and is developing [4]. So fast, timely and accurate diagnosis Covid 19 PCR is important for people with AIDS as well as starting effective treatment.

Keyword: Covid-19, AIDS, Symptoms, PCR, Rapid

A novel and feasible viral transport media for SARS-CoV-2 diagnosis with prolonged preservation time

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Abstract

Even as Covid-19 advances its inexorable march taking hundreds of thousands of lives and causing almost 4M confirmed death, early detection of patients or carriers could be considered as a best solution for the *control of* spreading disease. Variety of viral transfer media (VTM) have been suggested to stabilize clinical samples for the detection of SARS-CoV-2, particularly for low volume. Almost VTM solutions are based on balanced salt or saline solutions with a buffering capacity to maintain a 'near-neutral' pH supplemented with fetal bovine serum. Herein, we report a novel formulation of VTM that is cheap, high Performance, easy to produce, do not entail filtration for sterilization, and used reagents that were available from common commercial dealers. Several quality assurance assessments revealed that proposed VTM supported highly consistent amplification of the SARS-CoV-2 target (coefficient of variation = 3.5%) were performed using the San sure Real Time SARS-CoV-2 assay with long term preservation capacity. Moreover, our VTM was also showed to be compatible with multiple type of nasal and oropharyngeal swabs, able to maintain its functionality at room temperature for more than three months. Proposed media can be considered for large scale production of the VTM needed for high volume COVID-19 testing capacity.

Keyword: COVID-19, viral transfer media, molecular diagnosis, virucidal

The emerging role of biotechnological advances against COVID-19 disease

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Abstract

The emergence of SARS-Cov-2 viral disease (COVID-19) disease has caused to critical public health in all over the world. The rapid expansion and high mortality rate of this crisis has forced a huge pressure on scientific community to find effective diagnostic methods and proper therapeutic strategies against this viral infection. In this review article, we have studied the most recent biotechnology based strategies that have used to combat this new universal disease. We have concentrated on nanotechnology applications, vaccine production and bioactive molecules extracted from natural products. The goal of this review is to highlight the development of new opinions that can reduce the timelines for improving research quality, considering the in efficacy and security aspects of these modern biotechnological strategies.

Keyword: COVID-19, biotechnology, vaccine, natural products

Secondary metabolites of Myxobacteria as a source for anti-SARS-CoV-2 drug discovery

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Abstract

In recent years, a bacterial group known as Myxobacteria has attracted attention as microorganisms with the ability to synthesize secondary metabolites with diverse pharmaceutical characteristics. Antiviral compounds have also been found within their secondary metabolites. Until April 2021, derived metabolites from myxobacterial strains have shown effectiveness against human viral disease caused by Human Cytomegalovirus (HCMV), Human Immunodeficiency Virus (HIV), Ebola Virus Disease (EVD) and Hepatitis C Virus (HCV). The majority of the effective metabolites have been extracted from members of *Myxococcus* and *Sorangium* genera. Results obtained from genetic analysis performed using NCBI database proved that within 31 existing genera from the order *Myxococcales*, genomic sequence has been recorded for all species of 10 genera, half of the species of five genera, 30% of the species of seven genera and no genomic sequence is reported for 9 genera. Metagenomic investigations of Myxobacteria have shown that genomic sequences of these bacteria can be classified in more families and subgroups. Indicating that extensive number of the Myxobacteria species has yet remained unidentified. Since scientists are currently screening only the previously known antiviral compounds against covid-19, compounds with Myxobacterial origins can be significant candidates for inhibiting the COVID-19.

Keyword: Bioactive secondary metabolites; COVID-19; Natural products; drug discovery

Potentials of Crisper Technique in Diagnosis and Treatment of Covid 19 Infection

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Abstract

Coronavirus is one of the leading causes of human respiratory diseases, which was first observed in Wuhan, China in 2019 and spread rapidly around the world. Development of rapid, reliable, and affordable detection methods with improved therapeutic strategies have turned to a global concern today. Reverse transcriptase-polymerase chain reaction (RT-PCR) and quantitative type (qRT-PCR) have been useful in the diagnosis of COVID-19. PCR systems with high and automatic throughput can be employed. Serological observations also play a significant role in detection of the virus. CRISPR technology was considered as an antiviral therapy. CRISPR / Cas system is one of the genome editing techniques that is important due to accuracy, speed and cheaper than other techniques and is used in the diagnosis and treatment of diseases. With the onset of the corona pandemic, the use of CRISPR / Cas technology to develop drugs, vaccines, and generate beneficial mutations to inhibit pathogenesis was considered. The ability of CRISPR / Cas to identify genes involved in human disease, the capability to manipulate non-encoded regions, and the development of the entire genome library are some of the reasons for the importance of this method. In this review article, we aim to investigate the pathogenicity of COVID-19 and its detection methods, mechanisms of CRISPR technique and application of CRISPR technique in early diagnosis and treatment of COVID-19.

Keyword: COVID-19, CRISPR-Cas, PCR, Cas13

the Review of Recombinant Razi COVO Pars Vaccine Production

The SARS-COV-2 genome with 29.8 kbp, which encodes 27 proteins, contains 4 main structural proteins, including E, S, N and M proteins. Among these proteins, protein S has been suggested as the main antigen of Covid-19 vaccine due to its binding to cellular receptor (ACE-2). This protein has a molecular weight of 180-200 kDa and 1273 amino acids, consisting of a signal peptide of amino acids 1-13 in the N-terminal part and 2 subunits S1 and S2, which are responsible for the binding and entry of the virus into the cell through the receptor. Subunit S1 contains the RBD fragment, which is responsible for binding to the cellular receptor whose RBM (receptor-binding motif) portion is almost entirely conserved in SARS-COV-2, while subunit S2 contains HR, FP, and CT is responsible for fusion of the virus. Therefore, in designing a recombinant vaccine against this disease (potential targeting), it will be based on selecting parts of the virus that, in addition to minimal changes, can neutralize the antibody against them (neutralizing antibodies), including its attachment to the cell, which is the first step in infection.

For this purpose, Spike S1 and S2 glycoprotein subunit proteins matched with genes encoding amino acids 1-674 and 685-1211, respectively, from the SARS-COV-2 spike protein sequence (Gene Bank accession No.: MN908947) with the C-terminal human IgG Fc-tag was inserted into the expression vector. After expression in cGMP-confirmed eukaryotic cells, then purified in sequential steps. Finally, after achieving purity in accordance with the guidelines, the formulation was performed using the “Adjuvant system”, which is a combination of immune-stimulants to stimulate and modulate both arms of the immune system. Studies in safety and immunogenicity of the candidate vaccine showed high level of neutralizing antibodies associated with cell-mediated immunity in non-clinical and phase 1 clinical trials.

Genetic Engineering & Biosafety

Molecular Markers
Plant Tissue Culture
General Plant Biotechnology
Biosafety
Genetic Engendering
Genome Editing

Optimization of Nisin Gene Transfer by Agrobacterium in Carrot Plant

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Abstract

The production of transgenic carrots containing recombinant nisin protein was investigated by agrobacterium, LBA4404 strain and pBI121 plasmid. PBI121 plasmid contains NPTII reporter genes with promoter CaMV35S and terminator NOS, respectively. In order to prepare recombinant plasmid, nisin gene was first identified from *Lactococcus lactis*. After codon optimization, were cloned between bamHI and SacI in pBI121 vector. Nisin is a natural antibacterial that is used as a preservative in foods and drugs. Root, leaf, stem and nods explants of carrot were infection with agrobacterium suspension containing inoculation liquid with different concentrations of inoculation liquid (0.4, 0.8 to 0.6 and 1) at different times of co-cultivation (0, 1, 2 and 3 days) and they were also cultured in callus culture medium. The calli that were able to grow on the selection medium were selected and transferred to the regeneration medium containing kanamycin and cefotaxime at three concentrations of 100, 250 and 500 mg/L. Callus induction was observed in 40% of the explants. the produced embryos, After germination were transferred to the regeneration medium. The highest percentage of kanamycin resistant plants (45%) was related to the use of stem explant and bacterium concentrations of 0.6 to 0.8 and different co-cultivation times of 1 and 2 days and concentration of 250 mg/L cefotaxime .In order to ensure no contamination of plants to Agrobacteria and confirm the accuracy of gene transfer, polymerase chain reaction was performed using vir gene specific primers and the accuracy of gene transfer was confirmed. Polymerase chain reaction showed that 60% of hypothetical transgenic plants had at least one copy of nptII and nisin genes

in their genomes.

Keyword: Carrot, Indirect regeneration, Genetic fidelity, Marker

Pyramiding PVY resistance gene in transgenic potato (*Solanum tuberosum* L.) for resistance to PVY virus and potato tuber moth (*Phthorimaea operculella* Zeller)

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Abstract

Potatoes are very important in terms of food and industry, however like other agricultural products, it hosts many pathogens and weeds. In order to resolve this problem, two or more genes can be introduced into the plant using gene stacking technique and thereby transgenic plants with multiple resistance to different types of stress can be produced. These phenotypes are referred to as stacked or pyramided traits which are preferred by most of the farmers around the world. In this study, the gene silencing method was used to induce resistance against PVY virus. The aim of this project is to stacke of PVY virus resistance gene in transgenic potato plant which had resistance to potato tuber moth (PTM) (*Phthorimaea operculella*, Zeller) by re-transformation method. For this purpose, the RNAi construct were used. This construct was contains the coat protein (CP) fragment and the coat protein fragment with a region from the UTR (CP + UR) segment of the PVY genome. The construct was transferred to internode explants of potato by Agrobacterium mediated method. The regenerated shoots after growth and propagation were analyzed in the selection media containing the herbicide phosphinothricin. Putative transgenic plants were investigated using polymerase chain reaction (PCR). According to the results, 12% of the regenerated plants were transgenic.

Keyword: Gene pyramiding, PVY resistance, Transgenic potato, Potato tuber moth.

Comparative study of the efficiency of reverse PCR (Invers PCR) method with Southern blotting in determining the number of copies of cry1Ab gene

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Abstract

One of the major challenges of commercial use of genetic transformation in plants is the stability of the transgene expression over the years within the same individual. The number of transgenic copies in transgenic plants is usually determined by the method of Southern blotting. In the current study, putative transgenic potato plants (*Solanum tuberosum* cv. Marfona), which have been transformed for resistance to *Phthorimaea operculella* were investigated with the Southern blotting and Inverse PCR methods. The plant materials are the potato lines, which have been transformed by *Agrobacterium tumefaciens* harboring the plasmid encoding *cry*1Ab gene driving by the PEPC (Phosphoenolpyruvate Carboxylase) promoter. The presence of *cry*1Ab gene and its copy number was verified by Southern blotting and inverse PCR (I-PCR) in the potato transgenic clones. DNA was extracted from the leaf material. For I-PCR this DNA was digested with Hin6I and circularized by T4 DNA ligase. PCR was performed using gene specific primers. For Southern blotting DNA was digested with *EcoR*I and *EcoR*I+*Hind*III. The presence of *cry*1Ab gene and its copy number was confirmed by inverse PCR (Inverse-PCR) and Southern blotting in transgenic potato clone. The results obtained from Southern blotting and inverse PCR indicated two copies of the *cry*1Ab gene in the plant genome.

Keyword: Gene copy number, Transgenic, Inverse PCR (I-PCR), Inverse PCR.

Design and construction of a fusion expression plasmid containing the genes encoding etanercept and hydrophobin II

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Abstract

Rheumatoid arthritis (RA) is a common autoimmune systemic inflammatory disease and tumor necrosis factor-α (TNF-α) plays a pivotal role in RA. Etanercept (TNF-R2/Fc, trade name Enbrel®) is one of the anti-TNF drug which is produced by recombinant DNA technology in Chinese hamster ovary. In recent years, plants have been gained widespread acceptance as an attractive expression system for the economic production of recombinant pharmaceutical proteins. The two major challenges hindering the economical production of plant-made recombinant proteins include inadequate accumulation levels and the lack of efficient purification methods. It has been demonstrated that hydrophobin (HFB) fusions can increase the accumulation of target proteins and aid to simply purification of recombinant proteins. The aim of this study was to construct a plant expression plasmid containing the fusion cassette of HFBII-NF-R2/Fc. The *Nicotiana. benthamiana* codon-optimized *TNF-R2/Fc* gene was fused with *HFBII* gene using the flexible linker $(GGGGS)_4$ and cloned in binary expression vector pBI121. For high-level expression of the fusion cassette, a CaMV 35S promoter with a duplicated enhancer region and the 5′ untranslated region of *N. benthamiana* photosystem K subunit gene was also used in the construction. The endoplasmic reticulum (ER) retention KDEL signal was also added at C-terminal of TNF-R2/FC for localization of the fusion protein in ER and the possible formation of HFB-inducing protein bodies. To facilitate purification from plant extracts, a strep II-tag was engineered at the *C*-terminus of TNF-R2/FC for one-step purification using affinity chromatography. The structural integrity of recombinant construct; namely pBI121.HFBII-TNF-R2/Fc, was confirmed by PCR and restriction analyses. The resulting recombinant fusion plasmid can be used for expression and production of etanercept in a *N. benthamiana* platform in the future studies.

Keyword: Etanercept, Plant expression platform, Fusion protein, HFBII

Molecular cloning of *AtMYC2* transcription factor gene involved in the Taxol biosynthetic pathway

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Abstract

Taxol (Fig. 1, generic name paclitaxel) is an effective anticancer drug used widely in the treatment of a variety of cancers. Production of Taxol directly from yew trees remains a challenging problem due to the limited resources of *Taxus* spp. Plant cell culture is an attractive technology to solve these problems by securing the stable supply of Taxol without damage to the natural plant resources. Methyl jasmonate (MeJA) has been successfully used as an effective elicitor to enhance production of Taxol and other taxanes in *in vitro* cultured *Taxus* cells. MYC2 in Arabidopsis (Figure 2b), is the transcription factor of the core jasmonic acid signaling pathway. MYC2 transcription factor has the potential to activate the genes involved in the Taxol biosynthetic pathway. In the last few years, metabolic engineering strategies have been attempted to increase the production of plant bioactive secondary metabolites, by tackling either single key-limiting genes or regulatory transcription factors in medicinal plants. The aim of this study was to clone AtMYC2 transcription factor and construction of an expression vector. The full length cDNA of AtMYC2 was isolated and cloned into the binary expression vector pBI121. For high-level expression of the cassette, a CaMV 35S promoter with a duplicated enhancer region and the 5′ untranslated region of *Petroselinum crispum* chalcone synthase gene was also used in the construction. The structural integrity of recombinant construct; namely pBI121.AtMYC2, was confirmed by PCR and restriction analyses. This construction can be used to boost the expression of Taxol biosynthesis genes and overproduction of Taxol in *Taxus* spp. in the future studies.

Keyword: Taxol, MYC2 transcription factor, Cloning, Metabolic engineering

Increasing seed oil content in tobacco plants by transferring *DGAT1* gene

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Abstract

Genetic engineering is the fastest way to improve the oil content in plants. In this study, Diacylglycerol acyltransferase (DGAT) as a key gene in the kennedy pathway of fatty acid synthesis, was transferred to the tobacco plants by Agrobacterium mediated transformation using EHA105 strain. After molecular analysis of transgenic plants and stable presence of this gene, the content of fatty acids and their composition in seeds were examined. The content of fatty acid in transgenic plants was significantly higher than control plants. The average oil content of transgenic plants was 68.99%, which was 37.56% higher than non-transgenic control plants.

Keyword: Genetic engineering, Seed oil, DGAT1, Tobacco

A review of grain transfection methods using *Agrobacterium*

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Abstract

One of the most important mechanisms of gene transfer to crops is the use of different vectors, especially viruses and bacteria. With the help of these vectors and using appropriate gene transfer techniques, the yield of crops can be increased by artificial mechanisms. In recent years, this method has been used in research work on plants such as tomatoes, tobacco, safflower, sugarcane, soybeans, etc. *Agrobacterium* can also be mentioned. Today, one of the most common methods of gene transfer to rice plants is through *Agrobacterium*. In this review study, we discuss gene transfer techniques, their advantages and disadvantage.

Keyword: Gene transfer, Floral dip, *Agrobacterium*, Vacuum infiltration, Acetosyringone

Role of Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase 1 in Nodule Development of Soybean

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Abstract

Autoregulation of nodulation (AON), plays the central role in nodulation. The effect of *hydroxymethylglutaryl-coenzyme A reductase* 1 (*GmHMGR*1) gene, on nodulation and AON system in *Glycine max* (L.) Merr, was studied. Wild-type soybean (cultivar Bragg) and its near-isogenic supernodulating mutant (*nitrate tolerant symbiotic)* nts1007 were selected to identify the expression pattern of this gene in rootlets after inoculation by rhizobium. For further analysis, the full length of this gene and its promoter were amplified by inverse-PCR and BAC library screening. We made an intron hairpin RNA interference (ihpRNAi) and a *GmHMGR*1 promoter: β-glucuronidase fusion constructs, consequently for suppression of *GmHMGR*1 and histochemical analysis in transgenic soybean hairy roots induced by *Agrobacterium rhizogenes*. Results showed that the *GmHMGR*1 gene is functional at the early onset pathways leading to nodulation and the AON system has a negative effect on its expression and nodule formation in wild type rootlets. *GmHMGR*1 was expressed specifically in the developing phloem within the root, nodules and nodule lenticels. Expression of *GmHMGR*1 in transgenic hairy roots by RNAi silencing was suppressed about 85% related to their controls. This result is presenting that *GmHMGR*1 has a very important role in triggering nodule formation and its suppression caused the decrease of nodule formation while this phenomenon was induced synergistically in *nts* mutant lines with deficient AON system.

Keyword: Autoregulation of nodulation, *GmHMGR*1, RNAi, Soybean, *Glycine max* (L.) Merr.

Evaluation of Safety Aspects of Cisgenesis and Intragenesis in Comparison with Transgenesis

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Abstract

Cisgenesis and Intragenesis are useful technologies for gene transformation through crossable species using genetic engineering. These two technologies have been developed to respond to the considerations expressed in relation to transgenesis and, in contrast, have imposed technical limitations on transgenesis. In a way that it is not possible to transfer a gene to the recipient organism from species that their crosses are rare in nature. Cisgenesis and intragenesis are different in some aspects such as the removal of introns and the selection of promoters and terminators from other species. These restrictions have been met in comparison to transgenesis, in the hope of exempting from biosafety regulations. The issue of reduced biosafety evaluations of these technologies has been debated in various countries. Some countries accepting the reduced biosafety evaluations and others rejecting that for some reasons. Considering that definition of Living Modified Organisms (LMOs) produced in Cisgenesis and Intragenesis such as transgenesis is in full compliance with the definition of LMOs in Cartagena Biosafety Protocol, products of these technologies would not be exempted from Biosafety regulations in international level. In addition several studies have shown that gene transfer from distant species is possible in nature, and also some other concerns remained unanswered, exempting these products from biosafety regulations is unlikely to happen.

Keyword: Biosafety, Cisgenesis, Intragenesis, Transgenesis, Genetic Engineering.

Expression of exendin-4 fused to CTB in lettuce (*Lactuca sativa* L.) hairy roots

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Abstract

In this study, production and expression of recombinant Exendin-4 (EX4) gene bound to cholera toxin B subunit (CTB) in lettuce *(Lactuca sativa* L.) hairy roots by transgenic strains of *Agrobacterium rhizogenesis* of ATCC15834 and 1724 were obtained with extracted constructs from recombinant E. coli clones of TOP10 strain. Transgenicity of bacterial strains was confirmed through PCR cloning and then after inoculation of transgenic strains with lettuce leaf explants, hairy roots were obtained. Molecular extractions at the DNA level using specific primers of the *rol*B gene and specific primers of the *CTB-EX4* gene confirmed the hairy root origin as well as the transgenicity of the explants. After separating the transgenic samples from the non-transgenic ones, RNA was extracted from them and with the help of cDNA synthesis kit (Yekta Tajhiz Azma Company), expression of the samples were measured and compared by RT-PCR method, in three replications, using specific primers according to *Actin* reference gene with GelQuantNET software, and then the data analysis was performed with SAS 9.3 and Excel 2016 software. Also, control samples were considered in all stages of the experiment along with transgenic samples. Considering the results of this study, it was proved that insertion and expression of the recombinant *CTB-EX4* gene in the genome of transgenic hairy roots by *A. rhizogenes* strains are possible and ATCC15834 strain possessed higher yields of efficiency in transgenicity.

Keyword: Diabetes, Hairy root, Exendin, Recombinant protein, *A. Rhizogenes*

Efficient Strategy in Cellulose to Nanocellulose Conversion: Cellulose Targeting Using CRISPR/Cas9 System

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Abstract

Cellulose is the most abundant renewable and biodegradable polymer on the planet. It has been widely used in agriculture, industry, environment, and medicine approaches due to its high accessibility and unique physicochemical properties. Recently, by Introducing cellulose microfibrils in nanotechnology, instead of non-degradable and hazardous nanomaterials, which have negative effects on human health, livestock, and the environment, various nanocellulose-based industries have been developed with ecofriendly nanocellulose-based products. However, the recalcitrance of cell wall lignocellulose, time-consuming and none cost-effective procedures, the high cellulose DP, and crystallinity are the most determinant factors that hinder the development of nanocellulose investments and their massive production. Several reports reveal Numerous strong associations between the structure and arrangement of cellulose synthase subunits in CSC and the crystallinity and DP of cellulose microfibrils. However, the successful non-targeted mutant or RNAi-knockdown transgenic plants with a decrease in Crl and DP of cellulose microfibrils has not been reported, so far. Here, we have focused on the description of cellulose and nanocellulose characteristics and their applications in various industries. Furthermore, the main challenges in cellulose extraction were discussed to achieve high quality and high yield nanocellulose materials. With regard to the importance of genome editing methods in genetic engineering approaches, the feasibility of cellulose engineering using CRISPR/Cas9 system has been studied to develop GE plants that

produce cellulose microfibrils with reduced DP and Crystallinity index.

Keyword: Cellulose synthase A, CRISPR/Cas9 system, Gene editing, Genetic engineering, Nanocellulose

Construction of Recombinant CRISPR Vector for the Beginning Core Promoter of Bcl-x Apoptosis Regulatory Gene

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Abstract

The Bcl-x gene is located on chromosome 20 and has 3 exons. This gene is a member of the Bcl2 family, which play a major role in cell death. Their behavioral expressions are involved in the pathogenesis and progression of human cancers. Increase in Bcl-x copy numbers has been observed in a wide range of human cancers, including lung cancers. Unlike previous gene editing methods such as ZFNs and TALENs, the CRISPR was quickly and widely accepted by researchers because of its cost-effectiveness, scalability, and ease of use. The aim of this study was to induce knockout changes at the beginning of the Core Promoter of the Bcl-x apoptosis regulator gene using the crisper system. The sgRNAs are first designed using the Chopchop site for the target region. Plasmid pSpCas9 (BB) -2A-Puro, as an expression vector binds to double-stranded oligonucleotides. The recombinant vector was transferred to *E.coli* DH5α Susceptible host cell by heat shock method. Then, cells were cultured in medium containing ampicillin antibiotic. Finally, PCR colony was performed. Results of PCR and gel electrophoresis verified cloning of Bcl-x gene into the crisper expression vector. Construction of the recombinant CRISPR vector made by this work, it can be said that crisper could be used as a system for accurate modifications in different purposes, including gene therapy.

Keyword: crisper, mutation, cell death, cancer, gene therapy

Evaluation of Deviation and Codon Optimization in Persian Echium D6D enzyme for Recombinant Expression in Different Hosts

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Abstract

In recent years, genetic modification of oilseeds has become significantly suggested for industrial and nutritional fatty acids production, typically obtained from other sources. One of these essential fatty acids, which is considered very important in human and animal nutrition, is Gamma-linolenic acid (GLA, 18: 3n-6), which plays a vital role in biological structures and cellular functions. GLA is synthesized via unsaturation of linoleic acid (LA, 18: 2n-6) by the enzyme Delta-6 desaturase (D6D) which introduced the bond on 6-carbon position. In this study, in order to optimize the expression and recombinant production of D6D enzyme in different expression systems, the encoding sequence of Persian *Echium* (*Echium amoenum*) D6D (*Ea-d6d*) was evaluated and corrected based on the optimal codon tables of Arabidopsis, Saccharomyces cerevisiae, and Escherichia coli. After optimization, Codon Adaptation Index (CAI) increased to more than 0.85 in different hosts. Also, the lowest percentage of cytosine/guanine content (33.2%) and the highest initial sequence homology (81%) were obtained in Arabidopsis. After optimization, no codon with a frequency of less than 10% was observed in the studied hosts.

Keyword: Delta-6 desaturase enzyme, Persian *Echium*, Codon Optimization, Codon Adaptation Index, Recombinant Expression

Evaluation of Resistance to Fungal Diseases in Melon Transgenic Lines

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Abstract

Genetic engineering and transferring resistance genes from different sources is one of the modern ways to generate resistant plants to a range of pests or diseases in plants. After transformation, plants should be evaluated to confirm the expression and function of target genes. Also, transgenic plants ready for cultivation should be homozygous for the transgene. A construct of three genes including chitinase, glucanase, and PR-1 were transferred to melon cultivar “Khatooni” previously in order to induce resistance to fungal diseases. After transformation, two transgenic lines of K59 and K44 were developed. To obtain the first generation (T1), the T0 plants, which were confirmed to harbor the genes through PCR and southern blot test, were transferred to greenhouse condition and then were self-pollinated to produce seeds. Afterwards, the resistance of the T1 generation were evaluated to two different and common fungal diseases (charcoal rot and powdery mildew). Evaluation of plants for charcoal rot was carried out in soil contaminated with fungi and for powdery mildew by spraying spore suspension on leaves of plants. Analysis of data by chi-square test showed that in comparison with non-transgenic plants, K59 and K44 were significantly more resistant to above diseases. In T2 generation, the progeny of some plants in T1 showed segregation for resistance to charcoal rot and powdery mildew i.e. the parental plants have been hemizygous. Those with no segregation

were deduced to be homozygous for the transgene and they can be used for seed propagation.

Keyword: charcoal rot, powdery mildew, transgenic plants

Delivery systems of CRISPR/Cas-mediated genome editing

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Abstract

Clustered regularly interspaced short palindromic repeat-CRISPR-associated protein (CRISPR-Cas) systems are natural microbial immune systems that have been used as a tool for genome editing. CRISPR-Cas gene-editing components must be delivered to cells to reach the nucleus of targeted cells to be effective. There are many different methods of delivery that affect genome editing applications. Here we review some delivery systems and vehicles including viral and nonviral delivery systems. A viral delivery system is the use of viruses as vectors like adeno associated virus (AAV) vectors. The nonviral delivery comprise of different methods like physical delivery and chemical delivery. Each delivery methods have advantages and disadvantages. So, it is important to choose the efficient delivery system of CRISPR-Cas to achieve the best results.

Keyword: Delivery system ,genome editing, CRISPR/Cas

Study of the expression of recombinant L-Asparaginase II in tobacco hairy root lines at transcription level

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Abstract

Recombinant L-asparaginase II (ASN), Elspar, which is routinely produced in *Escherichia coli*, plays a decisive role in remission- induced and consolidation phase in the treatment of acute lymphoblastic leukemia (ALL). Among the expression systems for recombinant proteins, hairy roots have been considered by researchers due to their numerous advantages such as high growth rate, easy genetic manipulations, high level of recombinant protein production and potential as bioreactor culture. In a study of this group, *Agrobacterium rhizogenes* strain A13 carrying a binary vector pBI121 containing a tobacco-expression optimized *Asn*B gene (encoding recombinant L-asparaginase II) was used for hairy roots induction and several transgenic hairy root lines were produced. In this present study, gene expression in the transgenic hairy root lines were analyzed at transcriptional level by semi-quantitative RT-PCR method and *β-actin* gene was used as the internal control. Based on the results of this study, Nt.HR-*Asn*-28 line has the highest and Nt.HR-*Asn*-15 line has the lowest expression of *Asn*B transgene.

Keyword: *Asn*B gene, L-asparaginaseII, Tobacco hairy roots, Gene transformation, RT-PCR, Molecular farming

Tracing the presence and effect of *ssa* gene expression in the Backcross generations of hybrid parent of transgenic chickpea with non-transgenic chickpea

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Abstract

In the present study of transgenic chickpeas, the Jimbour variety containing the ssa gene producing SSA (sunflower seed albumin protein), which is rich in sulfur-containing amino acids such as methionine and cysteine, was used to cross with common kabuli cultivars in the country such as Azad and Bivanij. By cross-breeding, it is possible to transfer this valuable gene to these cultivars. For this purpose, T3 generation transgenic plants, whose transgenicity was confirmed by molecular analysis, were used as a generous parent in hybridization with two free crop cultivars. PCR reaction to confirm transgenicity of T3 generation plants as parent as well as greenhouse hybrids, Resulting from F1, F2 and reversal (BC1 and BC2). The results of amino acids analysis by HPLC method showed that the selected samples from the cross of E8 × Jimbour confirmed its sulfur-containing amino acids increased slightly compared to the non-transgenic control. The total amount of sulfur-containing amino acids including cysteine and methionine in the primary transgenic plant (E8 genotype) was 33.34%, in the F2 it was 0.17% and in the BC1 it was 0.12%. However, the amount of these two amino acids in the non-transgenic Jimbour control plant was zero percent.

Keyword: Hybridization, Chickpea, Sulfur-containing amino acids, Protein, SSA

The editing of *FAD2-1* gene using CRISPR/Cas9 system to increase oleic acid content in safflower plants

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Abstract

Safflower oil seed plant is native to Iran. In all Iranian domestic cultivars, the content of oleic acid is low. Oils with oleic acid levels above 55% having one double bond are more important than polyunsaturated fatty acids (having two or more double bonds) due to their oxidative stability against heat. Innovation of genetic engineering methods has made it possible to obtain oil seeds with high oleic acid content. With the development of new methods such as gene editing, this breeding process has been achieved purposefully with minimal manipulation in the plant genome. To date, the CRISPR/Cas9 system, as a genome editing method, has been widely used in gene silencing, gene replacement, multiple gene editing, gene function identification, and in regulating the replication process in animals and plant organisms. The aim of this study was the silencing of the *FAD2-1* gene using CRISPR/Cas9 system for increasing the amount of oleic acid in safflower plants. The gene construct of CRISPR/Cas9 system was designed and transferred to *Agrobacterium tumefaciens* after construction and were used for inoculation into safflower capitulum by in-planta method. After molecular analysis and screening of the edited lines for mutations leading to nucleotide and amino acid changes, the fatty acid profile was analyzed using GC mass to indicate the amount of oleic acid content. The results showed 18 point mutations in *FAD2-1* gene sequence, in some of the edited lines (*eg*. event 461A), which 6 cases of these mutation resulted in amino acid changes. Analysis of the fatty acid profile of these lines showed that the amount of oleic acid in the heterozygote lines increased from 12% to about 54%. This is the first report on a successful genome edited plant in Iran.

Keyword: CRISPR/Cas9, FAD2-1, Genome editing, In-Planta, Oleic acid, Safflower

Design and expression of some Malaria parasite immunogens in capillary roots of tobacco plant (Nicotiana tabacum L.)

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Abstract

Malaria is one of the most important parasitic diseases and one of the most important health issues in some countries, especially in the subtropical countries. The cause of malaria is a parasite of the genus Plasmodium and Plasmodium falciparum is mainly found in tropical regions of the world. Circumsporozoite protein (CSP) is a high-abundance protein on the surface of all sporozoites of Plasmodium species. This protein plays a major role in the process of parasite entry to hepatocytes (liver cells). This protein is the main candidate for recombinant vaccine. Malaria is in the pre-erythrocyte stage (blood stage). In here, a chimeric structure containing the CSP gene was designed with plant codon preference and cloned into a plant expression vector under the control of CaMV35S promoter were transferred. the presence of foreign genes in the genome of transgenic plants, PCR technique with specific primers. The authentic transgenic tobacco was transformed by Agrobacterium rhizogenesis strain A4 (non-recombinant) to produce hairy roots. The results showed that the transgenesis and production of hairy roots containing the desired genes were successfully performed.

Keyword: Malaria, Circumsporozoite protein, Chimeric, Agrobacterium rhizogenesis

Application of CRISPR for gene silencing with the aim of generating albino phenotype in plants

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Abstract

Genome editing tools such as zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats associated Cas9/gRNA system (CRISPR/Cas9) are some of the targeted genome editing methods. In the meantime, the advent of CRISPR technology has created a huge change in genome editing, and despite the fact that a short time has passed since the introduction of this technology, the salient features of this technique have made it the most widely used system for genome editing in recent years. Since its introduction, CRISPR has had several applications in plant genetic manipulation. In this study, a few candidate genes introduced which if be knocked out, plant would show the albino phenotype as an attractive visual morphology and a valuable choice to start using CRISPR technology in a plant genetic engineering laboratory.

Keyword: Genome editing, Gene knockout, CRISPR, Albinism in Plants

Identification of new KNL2 isoform in tomato plant (Micro-Tom)

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Abstract

Haploid plant production accelerates the plant breeding programs as the maximum homozygosity is achieved within a generation. In classical approaches like anther and ovule culture, besides being tedious and time-consuming, few crop species and genotypes are amenable to such methods. A promising alternative centromere-mediated genome elimination-based technology is developed that bypasses these restrictions. In this method, haploids could be obtained by manipulating the centromere-specific histone 3 variant, CENH3. In a given species, individuals with mutated CENH3 can induce haploidy when they are crossed to wild-type plants, which leads to the elimination of chromosomes with defective CENH3. The centromeric protein of KNL2 (KINETOCHORE NULL2) is involved in the centromeric localization of the CENH3 in eukaryotes. KNL2 localizes to the centromere and acts upstream of CENH3 deposition. The impaired expression of *KNL2* leads to reduced *cenH3* gene expression, errors in chromosome segregation, and reduced growth rate. Here, we isolate and characterize two isoforms of kinetochore component protein "KNL2" in *solanum Lycopersicum* (SlKnl2) for the first time. The results could be implemented in the production of haploid inducer line of tomato in the future.

Keyword: haploids, CENH3, centromeric, chromosomes.

Production of tolerant rice to roundup herbicide and drought stress

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Abstract

In this study in order to produce tolerant rice to roundup herbicide and drought stress, the pUhE-rN9 construct containing the Hygromycin marker, the glyphosate herbicide tolerance gene (*EPSPS*) under Ubiquitin promotor, and the drought-stress related gene *OsNAC9* under RCc3 root specific promotors were transferred to Hashemi cultivar. Positive plants were confirmed by PCR and Inverse PCR and the herbicide test on positive plants showed that these plants have high tolerance to herbicide compared to the control plants.

Keyword: Rice, Drought stress, Glyphosate, Transgenic, Genetic engineering.

Do transgenic food products have a higher nutritional value?

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Abstract

In early 2020, a document entitled “transgenic facts” were discussed in the Parliament of Iran in which questions were given and answered from the perspective of the author(s). The nature of the responses was against transgenic plants and using this technology in the country of Iran. This article discussed about one of these questions titled “Are transgenic food products have a higher nutritional value?” and the response provided in this report, will criticized by citations of the scientific articles of journals and the views of credible international scientists and centers.

Keywords: Transgenic Plants, GMO, Quality Improvement, Genetic Engineering, Agricultural Biotechnology

Transformation of DRO1 and CKX4 genes in order to modify rice root architecture and improved drought tolerance in rice

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Abstract

The engineering of plant root architecture system can be led to plant tolerance and maintained plant's yield during environmental stresses such as drought, Lodging, and nutrition. Furthermore, a greater root system through improving nutrient and water uptake could result to increase grain yield and optimal seed quality. Considering the water crisis in the country, the production of plants tolerant to drought will be valuable. In this study, to create a drought-tolerant rice plant, two genes involved in root length, mass, and root angle respectively, were cloned and constructed in a multi-gene vector and transferred to Hashemi cultivar. The OCKX4 and DRO1 genes that originated from the wild rice variety were isolated and cloned into the T-DNA region of binary vector under control of root-specific and constitutive cloning promoters respectively. The resulted recombinant vector called pUhrCkDro, introduced into the local cotton variety Hashemi by using an Agrobacterium-mediated gene transformation. Transformed calli were selected on MS medium containing 50 mg / l hygromycin. Putative calli were subsequently regenerated into full plants in the selective medium. Fully developed plants were to Yoshida solution and then to pots. Polymerase chain reaction was used to confirm the integration of OCKX4 and DRO1 transgenes in the T1 plant's genome. Independent events were analyzed by inverse PCR reaction to distinguish the events. The location of the transgene was identified in three of the eight events and is ongoing for other events. Comparison of root phenotype with untransformed control plant showed an apparent difference in root

structure but did not show any other differences. Transgenic plants were sown in the transgenic greenhouse of the Agricultural Biotechnology Research Institute and underwent more molecular analysis in T1 and T2 generations. The resulting multigene contract can also be used to transfer genes to other plants to change root structure and drought tolerance. It is hoped that the production of transgenic rice by increasing the root mass and changing the root architecture system can increase drought tolerance in this important crop and reduce water consumption in rice cultivation.

Keywords: Transgenic rice, Drought tolerance, Multi-gene vector, Root architecture

Standards and regulations of safety in nanobiotechnology

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Abstract

In the last decade, products based nanobiotechnology is increasingly growing in the Iranian and world markets. Due to the successful management and control of safety, ethical and environmental issues, the development of nanobiotechnology should be based on standards and regulations. In this study, national and international standards and regulations related to nanobiotechnology and organizations involved were investigated. The increasing trend of standard publications indicates an increase in attention to the safety issue of nanobiotechnology products over time. But the standards are mostly about the evaluation of nanomaterial toxicity and the safety of workers. The subjects such as interaction nanomaterial with biomolecules, medical device based on nanobiotechnology, environmental issues especially in the field of agriculture and novel product in the food industry need to be paid more attention in future. Investigation of domestic regulation about nanobiotechnology products showed that among related organizations, only food and drug administration consider health and safety criteria for Licensing to enter the market. The aim of regulations adopted from other organizations such as the Iran nanotechnology innovation council and Ministry of Agriculture – Jihad is the development of nanobiotechnology products in internal industries and growth of Iran's share of the internal and global market. Regulations must be adopted on the safety of nanobio-based products for the agriculture industry and monitoring of waste/wastewater from a manufacturing process or using the product. A present challenge in standards and regulations is the inordinate attention that has been focused on the size as the defining characteristic of the technology.

Keywords: Nanobiotechnology, Safety, Regulation, Standard

Venture Building and open Innovation Impact on Biotechnology Business Development and Investment

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Abstract

Biotechnology industry is a "complex Network" of corporate players, dominated by large firms with strong marketing capabilities and start-up firms that focus on research and development. According to Grand View Research, biotechnology global market size was valued at USD 752.88 billion in 2020 and is projected to expand at a CAGR of 15.83% from 2021 to 2028. The biotechnology industry faces a high-cost research and development, limited commercialization and constant technological change. On the other hand the emergence of open innovation models and trends such as digitization, machine learning and artificial intelligence have substantially influenced the creation and development of new business models and supply chain management in biotechnology industry. The industry has been an attractive field of investment for private venture capitals and corporations which has made fund raising more competitive for new startups. This review try to provide information about open innovation concept and introduce new business models for new comer VCs and companies and also to clarify the importance of venture building approach in order to manage investment risks and portfolio design in biotechnology industry.

Keywords: Biotechnology, Open Innovation, Business Models, Venture capital, Venture building

Identification and isolation of promoter region of *DBAT* gene involved in paclitaxel biosynthesis pathway in yew (*Taxus baccata* L.)

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Abstract

Cancers as one of the seven leading causes of death worldwide, causing many deaths worldwide each year. Paclitaxel is one of the herbal anti-cancer drugs and one of the sources of supply to the European yew species with the scientific name of *Taxus baccata*. Due to the very low amount of paclitaxel production in the bark of yew trees, it is necessary to use new methods to increase this valuable secondary metabolite. Increasing the expression level of key genes in biosynthetic pathways is one of the methods considered by research-In the present study, in order to predict and . ers by promoter engineering identify the DBAT gene promoter sequence, SRA database data related to the using genome of *T. baccata* were used. The resulting readings were assemble CodonCodeAligner software. PCR reaction was performed using specially designed primers to isolate the promoter sequence from the genome. The sequence of the 800 bp amplification fragment after sequencing was aligned with the predicted sequence. The results showed 97% identity between predicted and amplified sequences. The sequence of the isolated promoter from *T. baccata* genome was confirm via BLAST search. The results showed 95% identity in DBAT promoter sequence in *T. baccata* and *T. cuspidata* genomes. Based on the results, it seems that the use of this promoter will be effective in .increasing the production of paclitaxel

Keywords: Taxol, Cancer, SRA Database, Inducible promoter, Alignment

An efficient protocol for transient and stable gene transformation of duckweed using Agrobacterium tumefaciens

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Abstract

Over the past few years, attention to plant based bioreactors as an excellent and economically important expression system has shown increasing growth. Duckweed family known as Lemnaceae, the smallest flowering aquatic plant, shows promise ability to act as an eukaryotic bioreactor. Due to duckweeds unique features, such as, high protein content and rapid vegetative propagation, they can be introduced as a suitable host for the expression of recombinant proteins. To use this potential it is necessary to establishing an efficient and stable genetic manipulation system. Several methods were applied to these plants to increase the efficiency of *Agrobacterium tumefaciens* mediated transformation. Here the recombinant *A.tumefaciens* harboring the uidA gene for beta glucoronidase enzyme (GUS) was used as an standard reporter gene. .After transformation, the PCR techniques and GUS staining were applied for confirmation of integration and successful GUS expression. Due to natural limitation of monocotyledonous plants to receive foreign gene through *Agrobacterium*, establishing a general and efficient method for transformation of duckweeds, could open the way to manipulate genetically this monocotyledon aquatic plant.

keywords: Duckweed- *Agrobacterium*- Transformation- GUS assay

Animal Biotechnology

Animal Biotechnology
Marine Biotechnology

Comparison of the growth performance traits in F1 crossbred lambs between two strains Booroola Merino × Moghani and Booroola Romney × Moghani

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Abstract

The *BMPR1B* gene is one of the major genes controlling litter size in sheep. The SNP OAR6: 29382188A>G (NC_019463.1) is known as the Booroola/FecB^{B} fecundity mutation with additive effect on litter size. In the present work, Iranian Moghani ewes were artificially inseminated with sperm from two strains of homozygous Booroola carrier rams from New Zealand, Merino Tamlet and Romney. As expected for the first generation, F1 crossbred lambs of Booroola Merino × Moghani and Booroola Romney × Moghani were genotyped as heterozygous carriers of the Booroola mutation (FecB$^{B/+}$ genotype) using restriction fragment length polymorphism (RFLP) analysis. Growth of F1 lambs was followed by regular weighing and measuring from birth to 11 months of age. While birth weight was the same, the growth rate was significantly increased in F1 Booroola Romney × Moghani crossbred lambs compared to Booroola Merino × Moghani lambs after 3 months of age. In contrast, the body measurements showed no differences. These results suggest that the Booroola Romney × Moghani crossbreed may be appropriate for a strategie to create a composite breed based on local Moghani sheep with expected increased prolificacy and optimal lamb growth rates taking advantage of the good maternal qualities of the Moghani ewes.

Keyword: Cross breeding, Sheep, Booroola, *BMPR1B* gene, Growth traits

Meta-analysis of relationships between prolactin gene polymorphism and milk production in cattle

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Abstract

For More than several decades, many single studies have been conducted on genes that affect the milk-producing traits in cattle. Many studies look at the relationship between prolactin gene polymorphism and various milk production traits which often results are conflicting. This study was performed to examine the relationship between prolactin gene polymorphism and cattle milk production by meta-analysis method of various published research results. Meta-Analyses conducted in R with the metafor Package. The results for four models used for meta-analysis showed that in additive model (AA vs. BB) animals with AA genotype performed better than animals with BB genotype ($P<0.01$). It was also found that in additive, dominance, codominance and recessive models when Holstein breed cattle were studied, the difference in performance of animals with different prolactin genotypes was not significant, in these models, however, there is a significant difference when comparing non-Holstein dairy cows. It seen and shows the positive effect of allele A relative to allele B on milk production. It seems that allele A in comparison to allele B has a positive effect on milk production.

Keyword: Polymorphism, Meta-analysis, Milk production, Metafor

Transcriptome analysis of the laying hen magnum reveals differentially expressed genes involved in egg formation

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Abstract

The oviduct of a laying hen provides a favorable biological environment for the formation and fertilization of the egg. Synthesis of albumin mostly occurs in the tubular gland cells of magnum. The aim of this study was to analyze the transcriptome of the magnum in layers and non-layer hens for identifying the differentially expressed genes (DEGs) related to egg formation. The RNA sequencing analysis was performed on three laying and three non-laying hens. Totally, 92 DEGs were identified according the criteria and among these DEGs, 38 genes were up-regulated and 54 genes were down-regulate. Up-regulated genes were enriched for biological process which included acute-phase response and ion transport processes. Five hub genes were selected according to genes involved in GO analysis and the main module in protein-protein network including LYZ, ORM1, QSOX1, TF, and OVAL. All of these genes have important and vital role in egg formation in the magnum. The identification and functional analysis of DEGs may contribute to understand the development and formation of eggs in poultry. Therefore, these five hub genes were found to be the main genes in the magnum of laying hen compared to non-laying that can be used as markers of laying situation.

Keyword: Chicken, Egg formation, Albumen, magnum, RNA-Seq

Transcription of *Bcl-2* as an anti-apoptotic gene in two species of coral reefs, sensitive and tolerant to thermal stress

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Abstract

The programmed cell death or apoptosis is a process in which the community of cells in multicellular organisms are firmly regulated by controlling the rate of cell death. B-cell lymphoma 2 (will be mentioned as Bcl-2 hereafter) is from regulator protein families that regulates cell death or apoptosis. Any stress can affect the cellular and molecular conditions including the expression of genes such as *Bcl-2*. Coral reefs of the Persian Gulf are under thermal stress of tropical conditions and climate change as well. In this study two species of coral reefs, *Acropora downingi* (Wallace, 1999) and *Porites lobata* (Dana, 1846) were sampled from Qeshm Island and Larak Island respectively in February 2019. The samples were treated under heat shock of

34°C for 24 and 48 hours after two weeks adaptation at 25°C. The transcription of *Bcl-2* was studied in both species and in 3 times of control, 24h and 48h by qRT-PCR. The results showed that *Bcl-2* were up-regulated in both species at 24h after heat induction. The expression was down-regulated at 48h in *P. lobata*. In contrast, the expression of gene in *A. downingi* continued to be expressed up to 48h. These results might indicate that *Porites* cells are headed towards bleaching and death with increased temperature, while *Acropora* showed more resistance to high temperature. The results of this study, regarding the observed expression patterns, can clarify the response of different species to a thermal stress in coral reefs. The exposure of corals to acute conditions with high temperatures presented the behavior of the desired gene in the studied conditions.

Keyword: Coral Reef, Anti-apoptosis, *Bcl-2*, Thermal stress, *Aropora*, *Porites*

Investigation of protein complexes affecting milk production using network analysis

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Abstract

Proteins as individual units cannot perform their proper function, but what causes a particular phenotype is a set of protein interactions. Changes in the expression of certain proteins can cause significant changes in biological pathways. Some protein can play a more important role in biological pathways. These proteins are highly correlated at the level of molecular interactions and can cause changes in cellular function and major changes at the phenotypic level. One of the phenotypic changes in milk production is that milk and dairy products are the most important food sources. In the present study, the data used with the access number GSE33680 were extracted from the Arrayexpress database. Before analyzing the expression of genes, data quality control was performed using the Limma package in R software and using the basic components method. Then, the analysis was performed to identify different expression genes and to select more effective genes in milk production, the genes were introduced to DAVID software. Cytoscape software was used to construct the network. Protein clusters were determined and analyzed by MCODE algorithm. 118 genes were introduced to Cytoscape software and its output is 6 complexes. APLN, BCA1, ADCY5, LPCAT1, CDH17 ARC genes are seed genes, respectively, whose role in milk production was investigated.

Keyword: Bioinformatics, Protein complex, Network, Milk production

Sterilization of Sterlet (*Acipenser ruthenus*) by heat shock and microinjection of *Dead-End(DND)* gene knockdown agent

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Abstract

Chimera germ line production is so crucial for preservation of valuable and sensitive species such as sturgeon. A prerequisite for producing gametes derived from donor species is to have sterilized recipient species. Sterlet (*Acipenser ruthenu*) a small European species with a short reproductive cycle among sturgeon and can be an ideal recipient for chimera production. The aim of this study was to investigate two sterilization methods in sterlet. For this purpose, starlet newly fertilized embryos were exposed to heat shock of 37 ° C for 2-3 minutes at 20 minutes after fertilization. Alternatively, newly fertilized embryos in I to IV cell stages were sterilized by microinjection of knockdown agents against *dead end(dnd)*, which is responsible for migration and survival of primordial germ cells (PGCs). The results of larvae sterilization up to 5 days after hatching (5dph) indicated that the success of both methods in sterilization is the same ($p > 0.05$). In addition, hatching percentage of embryos in both methods did not show a significant difference ($p>0.05$). On the other hand, the mortality rate in sterilized embryos using microinjection of antisense Morpholino Oligonucleotide (MO) was higher ($p <0.05$). Manipulation of sterlet embryos in knockdown *dnd* gene method and the possibility of fertilization in triploid sturgeon are the most important disadvantages of these methods, however, antisense MO microinjection can permanently delete PGCs in the recipient species and thus increase the success of donor gamete-derived production.

Keyword: Chimera, Dead end (dnd) gene, Triploid, Sterilization.

The use of the Heter-LP algorithm to prediction of reposition antibiotics for *E. coli* mastitis

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Abstract

Mastitis, a disease with high incidence worldwide, is the most prevalent and costly disease in the dairy industry. Environmental mastitis pathogens, such as Escherichia coli (*E. coli*), are major etiological agents of bovine mastitis in well-managed dairy farms. Therapeutic success of bovine mastitis depends mainly on accurate diagnosis, severity of udder pathology, drug selection, and relevance of route of administration, supportive treatment, and elimination of predisposing factors. In the current research, Heter-LP, a new system biology-based method of drug repositioning, was applied to potentially identify novel therapeutic avenues for the treatment of *E. coli* mastitis. Public data repositories relevant to known diseases, drugs, and gene targets along with other specialized biological information for *E. coli* mastitis, including key genes with robust bio-signatures, drugs and functional related diseases were used as input data for analysis with the Heter-LP algorithm. Our analyses identified novel drugs such as Glibenclamide, Ipratropium, Salbutamol, and Carbidopa as possible therapeutics that could be used against *E. coli* mastitis. Predicted relationships can be used by pharmaceutical scientists or veterinarians to find commercially efficacious medicines or combination of two or more active compounds to treat mastitis.

Keyword: Drug repositioning, Drug target, *E. coli* mastitis, Gene regulator, Heter-LP algorithm, semi-supervised learning.

Nanog gene expression in mouse blastocysts influenced by embryo splitting

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Abstract

Embryo splitting actually resembles to a natural process to create identical twins. Split two-cell-stage embryo into blastomeres which are allowed to develop to fetus then adults, can produce monozygotic twins. These twins can be extremely efficient to understand how human twins could be different in phenotype very much, although they are near. Particularly, twin animal can be employed to investigate how much and which phenotypic discordance is related to epigenetic changes including DNA methylation caused by environmental and other effects, during the time. In this field of study on mouse embryo splitting, the developmental ability of the single blastomere, isolated from two-cell-stage embryo, has been examined and also their totipotency growing to adult has been reported. Moreover, isolating two-cell blastomeres could promote the development and growth of the blastomeres to full-term live fetus and consequently adult mice. During the last decades, there can be found several reported methods that can be used in embryo splitting. Depending on the growth stage of the embryo, blastomere biopsy and bisection can be employed for splitting embryo in cleavage stage and morulae or blastocysts, respectively. Mechanical division of the mouse embryos can predominantly causes cellular damage and consequently decrease the chance of the effective embryo splitting [Noli, 2017]. Bisection of the mouse morulae could successfully create twin embryos to transfer into the surrogate females. 25 percent of these cases could grow and develop to full-term live fetus. Besides, half-embryos of mouse developed from eight-cell-stage blastomeres which were isolated by biopsy, indicated non-significant results, after transferring the embryos to surrogate females. Molecular analysis of the gene expression could be an efficient approach to select embryo. During evolution of the mouse embryo, dividing cell lines is actually controlled by some gene regulation networks containing transcriptional factors which are specifically expressed in each cell type. The influence of the mouse embryo splitting on *Nanog*, as a pluripotent gene, expression were

evaluated in the present study.

Keyword: Embryo Splitting, Mouse Blastocyst, Two-cell Embryo, *Cdx2*, *Sox2*, *Oct4*, *Nanog*

Effects of new kisspeptin neuropeptide on gonadotropin secretion in goldfish (*Carassius auratus*)

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Abstract

Kisspeptins are secreted in the kiss neurons in the hypothalamus and affect GnRH-secreting neurons. Kisspeptins directly stimulate the secretion of GnRH from the hypothalamus and indirectly stimulate the secretion of gonadotropins from the pituitary gland. In this study, the effect of goldfish kisspeptin (KISS1) and human kisspeptin (HKISS) and a combination of them (KISS1+H) was investigated on the secretion of goldfish gonadotropins. These kisspeptins have been synthesized in the laboratory by solid-phase synthesis. These peptides were injected at a concentration of 100 µg/kg body weight and gonadotropin levels were measured and compared with control samples 6 hours after injection. It was observed that the gonadotropin secretion was significantly affected by the injection of kisspeptins.

Keyword: Kisspeptin, Ovaprim, Goldfish, Reproduction.

The effects of dietary betaine on colorectal inflammation and tumorigenesis on Balb/C mice

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Abstract

Betaine is a tri-methyl organic compound derived from Glycine which was first discovered in the juice of sugar beets in 19th century. The principal physiologic role of betaine is as an osmolyte and methyl donor (transmethylation). As an osmolyte, betaine protects cells, proteins, and enzymes from environmental stress (eg, low water, high salinity, or extreme temperature). As a methyl donor, betaine participates in the methionine cycle—primarily in the human liver and kidneys. Several studies on on betaine antioxidant and anti-inflammatory effects showed that it can suppress some inflammatory factors such as NF-κB. This study was conducted to investigate the preventative effects of dietary betaine on colorectal cancer in vivo. For this purpose 48 Balb/C mice were supplied after 2 weeks of adaptation and were divided into 4 study groups (control, betaine 50%, betaine 100% and betaine 200%) and were supplemented by betaine in their drink water in a daily basis for 40 day. Colon carcinogenesis in mice was induced by azoxymethane (AOM) and dextran sulfate sodium (DSS) protocol. At the end of study mice were euthanised and dital coorectal were prepared for histological studies. The results showed that mice in group betaine 200% performed better in face of inflammatory carcinogenesis while other groups showed tissue inflammation, hyperplasia and to some extent adenocarcinoma incidence. This can suggest betaine as an effective diatary supplement to protect cellular inflammation in colon and rectum. This hypothesis need to be confirmed by further molecular studies.

Keyword: Betaine, Colorectal cancer, Anti inflammation, AOM/DSS, Balb/C

Investigating the genetic diversity of Iranian native and Holstein cattle breeds using genomic data

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Abstract

In this study, genomic data of 590 cattle were used including Iranian indigenous and Holstein. Quality control and data filtration were performed using Plink 1.9 software (Purcell, 2007). After this quality control, individuals and SNPs with call rate (CR_{IND}) below 0.95%, SNP makers with minor allele frequency (MAF) > 0.01%, divergence from Hardy-Weinberg Equilibrium (HWE) (P-value >10e-6) were excluded. This procedure yielded 509 individuals with 13512 SNP marker. Then filtered data were used to genetic diversity and clustering analysis. Identification of genetic groups were performed using PCA analysis data by GenABEL software. With this explanatory variance, the studied populations are in 4 separate categories including purebred Sarabi population in the first group, crossbred from northwest of Iran in the second group, purebred Holstein populations from Iran, France and Ireland in the third group and native breeds of Iran including Sarabi, Najdi, Kurdi, Talashi, Mazandarani, Kermani and Pars and Sistani were in the fourth group. Further details of demographic differentiation were identified by Weir and Cockerham's fixation index. The range of differentiation in the present study varied from 0.18 between Sistani and Kurdish breeds to 0.700 to 0.004 between the Iran Holstein breed with Ireland and France Holstein breeds. The results showed that the highest difference between indigenous and Holstein breeds related to Sistani breed that had the highest difference with different Holstein breeds (0.128 to 0.138). With slight differences from other Iranian indigenous breeds, the Kurdish and Mazandaran breeds had the smallest genetic differences with the studied Holstein populations.

Keyword: Genetic Diversity, Fixation Index, Holstein Cattle, Indigenous Cross-bred

Genes related to distinct genomic regions between Holstein and Iranian northwestern crossbred cattle using XP-EHH and Rsb statistics

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Abstract

In order to detection genes related to distinct genomic between Iranian northwestern crossbred and Holstein cattle breed, respectively number of 100 and 60 sample from Iranian northwestern crossbred and Holstein populations were used. After ensuring the distinct structure of the studied populations, XP_EHH and Rsb statistics were used to identify the selection signatures. In total, 20 regions exceeding the threshold were identified as extremely differentiated. These selected genomic regions were surveyed to find encoding putative candidate genes and 135 genes were extracted from the corresponding areas in ARS-UCD1.2 Bos Taurus Genome Assembly. Some of detected genes in regions under selection were involved in metabolic pathways related to taste, smell, fat metabolic pathways, the immune system, reproduction performance, nervous system development, cell apoptosis, and other transmitters or receptors. The genes of the selected genomic regions were further examined for further analysis and finding of gene networks. These analyzes were performed by online software related to the genomic database (DAVID). Only one significant network was identified. This gene network communicates with taste receptors and specially detection of bitter tastes.

Keyword: Signatures of selection, Population differentiation index, Iranian northwestern native crossbred cattle, Holstein cattle breed

Review on Ability of CRISPR/Cas9 Gene Editing System in Animals

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Abstract

Various methods have been developed for targeted genome editing, including Zinc Finger Nucleases (ZFNs), Transcription-Activating Effect Nucleases (TALEN), and CRISPR / Cas9. Genome engineering tools are based on the failure of two strands at the target site of the target genome and the subsequent repair of these sections through homologous recombination pathways or the connection of non-homologous ends, thereby causing the desired genetic changes. In recent years, the CRISPR / Cas9 technique has been considered as a new and efficient method for genome editing and is based on bacterial immune system gene editing methods. It is noteworthy, this technique can be used to treat and control genetic diseases, improve food quality, make medicines, and in animals to improve growth and reproductive performance. The technology directly targets DNA and uses the Cas9 protein as a DNA-cutting molecule, which binds to the target sequence by a guide RNA that pairs with the DNA in question. This review discusses this new genome editing technique and its applications in mammals.

Keyword: CRISPR/Cas9, Genome Editing, Targeting Gene, Disease.

Single nucleotide polymorphism development markers involved in early sexual maturation from transcriptomes of rainbow trout (*Onchorhynchus mykiss*)

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Abstract

Rainbow trout (*Onchorhynchus mykiss*) is one of the most important freshwater aquaculture fish in Iran. It is necessary to development available molecular marker associated with genetic variation in maturation for *O. mykiss*. In this study, 52 novel SNP markers for *O. mykiss* were discovered and validated based on transcriptome sequencing. The observed and expected heterozygosities ranged from 0.177 to 1.000 and 0.239 to 0.638, respectively. The minimum allele frequency (MAF) ranged from 0.166 to 0.489. Among these SNP loci, 22 loci showed significant departures from the Hardy–Weinberg equilibrium after Bonferroni correction ($p < 0.05$) and significant linkage disequilibrium was found. These SNP markers would be used in genetic studies helping economic performance improvement and management of this species.

Keyword: Single nucleotide polymorphism (SNP), Rainbow trout, Next generation sequencing, transcriptome

Development and characterization of simple sequence repeats (SSRs) makers for *Capoeta aculeata* (Valenciennes, 1844) using NGS data

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Abstract

The species *Capoeta aculeata* (Valenciennes, 1844) is one of the most important freshwater species endemic to Iran. However, the investigation of a population genetic structure of this species is limited by the low number of molecular markers currently described. In this study, we implemented next generation sequencing technology to identify polymorphic microsatellite markers and investigate the population genetic structure of *C. aculeata* sampled from three geographical sites in Iran. We sequenced 60 individuals from three populations occurring in the Zagros basin. We characterized and developed 36 novel polymorphic microsatellite markers and these loci were examined in 120 individuals from three populations occurring in the Zagros basin. The average number of alleles per locus varied from 1.7 to 16. (average = 7.89). The results showed that, the polymorphism information content (PIC) of these SSR loci varied from 0.254 to 0.888. The observed heterozygosity (*Ho*) per locus ranged from 0.170 to 0.881, while the expected heterozygosity (*He*) per locus was from 0.170 to 0.881. Among these SSR loci, 20 loci deviated significantly from the Hardy–Weinberg equilibrium after Bonferroni correction ($p< 0.05$). These microsatellite markers could provide a valuable tool for future population and conservation genetics studies of *C. aculeate* populations and other closely related species.

Keyword: *Capoeta aculeate*, Microsatellite markers, Next generation sequencing (NGS), Genetic structure

Population genomics of *Capoeta aculeata* populations inferred from nuclear DNA markers

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Abstract

The present study aimed to investigate the genetic variation of *Capoeta aculeata* on the basis of DNA microsatellite loci from three rivers (Beshar, Khersan, Maroun) in Kohgiluyeh and Boyer-Ahmad Province in Iran. DNA from fin clips of 120 specimens extracted and was examined with 8 microsatellite markers. Genetic differences between the populations were discerned by pairwise comparison based on allelic distribution. The average numbers of alleles per locus ranged from 4 to 14, while the average observed heterozygosity (*Ho*) at various loci varied between 0.212 to 0.579, implying that a midway level of genetic variation. Among three populations, the Maroun River population displayed the highest level of variability in terms of heterozygosity. Tests of Hardy-Weinberg showed that the microsatellite loci deviated significantly in the populations. The results indicate that some of the populations were significantly differentiated from one another based on pairwise F_{ST} estimates. Genetic distance based measures supported the clustering of Maroun, Beshar and Khersan rivers may be genetically discrete from other *C. aculeata* populations. The neighbor-joining dendrogram topology constructed on the basis of genetic distances among populations supported observed division between the populations. The non-significant differentiation between *C. aculeata* samples from the Beshar and Khersan can be explained by a relatively disconnection of these two populations and/or small amounts of gene flow.

Keyword: *Capoeta aculeata,* Microsatellites, Genetic structure, Conservation genetics

Population genetic structure of *Pontastacus leptodactylus* in Caspian Sea basin

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Abstract

In this study, we evaluated the genetic variability of narrow-clawed crayfish (*Astacus leptodactylus*) from Caspian Sea, rivers or water reservoir, and estimated their genetic variation on the basis of DNA microsatellite loci. DNA from 194 specimens extracted and was examined with six microsatellite markers. Results obtained from the study showed that mean genetic variation indices (5.35 alleles per locus) and heterozygosity (0.53) were moderate and pattern of allelic richness was lower than anticipated (3.2). The results also demonstrated significant variability among collections of narrow-clawed crayfish from disparate locations. The mean observed heterozygosity at various loci varied between 0.222 to 0.732, implying that a midway level of genetic variation. Pairwise F_{ST} values affirmed that genetic distinctiveness among the collections. Within the Caspian Sea, the genetic hetrogeneity observed may be a consequence of genetic structure arising from long distance of broodstock. The weak differentiation between crayfish samples from the Anzali lagoon, Kiashahr and Masuleh can be explained by a relatively recent disconnection of these three populations and/or small amounts of gene flow. These results could give applicable information for more forceful management of this commercial species.

Keyword: *Astacus leptodactylus,* Microsatellite DNA, Stock structure, Caspian Sea

The mitochondrial DNA control region sequencing for genetic variation of the freshwater crayfish (*Pontastacus leptodactylus*)

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Abstract

In order to investigate population genetic structure of freshwater crayfish *(Pontastacus leptodactylus)* in Iran direct sequencing of mtDNA control region was used. A total of 132 samples were collected from the different locations. The quality and quantity of total DNA were determined by agarose gel electrophoresis ethidium bromide staining and spectrophotometery, respectively. The results showed that 38 haplotypes were observed between samples in sequencing analyses. The haplotype diversity (*h*) and nucleotide diversity (π) were 0.811±0.049 and 0.0127±0.0038 sequencing techniques, respectively. The results of F_{ST} and analysis of molecular variance (AMOVA) demonstrated that samples between Siahdarvishan River, Jafrood River and Astara region in the southwest Caspian sea statistically are significant in sequencing techniques ($P<0.0001$). Therefore three distinct population were identified. These results showed that haplotype distribution in different location were significant and populations of Siahdarvishan River and Astara region statistically were significant ($P < 0.0001$). These results suggests that the unique genetic structure of Siahdarvishan River, Jafrood River and Astara region represent a highly valuable genetic resource and provide useful information for identifying populations and genetic improvemnet. In addition, this study confirms useful application of molecular markers in investigating identifying of narrow claw crayfish.

Keyword: Freshwater crayfish, *Astacus leptodactylus,* mtDNA, Control region, Molecular markers

Application of RAD genotyping for genetic structuring and population assignment in *Pontastacus leptodactylus* (Eschscholtz, 1823)

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Abstract

The wild population of the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) have suffered an extreme decline in recent years. We developed single nucleotide polymorphism (SNP) markers for the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) using restriction site-associated DNA sequencing (RAD-seq) genotyping on the Illumina HiSeq4000 platform. In total, we sequenced 44 individuals from seven populations occurring in the Caspian Sea basin from Iran. Of the 86 SNP loci, 43 loci were found to be polymorphic and bi-allelic. The minor allele frequency (MAF) of these SNPs varied from 0.112 to 0.444. The observed heterozygosity (*Ho*) per locus ranged from 0.165 to 0.603, while the expected heterozygosity (*He*) per locus was from 0.202 to 0.506. The *Ne* varied from 1.5768 to 1.8850. Among these SNP loci, 14 loci deviated significantly from the Hardy–Weinberg equilibrium after Bonferroni correction ($p < 0.05$). These SNP markers provide a valuable resource for future population genomics and conservation genetics in the narrow-clawed crayfish.

Keyword: SNPs development, *Pontastacus leptodactylus*, RAD-seq, Genetic variation

Characterization of the complete mitochondrial genome of *Pontastacus leptodactylus* (Eschscholtz, 1823)

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Abstract

In this study, the entire mitochondrial whole of *Pontastacus leptodactylus* (Eschscholtz, 1823) was sequenced using restriction site-associated DNA sequencing (RAD-seq) genotyping on the Illumina HiSeq4000 platform. It is 16318 bp long and contains 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes, and one control region (D-loop). Twelve PCGs start with ATG, but *COX1* uses GTG as the start codon. In addition, all tRNAs display the typical clover-leaf structure. Phylogenetic analysis revealed that *P. leptodactylus* is closely related to *Astacus astacus*, and then clustered into a clade with other crayfish species. This work provides additional molecular information for studying *P. leptodactylus* conservation genetics and evolutionary relationships.

Keyword: Mitochondrial DNA, *Pontastacus leptodactylus*, RAD-seq, Phylogenetic

Population genetics of *Capoeta aculeata* as determined from mitochondrial DNA variation of the control region

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Abstract

Mitochondria1 DNA (mtDNA) control region sequences were analyzed to evaluate the population genetic structure of *Capoeta aculeata*. A total of 120 specimens were collected from the three rivers of the Kohgiluyeh and Boyer-Ahmad Province in Iran. mtDNA control region was amplified using PCR. Direct sequencing was performed according standard method. The results showed that 21 haplotypes were observed between 120 samples in the method. The highest numbers of haplotypes were observed in Maroun River in which five haplotypes (D1, D, F, G and I) among them were specific for the river and were not observed in the other rivers. The average haplotype diversity (*h*) and nucleotide diversity (π) were 0.822±0.073 and 0.0135±0.005, respectively. The results of F_{ST} based on kimura-2 parameters method and analysis of molecular variance (AMOVA) demonstrated that most variations occurred between samples from Maroun River and that the samples include two distinct population segment including Maroun River and Beshar River ($P<0.001$). As mtDNA control region is hypervariable segment, this can be provide potential marker for identifying probable populations and for determining their management and conservation units, leading to the useful application of molecular genetics in investigating conservation biology of *Capoeta aculeata*.

Keyword: *Capoeta aculeata*, Mitochondrial DNA, Genetic variation

Optimization of flow cytometry method to identify chromosom manipulation individuals in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Treatments designed to induce polyploidy are rarely 100% effective. Separation of polyploids within treatment groups is necessary for subsequent determination of performance levels. polyploidy has identified in a variety of ways: nuclear volume and cell volume of fish erythrocytes and other cells, chromosome counts, Detection of NORs by silver staining and The most effective and success method in identifying ploidy has been flow cytometry. In this study, the percentage of ploidy in 5 groups was measured using flow cytometry methods. The percentage of ploidy in these groups was 40-100%. Flow cytometry results were consistent with cytological results. This method is not widely used due to different methods of sample preparation, high cost and Lack of access to fish farms.

Keyword: Flow cytometry, Aquaculture, Polyploidy.

Experimental Studies of the Interactions of New Zealand Rabbit Blastema Cells and Mesenchymal Cells of Chick Embryonic Tissues (*Gallus gallus domesticus*) Under *in vitro* Conditions.

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Abstract

The study of cell behavior and their interaction with other cells and extracellular matrix is important to understand many physiological processes such as embryonic development or tissue repair and pathologies such as cancer cell metastasis and immune responses. Extensive studies have been done on the mechanisms of cell movement and migration, but most of these studies have been in two-dimensional matrices in the form of connection and separation from the matrix, and in fact three-dimensional conditions that normally prevail in the body of living organisms, do not exist. Therefore, the use of 3D models or natural textures can better simulate the conditions of natural textures. In this study, chick embryonic tissue in interaction with New Zealand rabbit blastoma tissue was used to investigate experimental studies of cell interactions in vitro. In this study, first the embryonic tissue of chickens was isolated and at the same time, blastma ring tissue was obtained from New Zealand rabbit ears. The blastoma rings were then assembled with thin strips of chick embryonic tissue. It was then transferred to the culture medium and examined on days 7, 10, 15 and 21 days after cultivation. Histological examinations were performed with hematoxylin-eosin, toluidine blue, Mason trichrome and DAPI fluorescent dye. The results of this study on different days after culture showed the penetration of blastoma tissue cells and the destruction of part of the embryonic tissue during cell penetration. It was also found that the best time for cell penetration in the scaffold was observed on the 15th day after culture. The results of this study show

that embryonic dynamic tissue can provide a suitable three-dimensional substrate for cell movement and migration and a suitable model to study the interaction between cells and extracellular matrix in vitro.

Keyword: Blastoma, Chick embryonic tissue, Interactions, *in vitro*

Identification of non-coding RNAs associated with fatty liver in laying hens

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Abstract

Following the food needs of a growing human population, the need for livestock products also expands. The liver in birds is involved in a set of metabolic functions in the body. FLHS Fatty Liver Bleeding Syndrome, a metabolic condition in birds, can reduce ovulation and even death. Previous studies have shown that many acquired traits related to fat metabolism in humans, pigs and mice are inherited. Alterations in lncRNA gene loci or related genomic sequences may affect many biological processes. In order to analyze lncRNAs in laying hen liver, RNA-seq data of six samples were used, half of which were the offspring of fat fathers and the other half were the offspring of fathers without fatty liver. Then, using the DESeq2 package, the difference in expression of lncRNAs in the samples was analyzed. The results of the analysis of differential expression of genes showed that there are 24,356 annotated genes, of which 1912 to the gene with a P-value of less than 0.05. Also 101 lncRNAs were found to be significant. Examination of gene loci revealed that the expression process of *GCGR*, *PDK3* and *PCK1* genes was in line with the expression of neighboring lncRNAs. The results of this study suggest that the lncRNAs found may have the ability to regulate genes in fatty liver and may partially justify fatty liver in laying hens.

Keyword: Chicken, non-coding RNA, transcriptome

Marine Biotechnology in Iran, a high priority issue for investment and strong consideration sector for extension

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Abstract

Marine Biotechnology has varied application for human and the society health as well as the production of various natural compounds for medicine, cosmetic, hygienic industries as well as conservation of biodiversity and the environment safety. Iran, despites having wide range of fauna and flora in the marine ecosystems in the north (the Caspian Sea) and the South (Persian Gulf and Oman Sea) as well as excellent diversity in inland waters bodies, unfortunately, Marine Biotechnology with its huge applications has focused mainly on limited number of commercial fish species but no dealt with other aquatic species. Wide range of techniques and methodology can be applied for aquaculture section: such as Extraction of high values biochemical compounds, enzymes, peptides, various identification kits, vaccine production, pre and probiotics as well as transgenic fish, sex and species identification kits etc., in which all can contribute for added value to chain production.

The world marine biotechnology products in 2020 was estimated about 4.8 billion USD while its predicted reach to 6.4 billion USD in 2025, however Iran has a negligible proportion or close to zero of this market at national or international trade.

There are several biomaterial compounds which are imported from abroad but there is huge optional for raw material as well as developed technology inside country which can be explore for commercialization. Here is some examples: Approximately, 420 tons of Omega 3 with the value of 20 million USD imported annually, while about 100 thousand tons of Kilka in the Caspian Sea and Sardine fish in Persian gulf catches every year and sold as one of the cheapest fish in Iran. The international market for omega 3 estimated about 4.9 billion USD. Gelatin and Collages are another compounds where imported for food and medicine industries, but again it can be produce from byproducts and by-catch fishes which is estimated 300 thousand tons annually. Several such compounds such as (Collagens, Chitin and Chitosan, Chondroitin Sulfate, Hyaluronic acid (HA), Biosilica, Alginate, calcium carbonate

and phosphorus, fucoidan, Hydroxyapatite etc.) imported and its raw materials were available inside the country. In some cases , these raw material is considered as waste and discarded to the environment and bring lots of costs for pollution removal.

Aquaculture with annual 5.3% growth rate is a fast growing sector in Iran as well as entire world as a reliable source for protein and healthy food production. Increase of Immune systems and cost reduction in farmed species by probiotic production and its use in formulated diet effects tremendously to reduce antibiotic usage in fish feeds. (e.g. Norway reduced 47 tons of Antibiotic in fish pellet annually). Application of new biotechnology for reduction of disease impacts via its treatment, friendly environment and diagnostic by molecular techniques can help aquaculture extension for a sustainable production.

Mass alga production (indoor and outdoor) is another source for Marine biotechnology involvement. Especially with high sunshine and appropriate temperature available for good quality production, national road map for alga were developed and few farm are active in this sector, but still we are far from industrial production.

The objective of this paper is to demonstrate the high potentials of marine biotechnology and introduce the field of investment and increase its share at the total volume in trade in the world market. At the same time wishes to call for immediate re-consideration for this new branch of biotechnology for governmental and private support in Iran.

Keywords: Marine Biotechnology, Alga, biochemical compounds, trade, investment.

"Effect of *Caralluma tuberculata* on regulation of genes related to carbohydrate metabolism"

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Abstract

Background and aim of study:

Caralluma tuberculata (*C. tuberculata*) has traditionally been used in Pakistan and other parts of the world as a folk medicine for treating diabetes mellitus. A few studies though have indicated its antihypergycemic effects in rodents and other animal models, the mystery remained unfolded as how did it modify the pathophysiological condition. Hence, this scientific study was aimed to explore the underlying mechanisms for its hypoglycemic activity in animal model of diabetes mellitus at molecular level.

Materials and Methods:

Methanolic extract (ME) of *C. tuberculata* as well as its hexane (HF) and aqueous (AF) fractions were prepared. Further, the experimental extract and fractions, under investigation, were explored for their effect on total glycogen content in liver and skeletal muscle of alloxan-induced rats by a standard method involving spectroscopy. In addition, the expression of various genes related to hepatic enzymes involved in metabolism of carbohydrates in liver were determined. At molecular level, mRNA expression of hepatic carbohydrate metabolizing enzymes namely glucose transporter 2 (GLUT-2), glycogen synthase (GS), glucokinase (GK), hexokinase 1 (HK-1), pyruvate kinase (PK), glucose 6 phosphate dehydrogenase (G-6-PDH), pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6 phosphatase (G-6-Pase) was determined by using quantitative real time polymerase chain reaction (qRT-PCR) following 35 days post-treatment of diabetic rats with ME (350 mg)/, HF(3 mg), AF (10 mg) and metformin (500 mg). All the above doses were calculated according to body weight of each animal in kg and administered via oral gavage tube at 12 hour time intervals.

Results:

A significant reduction ($P<0.001$) in hepatic and skeletal muscle glycogen con-

tent in fasting state was elucidated in post treatment diabetic rats. RT-PCR with specific oligonucleotide primers quantified the mRNA expressions for all nine selected genes and elucidated the effect of extract and fractions of plant on glucose transporter, gluconeogenesis, glycogenolysis, glycogenesis, glycolysis, and pentose phosphate shunt pathways involved in carbohydrate metabolism. The data of qRT-PCR revealed that expression of a gene responsible for transportation of glucose from blood to liver via GLUT-2 was significantly increased after administration of alloxan ($P < 0.0001$), whereas there was a substantial decrease after treatment with ME ($P < 0.0001$) and HF

($P < 0.001$), while it remained unaltered post-administration of AF. The qRT-PCR showed a profound increase in expression of genes related to GS, GK and HK-1 in liver tissue after administration of alloxan ($P < 0.001$) however, a significant decrease was observed in the expression of GS and GK ($P < 0.05$) and HK-1 ($P < 0.001$) after treatment with ME. Similarly there was a significant decrease in expression of GS ($P<0.005$), GK ($P< 0.01$) and HK-1 ($P<0.001$) genes following treatment with HF. Surprisingly, post-treatment with AF did not modify the gene expression of GS and GK, whilst it caused a profound decrease ($P < 0.01$) in expression of HK-1 gene. Contrarily, post administration of alloxan reduced the expression of gene related to PK, while it was significantly increased after treatment with ME, HF ($P < 0.01$) and AF ($P < 0.001$). Furthermore, the expression levels of G-6PDH remained unaltered after treatment with all the three experimental drugs. In addition, HF and AF did not cause any modification in PEPCK, whereas ME significantly caused a significant decline ($P < 0.001$) at expression level of gene. Treatment with all the drugs caused a decrease ($P < 0.001$) in PC while increase in G6Pase ($P < 0.001$).

Conclusion:

It is concluded, here, that all the three experimental drugs caused a substantial decrease in glycogen content in liver and skeletal muscle tissues of alloxan-induced rats. The analysis by qRT-PCR showed that glucose transport via GLUT-2 was overwhelmingly declined by ME and HF, whereas it did not modify by AF. Furthermore, ME, HF and AF decreased the biochemical phenomena of glycogenesis and gluconeogenesis. Contrarily, at expression level the gene quantified for G6Pase and PK were increased by the three treatments thus, indicating activation of the glycogenolysis and glycolysis respectively. It looks like that these drugs did not modify the expression of gene (G-6PDH) involves in PPSP.

چکیده دکتر صابر خدا بنده سخنران مدعو کارگروه دریایی

Marine organism possess different bioactive materials such as several toxins. The chemical diversity of natural toxins among marine animals is likely to be much higher than for terrestrial animal toxins, as the diversity of marine animals is greater. Investigations of marine toxins during the past few decades have provided a remarkable diversity of molecules new to science. These substances often possess such unique targets that they can serve not only as useful research tools, but in some cases can either be useful drug candidates in their naturally occurring forms or as leads for designing analogs. TTX is one of very important marine toxins. TTX derives its name from the taxonomic name of the fish family in which it was first discovered, namely the family Tettaodontidae.We extracted TTX from different organs of Persian Gulf pufferfish, Chelonodon ptoca, and used it for controlling of tumors growth in mice. Our researches on TTX bioactivity showed that, it can control breast tumors growth by controlling of NKA (Na, K-ATPase) activity and gene expression. NKA is the sodium pump enzyme that have critical importance to cellular metabolism: control of cellular pH, osmotic balance, and the transport of nutrients such as amino acids and vitamins into cells, excretion, migration and cell division. So, we concluded that by inhibiting of NKA by marine toxin TTX we can control cell metabolism and tumor growth.

Microbial & Food Biotechnology Industrial Biotechnology and Environmental Biotechnology

Microbial Biotechnology
Environmental Biotechnology
Industrial Biotechnology

Comparison of Polyhydroxybutyrate production with external potential application at Cathode and Anode poles compared to Control(Blank) with inexpensive whey substrate

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Abstract

Polyhydroxybutyrate (PHB) is one of the types of biodegradable polymers that has received much attention due to its properties similar to polypropylene. A major problem in commercializing PHB is its high product cost. Then a numbers of strategies for reducing the cost of PHB production has been done. One of the strategies for reducing cost and enhancing productivity in the fermentation process is, using oxidation reduction potential on PHB production by Ralstonia eutropha with inexpesive substrate (whey) of industrial wastes was studied during batch fermentaion. In this study, This operation was performed by reproducing the nicotin amide adenine dinucleotide phosphate (NADPH) cofactor using electrochemical reproduction method with application of external potential. In this method, by increasing the NADPH / $NADP^+$ ratio at the cathode, the input rate of Acetyl coenzyme A to the PHB biosynthesis pathway also increased. In this study, sampling was performed by applying different potentials in the range of 0.5 to 1.8 V at different times, so that the maximum amount of production was obtained after 70 hours of fermentation and applying a potential of 1.2 V. In this case, about 47% increase was obtained compared to the case without potential application and also, the highest amount of polymer produced at the cathode compared to the anode and control was observed. The results showed that the percentage of polymer in the cell also increased by 100% so that, not only helps to increase the efficiency of the fermentation process but can also play an effective role in reducing separation costs.

Keyword: Polyhydroxybutyrate, External potential, Regulation of metabolic pathway, Low cost sources

Investigation of phenol removal from groundwater by alginate-encapsulated consortium and calcium peroxide

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Abstract

Phenols are toxic products derived from a wide range of industrial activities. The entry of these contaminations into the environment especially groundwaters is seriously hazardous and must be treated.

This study explored impacts of biostimulation and bioaugmentation on phenol removal from groundwater.

A Bushnell Haas medium was used to isolate and optimize a phenol degrading consortium. To investigate the microbial diversity of the consortium, next-generation sequencing was performed. Sodium alginate provided a confined environment for the encapsulated consortium. Encapsulated-CaO_2 was also synthesized to supply oxygen. Subsequently, the coated consortium and encapsulated-CaO_2 were used in batch experiments.

The consortium was capable of growing in the range of 100 to 800 mg·L^{-1} of phenol. The highest growth rate was observed at 15°C and pH 7.5 in the presence of 100 mg·L-1 of phenol and ammonium nitrate. According to the results of NGS, Proteobacteria and Bacteroidetes were the dominant phyla in the consortium, respectively. According to the batch results, the application of 500 mg/L of encapsulated-CaO2 with the coated consortium could remove phenol completely within 40 d. While, the natural remediation of the contaminant resulted in 5% removal at the end of the experiments (60 d). Furthermore, encapsulated-CaO2 and coated consortium individually eliminated 100% and 24.1% of the initial phenol concentration after 60 days,

respectively. In conclusion, the innovative use of CaO_2 capsules in combination with an alginate-encapsulated consortium provides evidence for the successful application of this method in groundwater treatment processes.

Keyword: Phenol, Groundwater, Calcium peroxide, Consortium, Alginate

Evaluation of antagonistic yeast strains against *Penicillium expansum* and determination of their mechanism of action

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Abstract

Fungal spoilage can cause defects in the appearance and depletion of nutrients in fruits and vegetables and the main part of the spoilage occurs in the postharvest stage. *Penicillium expansum* is one of the most common and important fruit rot pathogens on a variety of fruits and it produces several toxic secondary metabolites. In this study, the antagonistic activity of 50 yeast strains belonging to 32 genera against *P. expansum* were evaluated using the dual culture method. Then, the ability of the production of volatile organic compounds and extracellular hydrolytic enzymes were investigated as putative mechanisms of biocontrol activity. Among the strains, 22 strains showed antagonistic effects against the pathogen ranging from 2.6-33.7% growth inhibition. Six strains with growth inhibition above 15% belonging to the species *Aureobasidium mangrovei*, *Coniochaeta euphorbiae*, *Tranzscheliella* sp., *Basidioascus persicus* and *Cryptococcus podzolicus* were selected for further studies. Three strains were able to produce volatile organic compounds at low levels (4-12%). Various enzymatic profiles were observed among the antagonistic yeast strains. *Aureobasidium mangrovei* IBRC-3026 and *Coniochaeta euphorbiae* IBRC-30188 showed the most growth inhibition via the production of diffusible and volatile organic compounds and have the most diverse enzymatic activities among the strains. Therefore, it seems that the strains may have the potential for application as biocontrol agents. However, further study should be carried out in the field and also on large-scale production, formulation, preservation condition, and application methods.

Keyword: Biological control, *Penicillium expansum*, Yeast

Optimization of coating formulation to increase the survival of probiotic bacteria during one-week storage in room conditions

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Abstract

Microcoating is the process of coating cells with a film of hydrocolloids that encloses and isolates them from the outside environment, allowing bacteria to survive in a variety of conditions and release them precisely where they are needed. The aim of this research was to look at different materials and formulations for probiotic microencapsulation in order to improve their survival in freezing and storage conditions. *Lactobacillus rhamnosus GG* was microencapsulated using alginate, sucrose, yeast, and skim milk for this purpose. With a survival rate of 1.8 percent, freezing, alginate, and sucrose formulations had the highest bacterial viability. For this reason, *Lactobacillus rhamnosus GG* was microencapsulated using alginate, sucrose, yeast, and skim milk. The freezing, alginate, and sucrose formulations had the highest bacterial viability, with a survival rate of 1.8 percent. As a result, the presence of sucrose and skim milk in the microcoating formulation is needed to increase bacterial survival during freezing and storage, according to the findings.

Keyword: Lactobacillus rhamnosus, Alginate, microcapsulation, probiotics, freeze drying

Isolation and screening of antimicrobial exopolysaccharide producing lactic acid bacteria

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Abstract

Exopolysaccharide (EPS) derived from lactic acid bacteria are promising sources of compounds with various activities like antimicrobial potential. In the current study, lactic acid bacteria were screened to find antimicrobial EPS against food borne pathogens. For this, dairy, fermentative and animal samples were collected from various cities of Iran and cultured on MRS agar. The isolates were evaluated according to their form, appearance, margin and elevation, catalase test and Gram staining. The isolates were screened for EPS production. Antimicrobial activity of produced EPSs against *Listeria monocytogenes, Yersinia eterocolitica,* and *Bacillus cereus* was tested and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antimicrobial EPS were determined. Finally, cytotoxicity of antimicrobial EPS was tested by red blood cell lysis assay. According to morphology of colonies, catalase test and gram staining, ٥٠ isolates were isolated from samples. Twenty isolate were EPS producing ones. EPS of AS١)٢٠) showed considerable antimicrobial activity against *Listeria monocytogenes* (MIC= 0.935 mg/ml, MBC= 0.935 mg/ml)*, Yersinia eterocolitica* (MIC= 12.5 mg/ml, MBC= 50 mg/ml)*,* and *Bacillus cereus* (MIC= 6.5 mg/ml, MBC= 12.5 mg/ml). This EPS showed negligible cell toxicity against red blood cells. Sequencing of 16 S rRNA gene revealed that AS20(1) isolate had most similarity with *Lacticaseibacillus zeae*.

Keyword: Exopolysaccharide, Lactic acid bacteria, antimicrobial activity

Zataria multiflora essential oil and oily fraction inhibit α-amylase and α-glucosidase activities

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Abstract

The *in vitro* anti-diabetic activities of *Zataria multiflora* essential oil and fatty acid against carbohydrate degrading enzyme were investigated. The gas-chromatography-mass spectrometry characterization revealed that the major constituents of essential oil were carvacrol (52.36%), thymol (14.95%), spathulenol (13.61%), caryophyllene oxide (4.37%), caryophyllene (3.29%), para-cymene (3.24%), globulol (1.47%), aromadendrene epoxide (1.29%), thymol methyl ether (1.25%), gamma-terpinene (1.23%), and alpha-terpinene (1.06%). The most abundant compounds in the oil fraction extracted from zataria multiflora were carvacrol (48.5%), 9,12,15-octadecatrienoic acid (15.5%), thymol (10.3%), hexadecanoic acid (8.7%), 9,12-octadecadienoic acid (7.6%), and 9-octadecenoic acid (6.6%). Essential oil manly composed from monoterpenoids and monoterpenes while oily fraction constituted from fatty acid and fatty acids including emega-3 and omega-6 polyunsaturated fatty acids. Both oily fraction and essential oil displayed anti-amylase, and anti-glucosidase activity. As a results we recommended oily fraction instead of essential oil from pharmacological and food applications.

Keyword: Essential oils; Fatty acid; Amylase; Glucosidase.

Inhibitory activity of *Oliveria decumbens* essential oil and oil on the amylase and glucosidase activity

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Abstract

Diabetes mellitus is mainly characterized by hyperglycemia, hyperlipidemia, and oxidative modification of sugar, lipid and protein including protein glycation and lipid glycation. The *in vitro* antioxidant and anti-diabetic activities of *Oliveria decumbens* essential oil (ODEO) and oily fraction (ODoil) against carbohydrate digestive enzymes like amylase and glucosidase were investigated. The gas-chromatography-mass spectrometry characterization revealed that main components of ODEO were carvacrol, thymol, gamma-terpinene, para-cymene, sabinene, and limonene, respectively. The most abundant compounds in the oils extracted from *Oliveria decumbens* were thymol, linoleic acid (9,12-octadecadienoic acid), linolenic acid (9,12,15-octadecatrienoic acid), palmitic acid (hexadecanoic acid), carvacrol, oleic acid (9-octadecenoic acid), and 7,10,13-Hexadecatrienoic acid, respectively. ODEO and ODoil displayed anti-amylase, and anti-glucosidase activities.

Keyword: *Oliveria decumbens,* Essential oil, Fatty acid, Amylase, Glucosidase.

Separation of carotenoids from microalgae extract using adsorption

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Abstract

Microalgae contain valuable pigments including carotenoids. The aim of this study is to separate carotenoids from other pigments incorporated into microalgae extract. Upon extraction of pigments using ethanol, carotenoids were separated from chlorophylls in an adsorption column loaded with graphene as the adsorbent. Graphene was selected for this purpose due to its high selectivity towards chlorophylls. In the adsorption column, the ratio of minimum mass of adsorbent (0.15 g) to extract volume (5 ml) was determined so that the separation ratio of R=0.003 (ratio of adsorbed carotenoids to adsorbed chlorophylls) obtained. The ratio of mass of adsorbent to extract volume could be up scaled for large-scale applications but the scale-up coefficient id not linear.

Keyword: Separation, Pigment, Microalgae, adsorption, Graphene.

Effect of hydro-priming and seaweed extract on germination traits of *Cupressus sempervirens* var. *horizontalis* seeds

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Abstract

The experiment was conducted to investigate the effect of hydro-priming of seeds and seaweed extract on germination traits of *Cupressus sempervirens* var. *horizontalis* seeds. *Cupressus sempervirens* var. *horizontalis* is a coniferous plant native to the north of Iran that has a strong and deep root system. It tolerates drought and cold of winter significantly. Germination as the first stage of plant development is one of the most important and sensitive stages in the life cycle of plants and a key process in seedling emergence. One of the ways to increase percentage and rate germination is to use priming method. This experiment was conducted in a completely randomized design with three replications in the greenhouse. For this purpose, the seeds were soaked in solutions with seaweed extract (at concentrations of 1% and 3.5%) and water (for hydro-priming) for 30 hours. The seeds were then dried in the shade and transferred to the cocopeat culture medium. 20 seeds were planted in each pot. After ending germination, the percentage, rate and average germination time were calculated and root length of seedlings was measured. The results of analysis of variance showed that the effect of the treatments on germination percentage and root length was significant but had no significant effect on germination rate and average germination time. The results of Duncan's mean comparison showed that hydro-priming had the highest germination percentage and also the highest root length was obtained by priming with seaweed extract in concentration of 3.5% and water.

Keyword: *Cupressus sempervirens* var. *horizontalis*, Priming, Germination, Root length, Seaweed.

The effect of different solvents on the separation of sapogenins from *Spirulina platensis* using thin layer chromatography (TLC)

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Abstract

Spirulina platensis is a photosynthetic, filamentous and spiral-formed blue-green alga that contain numerous bioactive ingredients with anti-cancer and anti-inflammatory, belonging to phenolic compounds, terpenoids, and alkaloids. Sapogenins also are one of the bioactive compounds that less known. The aimed of this study was to investigate the presence of sapogenins in Spirulina platensis and also the effect of different solvents on the isolation of sapogenins. Sapogenins were extracted from spirulina using acid hydrolysis and they were analysed by the TLC method with different solvents as a mobile phase. The results of all three TLC tests confirmed the presence of sapogenins in spirulina. Using the mobile phases of chloroform and methanol (10 - 1), chloroform and ethyl acetate (10 - 1), and petrol with ethyl acetate (10 - 1), 9, 6, and 3 sapogenin bands with different retention factors were observed, respectively. As a result, the presence of sapogenins in Spirulina platensis was proven and the use of mobile phases containing chloroform to isolate and identify different types of sapogenins by the TLC method will perform better.

Keyword: Spirulina platensis, Sapogenins, TLC, Retention factor

Isolation and identification of specific bacteriophagia against enteropathogenic Escherichia coli (EPEC)

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Abstract

In recent years, the increasing resistance of enteropathogenic Escherichia coli (EPEC) to commonly used antibiotics has made it difficult to choose the best treatment option. Bacteriophage therapy could be a potent alternative to antibiotic therapy for antibiotic-resistant bacteria. The aim of the present study was to isolate and identify a specific bacteriophage against EPEC and characterize bacteriophage in vitro and in vivo. The specific bacteriophage was isolated, and the effect of phage therapy on 48 mice (Balb/c) was investigated. Animals were divided into six groups, including A: PBS (negative control); B: bacteria (positive control); C: bacteria + ciprofloxacin (after 24 h); D: bacteria + bacteriophage (after 24 h); E: bacteria + ciprofloxacin + bacteriophage (after 24 h) and F: bacteriophage + bacteria (after 24 h). Specific bacteriophage against EPEC was isolated from hospital sewage. The bacteriophage had an icosahedral head (120 nm) and a tail (138 nm). The single dose of the bacteriophage (2 × 109 pfu ml−1) was able to control the infection. Unfortunately, because of the misuse of antibiotics by EPEC infected patients, the antibiotic resistant bacteria will become prevalent in the future and the treatment of EPEC infection is going to become more difficult than ever.

Keyword: EPEC bacteria, antibiotic resistance, bacteriophage, bacterial infection

Isolation and identification of potential lactobacillus probiotic species from neonatal feces

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Abstract

The aim of this study was to investigate the potential probiotic properties of Lactobacillus strains isolated from neonatal feces and to determine their antimicrobial activity against some anthropogenic bacteria. Stool samples were obtained from 120 infants less than 24 months old. A total of 105 Lactobacillus strains were identified by phenotypic testing. 30 isolates were randomly selected to investigate their potential probiotic properties. These isolates were investigated for acid resistance (pH: 2.5, 2 h) and bile (oxgall 0.3%, 8 h), adhesion to HT-29 cells, antibiotic susceptibility, and antimicrobial activities. Based on sequence 16S rRNA, 30 isolates identified as Lactobacillus fermentum (n = 11; 36.7%), Lactobacillus plantarum (n = 9; 30%), Lactobacillus rhamnosus (n = 6; 20%), and Lactobacillus paracasei (n = 4; 13.3%) All the tested strains survived under acid and bile conditions. Six lactobacillus strains revealed high adherence to HT-29 cells. Three strains including Fermentation L. (N2, N7), and L. plantarum (N20) showed good probiotic potential and inhibited growth of Yersinia enterocolitica ATCC 23715, Shigella Flexneri ATCC 12022, Salmonella Enterica ATCC 9270, and Enteropathogenic Escherichia coli (EPEC) ATCC 43877. Antibiotic resistance tests showed that all isolates are susceptible to tetracycline and chloramphenicol.Lactobacillus strains such as L. fermentum (N2, N7), and L. plantarum (N20), can be potential probiotics, but mostly in vitro and in live studies.

Keyword: Lactobacillus, Probiotics, Anthropogenesis, Antimicrobial

Biocontrol potential of yeast strains against *Botrytis cinerea* for controlling postharvest disease in fruits

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Abstract

Fruits play a crucial role in the human diet. *Botrytis cinerea* is the most common phytopathogenic fungus that can cause significant economic losses of fruits during storage and transportation. Antagonistic yeasts (also known as biocontrol yeasts) are promising substitutes for chemical fungicides in the control of postharvest decay owing to their widespread distribution, antagonistic ability, environmentally friendly nature, and safety for humans.

In this study, 50 strains of yeasts belonging to 32 genera were evaluated for their biocontrol activity against *B. cinerea* based on the dual assay method. Putative mechanisms of action associated with the biocontrol capacity of yeast strains against *B. cinerea* studied through in vitro assays including, the production of volatile compounds and the production of hydrolytic enzymes. Five yeasts species showed antagonistic capability against the pathogen ranging from ٪٤٦,١-٢٨,٦ growth inhibition.

Four of these strains were capable of producing volatile compounds which suppressed the pathogen growth from 53.9-68.7%. Various extracellular enzymatic profiles were observed among antagonistic yeast stains. In this study, *Aureobasidium mangrovei* IBRC-30265 was recognized as the potential biocontrol agent candidate with the greatest antagonist effect and various enzymatic activity including protease, chitinases, cellulase, *Beta-glucosidase, and* esterase activity.

Nevertheless, issues such as large-scale production, formulation, preservation conditions, shelf life, field studies, and application methods should be evaluated precisely prior to the introduction of strain as an effective biocontrol agent.

Keyword: yeast; biological control; postharvest decay; fruit; *Aureobasidium mangrovei*

The effect of traffic noise stress on growth and antioxidants activity of *Salvia splendens*

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Abstract

Traffic noise pollution is one of the most important environmental problems in today's societies, especially in populated cities around the world. The aim of this study was to investigate the effect of urban traffic noise on growth factors, oxidative stress parameters and antioxidant activity of *S. splendens*. Plants were divided into two equal groups (control and treatment) and each group was grown in two separate growth rooms for two months under the same controlled conditions. Traffic noise was recorded during peak traffic hours in the city. Frequency analysis was performed on the samples during the recording process. Plants in the treatment group were exposed to traffic noise for 15 days and 16 hours a day, while the plants in the control group were placed in complete silence. Exposure to traffic noise resulted in a significant increase in hydrogen peroxide content, malondialdehyde, followed by the inhibitory activity of free radicals (DPPH) and antioxidant enzymes such as catalase, peroxidase, and ascorbate peroxidase compared to the control group. On the other hand, traffic noise reduced dry and fresh weight in treatment plants. According to the results of this study, traffic noise can negatively affect the growth and physiology of *S. splendens* by causing oxidative damage and interfering with the activity of antioxidants (enzymatic and non-enzymatic).

Keyword: Antioxidant activity, Growth, Hydrogen peroxide, Traffic noise, *Salvia Splendens*

The effect of lactobacillus encapsulation of the lipid profile of rats fed a high-fat diet

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Abstract

Impaired fat metabolism leads to cardiovascular disease. Today, cardiovascular disease is the leading cause of death in industrialized countries. Treatment for lowering blood cholesterol levels includes diet management, exercise, and medication. The use of drugs in pregnant women is also very dangerous for people with liver and kidney failure. Probiotics are non-pathogenic living microorganisms that, if taken in sufficient amounts, have benefits for their host. The aim of this study was to investigate the effect of microencapsulated lactobacilli on lipid profile and weight of female rats fed a high-fat diet. In this study, 24 male wistar rats, which were grouped in 3 groups of 8 animals intended for study. Group1: The rats that received standard food. Group2: The rats that received high fatty food. Group3: The rats that received high fatty food via 109cfu / ml Lactobacillus. The results of analysis of variance with 95% probability showed that probiotic Lactobacillus can significantly reduce the level of triglyceride, total cholesterol, LDL and increase HDL levels in rats interfering with Lactobacillus ($P < 0.05$) and also Increases the rate of weight gain. Based on the findings, it was found that probiotic lactobacilli can reduce hyperlipidemia due to high-fat diet and also increase the weight of rats.

Keyword: Cholesterol, Triglycerides, Cardiovascular Diseases, Probiotics.

بومی در راستای توسعه سویه باسیلوسی برای *B. cereus*در باکتری Mpr حذف ژن پروتئاز تولید پروتئین‌های نوترکیب

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Abstract

Genetics has a long history of assisting in the development of microbial products. In industry, genetic manipulations are used to create strains that yield hundreds or thousands of times more than the original isolated strain.Mutagenesis and screening/ selection for higher yielding microbial strains, as well as the use of recombinant DNA technology, have led to significant improvements in fermentation efficiency and cost reductions.During Bacillus subtilis natural chromosomal transformation, recombination proteins guide the acquisition of homeologous DNA in different ways. A physiological condition that allows a bacterial culture to bind and take up high-molecular-weight exogenous DNA is known as genetic competence. The imported DNA replaces a homologous segment in the recipient genome via a homologous recombination process. Extracellular proteases are known to degrade secreted proteins. We used homologous recombination to remove the gene encoding the extracellular protease Mpr (991 bp)from *Bacillus cereus EG297*'s genome in order to develop *Bacillus* strains for optimal bioproduction.

کلمات کلیدی: توسعه سویه، پروتئاز، Mpr، *B.* cereus، نوترکیبی همولوگ

MPr protease gene deletion in a local strain of Bacillus cerues to improvement of strain for recombinant protein production

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Abstract

Genetics has a long history of assisting in the development of microbial products. In industry, genetic manipulations are used to create strains that yield hundreds or thousands of times more than the original isolated strain.Mutagenesis and screening/ selection for higher yielding microbial strains, as well as the use of recombinant DNA technology, have led to significant improvements in fermentation efficiency and cost reductions.During Bacillus subtilis natural chromosomal transformation, recombination proteins guide the acquisition of homeologous DNA in different ways. A physiological condition that allows a bacterial culture to bind and take up high-molecular-weight exogenous DNA is known as genetic competence. The imported DNA replaces a homologous segment in the recipient genome via a homologous recombination process. Extracellular proteases are known to degrade secreted proteins. We used homologous recombination to remove the gene encoding the extracellular protease Mpr (991 bp)from *Bacillus cereus EG297*'s genome in order to develop *Bacillus* strains for optimal bioproduction.

Keyword: Homeologous recombination, *Bacillus cereus*, Mpr, Protease, Strain develepement .

Molecular isolation and identification of endophytic bacteria from *Caparis spinosa* L. effecting on plant pathogenic fungi *in vitro* condition

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Abstract

Biological control remains as a not exploited issue in most countries, such as Iran. Therefore, the present study focused on the diversity of culturable endophytic bacteria in the internal tissues of *Caparis spinosa* L. In order to evaluate their antagonistic potential, four plants (including 4 samples from each part of roots, stems, leaves and fruits) of this plant were selected from 15 natural habitats and endophytes were isolated according to the standard protocol. After isolation of endophytic bacteria, their molecular identification was performed based on 16SrDNA gene. For screening biocontrol endophytic bacteria, they were evaluated based on antagonist method with three pathogenic fungi including *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in vitro condition. Results showed that, ninety three endophytic bacterial isolates were recovered from different tissues. Finally, 18 isolates (19.35%) based on molecular detection of the 16S rDNA gene sequences were identified that had antagonistic activity on studied pathogens. Twelve endophytes (66.67%) had gram positive nature and were included in the Firmicutes cluster (including *Bacillus*, *Paenibacillus*) and 6 strains had gram negative nature and were included in the Proteobacteria cluster (including *Alcaligenes*, *Stenotrophomonas* and *Aeromonas*). The

most superior bacterial species and the majority of the most bioactive strains effective on studied pathogens belonged to *B. subtilis* (CSI4) and *S. rhizophila* (CSZ10). The majority of isolated endophytic bacteria affecting on *F. oxysporum*, *R. solani* and *S. sclerotiorum* was related to Dehloran (Ilam) location. Also, The rate of colonization was 37.50%, therefore, as seen, most of the bacterial strains affected on all studied pathogenic fungi were recovered from the stem (44.44%). This can be utilized in future application, such as effect of crop seed treatment with endophytic bacteria, delivery of degradative enzymes for controlling certain plant diseases. These findings can be advantageous for agriculture, pharmacy, medicine and the biological and biotechnological industries.

Keyword: Molecular Identification, Endophytic bacteria, Capparis spinosa, Plant pathogenic fungi.

Isolation and molecular identification of endophytic fungi in Saffron (*Crocus sativus L.)*

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Abstract

Endophytic fungi are an important component of biodiversity related to plants and have beneficial effects on their hosts. Endophytes are ubiquitous and have a high biodiversity. This group of fungi is located inside plant tissues without causing symptoms. The aim of this study was to identify endophytic fungi into saffron in Hamadan province. Sampling of healthy plants was carried out randomly from two saffron fields (ten years and two years) in the fall of 2020. Sampling from three different tissues; The leaves, roots and tubers of the plant were taken. Isolates were identified based on morphological characteristics and sequences of ITS regions (ITS1 - ITS4). A total of 49 isolates were obtained which all isolates were identified and belonged to the Ascomycota group and include the following 10 genera, which including: *Cadophora malorum*, *Aspergillus niger*, *Penicillium canescens*, *Aspergillus europaeus*, *Alternaria chlamydosporigena*, *Ascochyta rabiei*, *Aspergillus* sp. Most endophytic fungi were isolated from glandular tissue (64.94%) and ten-years field (36.73%). Among the isolated species *Alternaria chlamydosporigena*, *penicillium canescens*, *Aspergillus europaeus* are reported for the first time from Iran and species *penicillium canescens*, *Aspergillus europaeus*, *Ascochyta rabiei*, *Aspergillus* sp. are reported for the first time as endophytes from this host in the world.

Keyword: Endophyte, Saffron, Biodiversity, Molecular identification.

Investigation of the possibility of crocin synthesis by endophytic fungi isolated from Saffron (*Crocus sativus*) using HPLC technique

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Abstract

Ssaffron is a Perennial plant, belongs to the lily family and lilies order. Three main secondary metabolites of Saffron are Crocin and its derivatives, which are responsible for the color, Picrocrocin is responsible for the bitter taste and safranal is responsible for the aroma of saffron. Endophytes are microorganisms that spend at least one stage of their life cycle in plants without causing any symptoms. These microorganisms have been found in many plant species and are recognized as a potential source of effective natural compounds for use in medicine, agriculture and industry. This information led us to the conclusion that Saffron can be another source for endophytic fungi with biological activity, therefore, the aim of this study was to investigate the possibility of Crocin synthesis by endophytic fungi from Saffron using HPLC technique. Nine endophytic fungi isolated from Saffron were examined. The results of HPLC analysis showed that only the endophytic fungus *Penicillum Canescens* FT16 (MW922804) isolated from Faffron leaves was able to produce Crocin in the amount of 2.648 ppm and the other endophytic fungi were not able to synthesize Crocin (or less than the amount of 2 ppm). The results also showed that saffron leaves and stigmas contained crocin in the amount of 3.299 and 1238.92 ppm, respectively, while this component was not found in the Corms of this plant.

Keyword: saffron, endophytic fungi, crocin, HPLC, picrocrocin.

Investigation of the possibility of flavonoids synthesis by endophytic fungi from N*epeta crispa* using HPLC technique

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Abstract

Studies on plant extracts, endophytic fungi and medicinal plants have been shown that plants, especially medicinal plants, are an available, abundant and reliable source of endophytic fungi. Endophytic fungi are often mentioned to asymptomatic fungi that can be present in all plants. They live in the intracellular space of stems, Auricles, roots and leaves of plant without any visible negative effects. The secondary metabolites produced by endophytes are in imitation of their hosts and they produce Accurately the same metabolites produced in that particular plant. Therefore, due to the availability of *Nepeta crispa* and being native in Hamadan province and its high medicinal benefits, the aim of this study was to investigate the possibility of flavonoids synthesis by endophytic fungi from *Nepeta crispa*. For this purpose, methanolic extract of this plant and endophytic fungi isolated from it were prepared by maceration method and examined by HPLC technique. The results showed that *Quercetin* and *Apigenin* were not observed in any endophytic fungi, also in stem and leaf extracts of this plant. The results also showed that *Chaetosphaeronema achilleae* FB5 (MW940813) (endophytic fungus) had the highest production of Rutin (flavonoid) with an amount of 179.290 ppm and the *Penicillium* sp. FB16 (MW940821) (endophytic fungus) showed the lowest production of *Rutin* with a value of 1.35 ppm. The leaf extract *Nepeta crispa* showed a content of 46.088 ppm of *Rutin*. Also, the stem extract of this plant showed 21.469 ppm of *Rutin*.

Keyword: *Nepta crsipa*, Endophytye, HPLC, Methanolic extract.

Phytochemical analysis of methanolic extract of *Nepeta crispa* L. using GC.MASS technique

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Abstract

Due to the medicinal properties of *Nepeta crispa* in helping to treat gastrointestinal and respiratory diseases, this plant can be a good alternative to chemical drugs. *Nepeta crispa* as a medicinal plant contains substances such as glycosidic flavonoids, coumarin, glycosides, beta and gamma cytosterols, oils, 1,8 cineole, pentalactone, - 7α-7α-4aβ, pentalactones and anthraquinones and anthocyanins that have antioxidant, antifungal and antibacterial properties and prevent adverse reactions in cellular and extracellular environments. Medicinal plants and their derivatives (essential oils and plant extracts) are widely used to prevent oxidative spoilage of food due to their strong and diverse antioxidant compounds. Therefore, the aim of this study was to investigate the components of this plant using GC. MASS technique and the results showed that the combination of gamma. -Sitosterol with 37.86% in leaves and 53.75% in stem is the most common compound detected in this plant and Cis-8-methyl -exo-tricyclo [5.2.1.0 (2.6)] decane with 0.79% in leaves and cis-. alpha.-Bisabolene with 0.28% in stem are the lowest compound, respectively.

Keyword: Phytochemical, *Nepeta crispa*, GC. MASS, Methanolic extract.

Isolation of the symbiotic mold from *Juniperus* sp. as Paclitaxel producing fungi

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Abstract

Due to the growing need for Paclitaxel as an effective treatment for advanced cancers, using a method that is a cost-effective and environmentally friendly resource and is not affected by environmental changes, can increase the need for this drug to supply. Microbial fermentation of Taxol-producing endophytic fungi is one of these sources, but due to low production rate and production instability in laboratory conditions, it has not yet entered industrial-scale production. Isolation Taxol-producing endophytic molds from plants that have not been studied in this case and determined the amount of Taxol production in them, along with other optimizations, can solve these problems to some extent. In this study, for the first time, a symbiont mold with *Juniperus* sp. According to the results of High Pressure Liquid Chromatography (HPLC), the amount of Taxol production is 12.4 mg / l, which is the highest amount of Taxol production with endophytic fungi reported so far in the world.

Keyword: Taxol, Symbiont mold, *Juniperus* sp., Cancer.

Optimization of heat-resistant cysteine protease production

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Abstract

Summary of Introduction: Heat-resistant proteases are an important group of protease enzymes that have various industrial applications including food production, detergents and other chemical, pharmaceutical and food industries. Thermal denaturation is a common cause of enzyme inactivation, so one of the important characteristics for the commercialization of protease enzymes is their stability and resistance to heat, which is usually enhanced by the presence of additional hydrogen- bonds, ions, hydrophobic and some chelators. Optimization of an economic culture medium for the production of recombinant cysteine protease enzyme in *Escherichia coli* using experimental design method. In this study, the variables affecting enzyme activity were first screened by Taguchi statistical method and the most important ones were selected. Then the effect of selected variables was optimized using the response surface design method (central composite design). To do this, a clone of recombinant *Escherichia coli* was grown on a Kanamycin agar LB plate and inoculated into 5 mL of basic culture medium including molasses, yeast extract, sodium chloride, wheat, corn and rice extracts. From this inoculum prepared in equal amounts to 50 mL of 8 culture media designed in inoculation screening experiments and the amount of activity and specific activity of the enzyme were measured. In the next step, the effective variables were identified and all these steps were performed for 16 culture media designed for optimization experiments. The results of the screening and optimization of effective variables with the aim of increasing the amount of enzyme activity in Table 1 and the process of increasing it are presented in Figure 1. During the optimization process, the activity of the enzyme in relation to LB medium and screening medium increased by 2.3 and 1 units / mL, respectively.

Keyword: Optimization, Recombinant cysteine protease, Protease activity, Enzyme stability with nanoparticles, immobilized enzyme.

Bacterial magnetosomes: Biological smart weapons for targeted treatment of cancer

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Abstract

Magnetic bacteria are a group of polyphyletic bacteria that are able to orient in an external magnetic field due to their ability to produce structures called magnetosomes. Magnetosomes, which are present in most magnetic bacteria in nanometer size, are intracellular organelles composed of magnetic iron mineral crystals that are individually surrounded by a phospholipid layer. Due to unique properties of Magnetosomes, such as their small size, diverse composition, and good stability, they have become an attractive topic in various fields of research, such as targeted drug delivery and cancer treatment. Unlike many bacteria currently being tested in clinical trials for cancer treatment, magnetic bacteria are not pathogenic and can be engineered to kill specific tumor cells. This group of bacteria can specifically target tumors and cause toxicity in a controlled manner. These drug loaded nanoparticles with specific ligands can bind to surface receptors on the tumor cells and accumulate at the tumor site. Magnetosomes vary in their magnetic properties and distribution at the tumor site and are used for cancer diagnosis and targeted therapy.

Keyword: Magnetic bacteria, Magnetosome targeted therapy, cancer .

Molecular docking studies of two synthetic peptides as hexokinase II enzyme inhibitors

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Abstract

The expression and activity of some glycolysis pathway enzymes, such as hexokinase, are greatly increased in cancer cells. Therefore, inhibition of hexokinase is considered as one of the important strategies in the selective and targeted treatment of cancer. The aim of this study was to investigate the docking of two synthetic peptides derived from the yeast *Saccharomyces cerevisiae* as potential inhibitors of human hexokinase II. The research was conducted in a descriptive-analytical manner. To investigate the mode of GA-8 and PAR-3 peptides coupling with hexokinase, their chemical structures were first designed using the Discovery Studio software. Then, for energy optimization, the results exported into the Hyperchem software. Molegro Virtual Docker software was used for docking studies and finally docking results were analyzed with Molegro Molecular Viewer software. The results showed that the most important bonds and forces involved in binding these peptides to the enzyme are hydrogen bonds and hydrophobic forces. GA-8 peptide interacted with both C-domain and N-domain residues of the enzyme, but PAR-3 peptide only bound to some C-domain residues of the enzyme. Met247 residue was the only common residue in these interactions. In general, according to the information obtained from docking studies, it can be concluded that GA-8 peptide with a more negative binding energy of -167.893 kJ/mol compared to PAR-3 peptide with a binding energy of -100.455 kJ/mol can be suggested as a more effective inhibitor of hexokinase II, but further studies is needed to confirm this claim.

Keyword: Cancer, Hexokinase, Peptide, Molecular docking.

Isolation, screening, and identification of biosurfactant-producing native probiotic strain

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Abstract

Probiotics are living microorganisms that provide beneficial effects while colonizing the host. Lactic acid bacteria species and Bifidobacterium are among the best-known probiotics. One of the compounds produced by probiotic bacteria are biosurfactants (BSs). BSs capable to emulsify and decrease surface tension and interfacial tension. Due to their interesting properties such as higher biodegradability, lower toxicity and higher activity at extreme conditions, BSs have become the interests of researchers as promising alternative of a number of synthetic surfactants. In this study, isolation of probiotic bacteria from local dairy products was carried out in order to search for biosurfactant producing bacteria. The 70 dairy samples were collected aseptically of the various environments of Iran. The bacteria were isolated by serial dilution method. The bacterial isolates were purified by repeated subculturing on MRS medium at 37°C. Production of BSs of the isolates was done by fermentation in flasks containing MRS broth for 48 h at 37°C. Primary screening was performed by using oil spreading assay in order to find promising producers. Eighty bacteria were isolated from 70 dairy samples. Results showed 25% of isolates were positive for the oil-spreading assay. Three of isolates showed the highest oil spread in activity whit more than 6 cm clearing zone diameter. Based on the results of probiotic tests and BS production, F20S2 isolate was identified by 16S rRNA method as *Lactobacillus brevis* and introduced as valuable candidate for producing biosurfactant.

Keyword: Biosurfactant; Probiotic; Oil Spreading assay.

Virulence assessment of indigenous entomopathogenic fungus, *Beauveria bassiana* (Ascomycota: Hypocreale) against *Trogoderma granarium* (Coleoptera: Dermestidae)

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Abstract

Khapra beetle, *Trogoderma granarium* a destructive pest of stored products, are mainly controlled in warehouses by pesticides which are harmful to consumers and the environment because of their residues and side effects. Nowadays, biological control, especially with entomopathogenic fungi has highly regarded for controlling the pests in storages which can be promising for food security. In this study, pathogenicity, and virulence of the indigenous isolate fungus, *Beauveria bassiana* DE (origin: Soil of Dezful fields, Khuzestan Province) were assessed on adults of this pest. Bioassay experiments were conducted by impregnating wheat seeds with fungal conidia. After the primary test five doses (250, 410, 660, 850, and 1750 mg conidia/kg seeds) between 10%-90% mortality were prepared. Each of the doses was prepared by putting 30 g of the seed-fungus mixture in the 50 ml-plastic jars and 15 one-day-old adults were placed into the jar. In the same way, untreated-wheat seeds were used as the control. Each dose was replicated 4 times. Mortality was recorded 5, 7, 10, and 14 days after treatment. Based on the probit analysis, the values of 167.7 and 295.32 ppm were obtained as 2-weeks LD_{25} and LD_{50} which are indicating high virulence of this isolate. The minimum and maximum doses caused 52.5 and 100% mortality, respectively 14 days following the treatment. According to results, considering the high potential of *B. bassiana* -DE in the controlling Khapra beetle population, it can be used in the integrated management programs of this pest and other storage coleopteran pests.

Keyword: Entomopathogenic fungus, Virulence, Storage pests, bioassay.

Iranian and foreign diatomaceous earths insecticidal activity against the khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae)

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Abstract

Diatomaceous earths (DEs) are fossilized diatoms (Ochrophyta: Bacillariophyceae) shells that are used as the best physical control agent for stored pests due to selective, healthy, and cost-effective function. Insects epicuticle is scratched after contact with DE and its wax layer is absorbed, finally, they die of dehydration. The insecticidal activity of two DE commercial products, Iranian Sayan®, (Kimia Sabzavar company), and foreign Celite®610, (Brandt company) was assessed against *Trogoderma granarium*, an important pest of storage products. To bioassay, five doses of each dust were prepared by combining wheat grains with dust. Each dose was divided into four parts of 30 g and each poured into a 50 mL-plastic vial containing 15 adult insects, then their lids were immediately sealed with the polyester net. Untreated seeds were used as control. The dead insects were counted 5, 7,10, and 14 days later and mortality was corrected using the Abbot formula. Results showed that mortality had a dose-dependent increasing trend. The maximum mortality (67.5±2.5% and 74.4±2.6%) occurred at the highest dose of Sayan (7000 mg conidia/kg seeds) and Celite (2000 mg conidia/kg seeds), respectively. Sayan and Celite 14-day LD_{50} values were 4339.4 and 992.2 ppm, respectively. According to the lethal dose ratio, the two formulated dusts were significantly different in terms of toxicity. The sufficient amount from Celite to kill half of this insect population was 4.47 times more than Sayan. Therefore, because of reducing the possibility of seeds bulk density, the foreign product can be more effective in managing stored coleopteran pests.

Keyword: diatomaceous earth, Sayan®, Celite® 610, coleopteran stored pest, warehouse.

The effect of pentosephosphate pathway key genes' overexpression on the production of GFP in Pichia pastoris

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Abstract

The commercial success of a recombinant protein production system depends on its ability to achieve cost-effective production in the large scale. The used host is one of the effective key factors in improving the quantity and quality of production. *Pichia pastoris* is one of the widely used host that is considered for its special properties. Host engineering is amongst the effective methods in recombinant protein production. A common strategy in metabolic engineering is to direct the carbon flux to the desired compound. In this research, we tried to engineer the host by overexpression of two key genes in the pentosphosphate pathway. For this purpose, the sequences of glucose 6-phosphate dehydrogenase (ZWF1) and 6-phosphogluconolactonase (SOL3) genes were extracted from the yeast genome of *Pichia pastoris* and cloned again into the *Pichia pastoris* expressing the green fluorescent protein (GFP), as the model protein. All the three genes, GFP, ZWF1 and SOL3, were expressed under the control of inducible AOX1 promoter. The results showed that overexpression of ZWF1 and SOL3 results in 2.2, and 4 fold increase in the GFP model protein, respectively. Based on these results, the pentose phosphate pathway is introduced as a suitable target for metabolic engineering of *Pichia pastoris* yeast.

Keyword: *Pichia pastoris*, recombinant protein, pentose phosphate,.glucose, 6 phosphate dehydrogenase, 6-Phosphogluconolactonase.

The effect of pentosephosphate pathway key genes' overexpression on the production of GFP in Pichia pastoris

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Abstract

The commercial success of a recombinant protein production system depends on its ability to achieve cost-effective production in the large scale. The used host is one of the effective key factors in improving the quantity and quality of production. *Pichia pastoris* is one of the widely used host that is considered for its special properties. Host engineering is amongst the effective methods in recombinant protein production. A common strategy in metabolic engineering is to direct the carbon flux to the desired compound. In this research, we tried to engineer the host by overexpression of two key genes in the pentosphosphate pathway. For this purpose, the sequences of glucose 6-phosphate dehydrogenase (ZWF1) and 6-phosphogluconolactonase (SOL3) genes were extracted from the yeast genome of *Pichia pastoris* and cloned again into the *Pichia pastoris* expressing the green fluorescent protein (GFP), as the model protein. All the three genes, GFP, ZWF1 and SOL3, were expressed under the control of inducible AOX1 promoter. The results showed that overexpression of ZWF1 and SOL3 results in 2.2, and 4 fold increase in the GFP model protein, respectively. Based on these results, the pentose phosphate pathway is introduced as a suitable target for metabolic engineering of *Pichia pastoris* yeast.

Keyword: *Pichia pastoris*, recombinant protein, pentose phosphate,.glucose, 6 phosphate dehydrogenase, 6-Phosphogluconolactonase.

Identification of microbial population and their interaction with Radish in biological nitrate reduction

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Abstract

Surplus nitrate has severe consequences on human health and the environment. The purpose of this study is to investigate the interaction of bacteria and radish in biological nitrate reduction and the fate of excess nitrate in the environment. This research was done in three different concentrations of nitrogen fertilizer (0, 50, and 500 ppm). The total amount of nitrate in plant and media in treatments obtained 177.2, 213.5, and 593.33 ppm, respectively. Between purified bacteria, three specimens identified by the 16SrRNA gene sequencing method. Two specious of denitrifiers were identified by nitrate reduction test. The final result demonstrated that the microbial population and nitrate removal were at the highest level in the presence of the radish. To conclude, the interaction of microorganisms and the plant in removing nitrate is a promising approach for crop cultivation, given that in oral consumption of the plant, it would be detrimental to human health.

Keyword: Denitrification, Bacterial population, Denitrifier, phytoremediation, bioremediation.

The hazard of remaining nitrate in food products and drinking water

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Abstract

Although nitrate is the essential nutrient of our body, but the excess amount of it as residual strongly causes disease. Nitrate comes from plant and animal foods that are vital to our metabolism. The nitrate is absorbed through the soil by plant and becomes an herbal protein that is placed in the food chain. If the nitrate is absorbed more than the plant requirement, it will be accumulated into the plant's organs. Since nitrogen fertilizers increase weight yield, farmers consume large amounts of that. The excess nitrate remains in the plant's body, which feeds into our body after consumption. In addition, excess nitrate in the soil enter to the drinking water after leaching or directly transported from groundwater to surface water and groundwater. The entry of nitrates from food products and drinking water into our bodies causes dangerous diseases such as cancer, methemoglobinemia, osteoporosis, kidney stones and some other new diseases. The present article, while demonstrating the state of nitrate accumulation in food and water as well as its standards, deals with diseases caused by it and find a solution to reduce the risks.

Keyword: Chemical fertilizers, Nitrate accumulation, Residual nitrate in food and water, Diseases.

Expression of Intimin-H7 chimeric candidate immunogen against Enterohemorrhagic *Escherichia coli* O157:H7

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Abstract

Background and Objectives: Enterohemorrhagic *Escherichia coli* O157:H7 It is one of the main causes of food poisoning and diseases such as hemorrhagic colitis and hemolytic uremic syndrome. Human infections caused by this bacterium are caused by consuming contaminated water and food. Thus vaccination against the pathogen of great importance. The aim of this study was to produce the recombinant Chimin protein Intimin-H7 as a candidate for *E. coli* O157: H7 vaccine. Nucleotide and amino acid sequences of Intimin and H7 were obtained from the NCBI database. For maximum expression, the gene was optimized according to *E. coli* codon preference. The synthetic gene was cloned into the expression vector pET28a + and then transfected into the *E.coli* BL21 DE3 host. Recombinant protein expression was induced at a concentration of 1 mM IPTG. For maximum expression, three parameters including IPTG concentration, time and temperature were optimized. The recombinant protein containing His-Tag was purified by nickel affinity chromatography column. Purified protein was observed in SDS-PAGE gel. In this study, blf1 gene in cloned pET28a(+) expression vector, was approved by PCR and enzymatic analysis. PAGE-SDS test showed the presence of a 55 kDa protein of suitable purity. The protein was purified by imidazel gradient in imidazel 250 with a nickel affinity chromatography column. Due to the fact that in the present study, specific sequences of Intimin and H7 proteins from *E. coli* O157: H7 were cloned and the corresponding protein was produced and purified. Finally, this protein can be used as a suitable candidate against hemorrhagic colitis caused by this bacterium.

Keyword: Intimin-H7, *E.coli* O157:H7, Recombinant vaccin, Expression.

Fungal degradation of polymers including natural rubber, polyethylene, styrene butadiene and neoprene

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Abstract

Polymer contamination is an important issue that needs to be addressed as it poses risks to the environment and living organisms. So far, methods have been proposed for the disposal and reuse of polymers. It is natural that methods such as pyrolysis and incineration of waste pose risks and pollution to the environment. Also, if the waste is left in the environment, it will cause a fire. On the other hand, rubber waste is a good place for insects such as malaria. The best method is biodegradation, where research is ongoing. Therefore, the purpose of this study is to use Trichoderma fungal strain and test the enzymatic activity of this strain to degrade different types of polymers. In this study, after isolating the Trichoderma sample, it was cultured in salt medium containing culture medium containing 0.3 g NH4NO3, 0.5 g K2HPO4, 0.1 g NaCl, 0.02 g MgSO4.7H2O, 2 g agar. Then, a certain amount of polymer was poured into a 50 ml container with the expressed culture medium and incubated for 6 weeks at a temperature of 30 and 110 rpm. After 6 weeks, sampling was performed every 7 days for 6 weeks and the results of its enzymatic activity of laccase and manganese peroxidase were reported. The results showed that the enzymatic activity of the fungal strain was better in the degradation of natural rubber and polyethylene. In general, the use of fungal strains for biodegradation of polymers can be effective.

Keyword: Biodegradation, Polymer, Trichoderma strain, Laccase, Manganese peroxidase.

Application of Cold Atmospheric Plasma for expression improvement of Green Fluorescent Protein in Recombinant Yeast

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Abstract

Yeasts are appropriate host in the in recombinant protein research and industries, and improving its capabilities are the focus of many researches. Cold atmospheric pressure (CAP) plasma has found remarkable applications in different biological and medical sciences, such as wound healing, cancer treatment and cell function modification. The goal of present study is the evaluation of plasma in the recombinant protein production capacity. For this purpose, the yeast *Pichia Pastoris* host expressing green fluorescent protein (GFP) model protein was applied, and the effect of CAP on the expression level of this protein was assayed. Fluorescence intensity of supernatant containing expressed GFP was measured by fluorimetry. Bradford test was used for protein quantification. Cell growth was assessed using Optical Density (OD) measurement at 600 nm wavelength. The results showed significant fluorescence augmentation and protein concentration increment in CAP-treated clones. According to the obtained results, CAP plasma is introduced as an applicable instrument in the researches related to the improving recombinant protein expression.

Keyword: *Pichia pastoris*, Recombinant Protein, Cold Atmospheric Pressure Plasma, Green Fluorescent Protein, Gene expression.

The study of inulin levels in chicory and Salsify plants

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Abstract

Chicory (*Cichorium intybus* L.) and Yellow salsify (*Tragopogon dubius* L.) are biennial plant of the Asteraceae family and are among the plants whose roots contain considerable amounts of inulin. Linear inulin is one of the simplest types of fructan, which is widely used in the food industry worldwide due to its beneficial nutritional and functional properties such as fat replacement, texture improvement of foods and prebiotic effects. The inulin of both plants was extracted under the same conditions and the amount of inulin and their extraction efficiency were compared with each other. The results showed that the amount of inulin in both plants is almost the same and there is no significant difference between them.

Keyword: Chicory; Yellow salsify; inulin; fructan.

Biotransformation of azo dye sunset yellow by *Klebsiella* spp.

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Abstract

Azo dyes are classified among the synthetic dyes widely used in many industries. Removal of these dyes from the wastewaters has been of great importance for many years. Sunset yellow is an azo dye widely used in food industries and therefore is found in different industrial wastewaters.

The present study investigates the capability of *Klebsiella* spp. KY357316 for biodegradation of sunset yellow. Biodegradation was conducted anaerobically in YS medium including sunset yellow at concentration of 50 mg/l. Spectrophotometry showed that *Klebsiella* spp. was able to completely remove sunset yellow from YS medium during 100 h. Moreover, thin layer chromatography (TLC) revealed that sunset yellow was biotransformed into aromatic amines during decolourization by *Klebsiella* spp.

Keyword: azo dye, biodecorization, *Klebsiella* spp., biotransformation.

Molecular identification of *spirulina* species susceptible for food supplement

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Abstract

Human beings are always attending to produce nutritional supplements to improve nutritional sources and maintain physical and mental health. Biological resources are the best option for producing dietary supplements. Microalgae are potentially a great source of compounds that can be used to produce dietary supplements. Spirulina is a species of microalgae that has a very high nutritional value and is currently produced in many parts of the world. Spirulina is a rich source of protein containing essential amino acids for the body. In this study, spirulina species were isolated from samples that were collected from Mazandaran province. Samples were purified by using the solid culture method. Morphological identification was performed based on identification keys, then specimens were separated based on the apparent similarity to spirulina. There is a need for a method that shows the highest growth over time for the targeted screening of samples in industrial cultivation, so samples growth conditions were examined over time. The species that showed the highest growth rate was selected and used for accurate molecular identification. First of all, some primer was designed according to the protected areas in Spirulina species. At the second, the desired fragment was amplified using polymerase chain reaction and use for sequencing. Finally, The results were BLAST in the Gene World Bank, thus the phylogenetic tree was drawn. The highest similarity was obtained for selected spirulina with 99% similarity to *Spirulina platensis*.

Keyword: Spirulina, Dietary supplement, Growth rate, Molecular identification.

Isolation and identification of probiotic bacteria from yogurt samples in Sabzevar city

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Abstract

Lactic acid bacteria are a diverse group of microorganisms that are very important in the dairy and fermentation industries. Screening to find lactobacillus strains with desirable properties is ongoing. With the expansion of industrial production of dairy products, it is possible to lose the native bacteria found in traditional dairy products. Therefore, isolation, identification and determination of useful properties of these bacteria are necessary for future applications. In this study, native strains of Lactobacillus acidophilus were isolated from 32 yogurt samples in Sabzevar. In order to identify this bacterium, non-molecular methods including gram and catalase tests, growth study at 15 and 45 degrees, and the possibility of fermentation of sugars were used. Using these experiments, 5 Lactobacillus isolates of *Streptococcus thermophiles* were isolated and identified from the yogurts of Davarzan and Khoshab regions and their probiotic potential (gastric acid resistance) was evaluated. The identified samples provide the quality of dairy products in this region and have the potential to be used in industrial products and can be used as probiotic primers in the preparation of dairy products.

Keyword: Probiotic, Lactobacillus, Traditional yogurt.

Investigation of biogas production from sugar beet waste along with cattle manure

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Abstract

Anaerobic digestion process is one of the acceptable technologies for energy production from organic waste. One of the significant sources of organic waste production is the factory of food processing industries and organic waste in municipal waste. The aim of this study was to evaluate the process of methane production from sugar beet waste along with bovine manure by anaerobic digestion. To perform the experiment, a laboratory digester for biogas production of one-stage type with the ability to automatically control the environmental conditions in terms of temperature was designed and built. Experiments on co-digestion of sugar beet and beef manure were evaluated at five levels. After 28 days of digestion, cumulative changes in biogas production were measured for each compound. Finally, anaerobic digestion test was performed on cattle manure alone. The amount of biogas produced at this temperature averaged 240 liters of biogas per kilogram of volatile dry matter. The frequency of production of perishable materials and animal waste in different parts of the country and the need to take the necessary measures to eliminate them, as well as the ability to produce biogas from these sources, the need for this research.

Keyword: Biogas, Beef manure, Sugar beet, Anaerobic digestion, Methane gas.

Effect of light spectrum on protein and protein pigments on Spirulina Platensis

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Abstract

The national academy of medicine recommends that adults should get a minimum of 0.8 g of protein for every kilogram of body weight per day. Spirulina platensis is one of the photosynthetic cyanobacteria that has been used in various studies to investigate. Cyanobacteria are one of the promising cyanobacteria to produce high-valued compounds such as proteins nucleic acids, lipids, vitamins and pigments which are extensively used in food, drug and cosmetics industries. So, cyanobacteria are an ideal food which contains 46-63 % proteins, 8-14% carbohydrates and 4-9% lipids based on its dry weight. In the present study, Spirulina platensis as a blue-green cyanobacteria which was cultivated in Zarrouk medium for 14 days under the white LED and its protein contents have been extracted by Lowry method. Spirulina platensis is commercial cyanobacteria. Spirulina cells are sensitive to spectrum of light. Because of high value- nutrient used to conduct various studies to investigate and understand the biochemical and biophysical properties of cyanobacterium cultivated under LED light. This study is carried out based on protein and protein pigment (phycocyanin) in Spirulina platensis under various color LEDs (white and red (620-680 nm)).

Keyword: Spirulina platensis, protein, Light spectrum, Phycocyanin, cyanobacteria.
IB-114.

Development of an inexpensive culture medium for over-production of bacterial cellulose-based on the response surface statistical design method

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Abstract

The application of bacterial cellulose (BC) has been considered in various industries, especially in the medical and pharmaceutical industries for its excellent properties compared to plant cellulose, but due to the high production costs of BC compared to plant cellulose, its industrial applications have been limited.

Considering the high cost of culture medium which represents approximately 30% of the production cost, reducing the cost of the culture medium is one of the most important strategies for the economic production of bacterial cellulose. Therefore, this study examined an inexpensive culture using only two materials, glucose syrup and Corn Steep Liquor (CSL) for high production of bacterial cellulose.

For this purpose, the effect of glucose syrup and CSL concentrations on 5 levels were investigated using the central composite design (CCD) of response surface methodology (RSM). The response in the form of contour plots has been used to find the optimal culture concentrations and the maximum yield of bacterial cellulose production.

Under optimal conditions of 110 g/l CSL and 35 g/l glucose syrup at 30 ° C, after 10 days, by using more than 90% of the culture medium, 15 g dry weight of BC per liter of culture, under static conditions and without aeration were obtained.

The amount of production from this culture medium is one of the highest values ever reported for the production of bacterial cellulose.

Keyword: Bacterial cellulose, CSL, glucose syrup, central composite design.

Screening Bacillus spp. producing vitamin K_2 from different soil sources

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Abstract

Vitamin K_2 is one of the three types of vitamin K, which produced by bacteria. It has an important role in bone and cardiovascular mineralization and prevents osteoporosis and cardiovascular disease that are of the major matters in public health concern. This vitamin is rare in dietary uptake. Thus, finding high vitamin production in bacterial strains can be valuable. Ten soil samples from different regions of Iran includes Golestan, Mazandaran, Markazi, and Tehran provinces were collected. These include forest, garden, farm, forest park, and red soil fields during April and May 2019. Bacillus strains were isolated and investigated for vitamin K_2 production through liquid state fermentation in specific media contain soy peptone, yeast extract, glycerol, and K_2HPO_4. The vitamin concentration measured in 248nm UV-spectroscopy following extraction by n-hexane: 2-propanol. High vitamin producing strains were biochemically characterized. From 97 isolated strains, 20 strains have higher absorbance value in comparison with a standard calibration curve. According to the biochemical characteristics, high vitamin producer isolates were *Bacillus subtilis*, *Bacillus cereus*, and closely related bacteria. Isolating high vitamin K_2 producer strains is a global matter and soil is widely used as a major source of *Bacillus* species that are candidates for high vitamin K_2 producing strains.

Keyword: Vitamin K_2, fermentation, optical density.

Rapid detection and quantification of bacteriophage contamination of lactic acid bacteria in yogurt

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Abstract

One of the main microbiological problems of the dairy industry is the susceptibility of starter bacteria to virus infections. starter cultures used in the manufacture of several fermented dairy products, including yogurt, is also sensitive to bacteriophage attacks. To avoid the problems associated with these viruses, quick and sensitive detection methods are necessary. In the present study, a fast real-time quantitative polymerase chain reaction assay for the direct detection and quantification Bacteriophages in milk was developed. A set of primers was designed, based on the Mur and Host gene sequence of different Bacteriophages. The results show the proposed method to be a rapid (total processing time 30 min), specific and highly sensitive technique for detecting bacteriophages in dairy products.

Keyword: Bacteriophages, Real-Time PCR, Dairy Products, starter.

Evaluation of promoter genes of histidine biosynthetase (hisI) gene, glutathione synthetase gene (gsh), P-type ATPase heavy metal transporter gene in algae Clatrix to remove nickel heavy metal

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Abstract

Introduction: In recent years, the use of different biomass to remove heavy metals from effluents has been considered. Among these, the use of microorganisms such as cyanobacteria that have the potential to remove heavy metals has found a special place, including the algae Clatrix. In this algae, histidine biosynthetase (hisI) genes, glutathione synthetase gene (gsh, P-typeATPase heavy metal transporter gene) had a significant effect on removing nickel heavy metal contamination in order to understand the mechanism of action of these genes and Information on how they work is to examine the regulatory areas of the promoters.

Objective: Promoter analysis and identification of common cis-elements between the three proposed genes. **Materials and Methods:** In this study, upstream sequences of these genes were prepared from EcoGene database and promoter analysis was performed by PlantCARE site and the motifs in each gene and their role were identified.

Results: Studies have shown that in all three genes involved in the same process, light-response systems and biological and non-biological stresses probably regulate the expression of these genes. This is the first time this has been done in this algae. It is hoped that further studies on this algae will be helpful in confirming this.

Keyword: Histidine biosynthetase gene *(hisI*), Glutathione synthetase gene (*gsh,*) P-typeATPase heavy metal transporter gene, Promoter analysis.

Effect of different carbon sources on the production of hyaluronic acid by *streptococcus zooepidmicus*

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Abstract

Hyaluronic acid (HA), is a high molecular weight linear and natural glycosaminoglycan or mucopolysaccharide that has wide applications in various industries due to its biocompatibility and viscosity properties and high water absorption capacity. Due to the economic importance of this product, our aim in this study is to produce this biopolymer by *Streptococcus zooepidemicus* with several different carbon sources including glucose, sucrose, lactose, potato starch and sago starch. The amount of hyaluronic acid was measured by turbidimetric method of acetyl trimethyl ammonium bromide. According to the results, the average concentration of hyaluronic acid produced in five different carbon sources was 0.35 g/l. The three sources of carbon glucose, sago starch and potato starch with the highest production, respectively (0.51g/l, 0.88g/l, and 0.63g/l). Optimal pH and hyaluronic acid concentration for five carbon sources; glucose (pH 5.51, 0.51g/l), (sucrose pH 5.31, 0.22g/l), lactose (pH 7.52, 0.14g/l), potato starch (pH 6.52, 0.63g/l) sago starch (pH 6.6, 0.88g/l). The results show a direct relationship between carbon sources and pH on the efficiency of hyaluronic acid, which can increase production. One of the important reasons for high production of hyaluronic acid by glucose is its direct absorption by bacterial harvesting mechanisms Species that become glucose precursors are effective in increasing hyaluronic acid production.

Keyword: hyaluronic acid, *Streptococcus zooepidemicus,* exopolysaccharide, carbon sources**.**

Effect of different carbon sources on the production of hyaluronic acid by *Streptococcus zooepidmicus*

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Abstract

Hyaluronic acid (HA), is a high molecular weight linear and natural glycosaminoglycan or mucopolysaccharide that has wide applications in various industries due to its biocompatibility and viscosity properties and high water absorption capacity. Due to the economic importance of this product, our aim in this study is to produce this biopolymer by *Streptococcus zooepidemicus* with several different carbon sources including glucose, sucrose, lactose, potato starch and sago starch. The amount of hyaluronic acid was measured by turbidimetric method of cetyltrimethylammonium bromide. According to the results, the three sources of carbon glucose, sago starch and potato starch with the highest production, respectively 0.51g/l, 0.88g/l, and 0.63g/l. While, maximum hyaluronic acid concentration for two other carbon sources, sucrose and lactose, was, respectively 0.22g/l and 0.14g/l. The results show a direct relationship between carbon sources and pH on the efficiency of hyaluronic acid, which can increase production. One of the important reasons for high production of hyaluronic acid by glucose is its direct absorption by bacterial harvesting mechanisms Species that become glucose precursors are effective in increasing hyaluronic acid production.

Keyword: Hyaluronic acid, *Streptococcus zooepidemicus*, Exopolysaccharide, Fermentation.

Applications of Bacterial Cellulose in Tissue Engineering and Regenerative Medicine

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Abstract

Tissue engineering and regenerative medicine is an emerging science and technology that has now become an important industry in the field of medicine. This science focuses on the development of treatment methods based on the repair and replacement of damaged tissues and organs. One of the important foundations in tissue engineering is biocompatible scaffold. Bacterial cellulose has unique physical and chemical properties such as high purity, high mechanical strength, insolubility, high biocompatibility, high porosity, high crystallization, high degree of polymerization, non-toxicity and suitable flexibility; These properties make bacterial cellulose a suitable material for the production of substrates for medicine, tissue engineering and food industries.

Keyword: Bacterial Cellulose, Tissue Engineering, Plant Cellulose, Biocompatibility, Mechanical strength, Flexibility.

Herbal Preservative for Mayonnaise Sauce Based on Extract of *Heracleum persicum* Fruit on *Salmonella typhimurium*

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Abstract

Heracleum persicum is a medicinal plant with many nutritional and medicinal applications which is native in Chahardangeh village, Sari city, Mazandaran province, Iran. The objective of this research was to introduce an herbal preservative for mayonnaise sauce using antibacterial effect of the methanolic extract of H. persicum fruit on Salmonella typhimurium. Standard micro dilution method was used to obtain the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) against bacterium growth. In addition, Kirby-Bauer disk diffusion method was used to measure the inhibition zone diameter. MIC and MBC were obtained 75 and 100 mg ml^{-1}, respectively. In addition, mean inhibition zone diameters were obtained 12.75, 14.25, 15.5, 15.75, and 17.5 mm in mayonnaise sauce for 25, 35, 50, 75, and 100 mg ml^{-1}, respectively after 24 h incubation. Furthermore, Triamcinolone N.N was used as a positive control in this research whose mean inhibition zone diameter against S. typhimurium was obtained 18.5 mm. The obtained results showed the strong antibacterial effect (17.5 mm) near the mean inhabitation zone of positive control (18.5 mm) in mayonnaise sauce. Therefore, it was concluded that concentration range of 75 to 100 mg ml^{-1} of H. persicum extract can be suggested as an alternative for dangerous preservatives such as sodium benzoate in mayonnaise to remove S. typhimurium which exists in it for using raw egg.

Keyword: Antibacterial, Heracleum persicum, Mayonnaise sauce, Methanolic extract, Salmonella typhimurium .

Identification viabl*e but Not culturable Bacteria in water sources (PCR and culture) Kermanshah , Iran

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Abstract

As the world population grows, it is predicted that freshwater supplies will be rare in the 21st century. Despite abundant advances in water and wastewater treatment, waterborne diseases still threaten the health of the people of the world.*Listeria monocytogenes* bacterium is a pathogen that causes listeriosis. This pathogen can also cause meningitis, poisonous sepsis, and abortion in humans. One way of transmitting this microorganism is water and foodstuffs. Quick and accurate identification plays an important role in preventing infections. Also, due to the importance of *Campylobacter jejuni* in water and food industries and causing infection, toxication, and digestive problems in humans, the identification of this bacterium can be an effective step in preventing water contamination with *Campylobacter jejuni*.The aim of the present study was to identify *Listeria monocytogenes* and *Campylobacter jejuni* through culture and PCR and compare them in the water supply of Kermanshah city.

18 samples were collected from different water supplies of Kermanshah. DNA was extracted from standard *Campylobacter jejuni* and *Listeria monocytogenes* using a DNG-Plus kit. PCR reaction was optimized using specific primers. After determin-

ing the specificity and PCR detection limit, the collected water samples were examined and at the same time, the samples were cultured and examined.From 18 samples of water supply sources in Kermanshah by PCR, *Campylobacter jejuni* was isolated from all samples, and *Listeria monocytogenes* was isolated from 17 samples, and also 4 cases of *Campylobacter jejuni* and 2 cases of *Listeria monocytogenes* were isolated by culture method.The results showed that PCR has a better performance than culture for detecting *Listeria monocytogenes* and *Campylobacter jejuni.*

Keyword: *Listeria monocytogenes, Campylobacter jejuni*, PCR, drinking water.

A review of the importance of bioactive peptides in dairy products and their production

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Abstract

Bioactive peptides are hydrolysates with specific amino acid sequences that have a positive physiological effect on the body. They are neutral in the main protein, but when microbial enzymes or digestive enzymes are added to them, they are separated from the main protein during the digestive process and show their useful properties. Dairy products, especially fermented products, are potential sources of bioactive peptides. Many of them have superfood physiological functions that make them eligible for classification as "functional foods". In this article, we will examine bioactive peptides in dairy, their production methods and their positive effects on health.

Keyword: Bioactive peptides, Dairy, Functional foods, Protein.

In Vitro Cell Viability Investigation of Human Dermal Fibroblast Cells under the Effect of Active and Inactive *P. acidilactici* Bacteria

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Abstract

Although the benefits are undeniable, the increased application of ionizing radiation in the medical field results in greater potential risk for the patients. Through decades, a broad spectrum of compounds and their derivatives were tested for protection properties, but an ideal radioprotector is still unmet. Probiotic bacteria were examined as potential radioprotective agents. The aim of this study is to evaluate the safety of different numbers and concentrations of active and inactive probiotic *Pediococcus acidilactici* respectively in cell viability assays for testing *P. acidilactici* radioprotective properties. The experiments were established using the MTT colorimetric assay for assessing normal human dermal fibroblast viability through metabolic activity. After 24hour incubation of the cells with active and inactive *P. acidilactici*, no significant viability changes were observed. Instead, it was found that high number of active *P. acidilactici* and high concentration of heat killed *P. acidilactici* increased cells' growth by 2 and 4 folds respectively. In conclusion, the results suggest that the application of high numbers of active *P. acidilactici* as well as high concentrations of inactive form is considered nontoxic.

Keyword: Probiotics, *Pediococcus Acidilactici*, viability assay, radioprotectio.

Biochemical, physiological and molecular identification of tomato stem soft rot in West Azerbaijan province

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Abstract

Tomato is one of the most important and economic crops. The different pests and disease agents attack tomato plants. Bacterial pathogens have an important role in yield reduction of this high consumption product. To identification the agent of stem tomato soft rot, survey of tomato fields was performed in different counties in West Azarbaijan province during summer 2016-1017. Sampling was done from plant with soft rot symptoms in stems. After isolation and purification of bacteria, the isolates which able to rot the potato slices, determined as pathogens. Identification of pathogenic bacteria was performed via morphological, biochemical and physiological tests based on valid plant bacteriology references. Pathogenic isolates were gram-negative, rod shaped, motile and facultative aerobic. The rresults of oxidase, hypersensitive reaction on tobacco leaves, arginine dihydrolase, fluorescent pigment production on King'B medium, starch hydrolysis and phosphatase were negative and catalase, gelatin hydrolysis, aesculin hydrolysis, casein hydrolysis, nitrate reduction, lecithinase and NaCl 4% to 7% tolerance were positive. Molecular identification of isolates was carried out using the specific primers of this bacterial species. A 420 base pair fragment was amplified by all isolates using ADE1/ADE2 primer pair. According to the phenotypic and molecular results, *Dickeya chrysanthemi* was determined as the bacterial agent of stem tomato soft rot in infected plants. The mentioned bacterial pathogen includes the first reports of tomato plants from Iran.

Keyword: Pathogenic bacterium, Phenotypic features, Molecular identification, Tomato.

Selection of hybrid-biological scaffold consisting of Hydrogel /Nanofiber framework for Beta cell transfer

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Abstract

Type 1 Diabetes Mellitus (T1DM) is characterized by the autoimmune destruction of Beta-cells in the pancreatic islets. In this regard, islet transplantation is performed with the aim of replacing damaged cells through minimally invasive surgery. Unfortunately, this method still has limitations in its widespread clinical application, including the need for long-term immunosuppression, a shortage of pancreas donors, and the loss of a large percentage of islets after transplantation. The islets of Langerhans also need to maintain their circular morphology to maintain functional insulin secretion in response to glucose stimulation. For this purpose, islands can be encapsulated in hydrogel-like biomaterials to reduce island loss. The purpose of this study is to provide updates on various hydrogel-based encapsulation strategies in insulin-producing cells and to provide the most appropriate method. For this purpose, composite composites consisting of nanofibers and hydrogels were made to grow cells in a cell-friendly environment mimicking the extracellular matrix. Electrospan nanofibers were spun under optimized conditions consisting of polycaprolactone (PCL) and gelatin and then incorporated into alginate-containing hydrogels. Nanofibril (NF / hydrogel) hydrogel hybrids bind with calcium and with / without cell presence. The mechanical properties of the incorporated NF / hydrogels are expected to increase significantly in proportion to the NF content in the NF / hydrogels. Fibroblasts cultured in NF / hydrogels show higher adhesion to the matrix than those without NF. Cells in NF / hydrogels show higher levels of insulin release by increasing NF content. Therefore, NF / hydrogels are also expected to serve as a cell culture matrix to facilitate cell-matrix interactions by combining nanofibers and supportive hydrogels. Previously, several studies had reported that when anchor-dependent cells grow at more difficult levels, cell function is greatly increased.

Keyword: Insulin, Hybrid Scaffold, Beta cell, Diabetes.

Identifying Functional Cytochromes P450 (CYPs) in *Alcanivorax* to Engineer a Marine Bacterium with a High Oil-Biodegrading Activity: a Bioinformatics Approach

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Abstract

Cytochromes P450 (CYPs) are a class of enzymes present in diverse organisms with the ability of converting Xeno-organic molecules and indigenous biomolecules into hydrophilic counterparts. The genus *Alcanivorax* represents a group of marine bacteria with ability to grow on hydrocarbons present in oil. Their ability to utilize hydrocarbons as a sole source of energy, makes them potential candidates for bioremediation of marine environments contaminated upon oil. Here, the genome and proteome related to the members of this genus were scanned to find the low molecular weight P450s to evaluate their potential functionality for future biotransformation studies based on sequence homology and molecular modeling. Our results revealed more than 20 P450s with length ranging from 230 to 288 residues. Molecular modeling uncovered the conserved residues responsible for heme-binding and catalytic activity of “mini-P450s”; so we had to simulate the selected sequences. With a high probability, the final selected truncated CYP could be used rather than “regular P450s” in the bioremediation production.

Keyword: bioremediation, sequence homology, molecular modeling, Biotransformation.

Production and characterization of specific fusion scFv-AP protein against *Fig Mosaic Virus*

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Abstract

Alkaline phosphatase (AP) is a group of hydrolase enzuymes that remove the phosphate group from proteins, nucleotides and alkaloids (dephosphorylation). Moreover, analytic detection in immunoassays is carried out with primary or secondary antibodies that are labeled with sensitive reporter molecules like (AP). An attractive alternate to the chemical coupling of these antibodies is the construction of genetically engineered fusion proteins consisting of an enzyme and a specific single-chain variable fragment (scFv). In this study recombinant (scFv) antibody of *Fig mosaic virus* Nucleoprotein (FMV-NP) fused to AP protein is generated for direct detection of the virus in infected plants. For this aim, scFv(FMV-NP)-AP construct was inserted in bacterial expression vector, pET28b, and recombinant protein was produced in bacterial host *Escherichia coli*. Purification of recombinant fusion protein was performed by affinity chromatoghraphy. Large scale expression of recombinant protein was performed in BL21.de3 strain of *E. coli* and purification was carried out through Immobilized metal ion affinity chromatography (IMAC) in column containing Ni-NTA agarose beads. Successful expression and purification steps were confirmed by

SDS-PAGE followed by western blotting analysis. Results obtained from serological analysis proved that generated recombinant fusion antibody, scFv (FMV-NP)-AP, is able to detect FMV in infected fig samples. Fusion protein developed in this research reduced the immunochemical detection of FMV by omitting the use of enzyme labeled secondary antibodies.

Keyword: Alkaline phosphatase, fig mosaic virus, recombinant antibody, (scFv-AP) constructs, fusion protein.

A step towards using modern biotechnology methods in the protection of Persepolis World Heritage

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Abstract

Biodegradation and its destructive effects on cultural heritage and monuments have been considered by many scientists around the world .Biological degradation of organic and inorganic materials is caused by the metabolic activity of living organisms and their growth (Charola et al., 2011). The most important step in studying this field of work is to know the techniques and methods of studying the microorganisms involved in this process. In the last few decades, biotechnology has made possible the development of revolutionary techniques useful for diagnosing deterioration of cultural heritage induced by micro-organisms and devising effective conservation/restoration strategies. Massively parallel sequencing, or next-generation sequencing, has revolutionized biological science (Wright et al., 2019). With its large throughput, scalability, and speed, NGS allows researchers to study a variety of biological systems. With biotechnological approaches, sample amounts are minimized, and that makes it easier to spot contamination and complex microorganisms in cultural assets, as well as reveal uncultivable species on both organic and inorganic substrates (Gadd et al., 2012). This approach, based on genomic DNA analysis, and metabolic byproducts. It is a well-known fact that microorganisms in nature play an important and vital role in biodeterioration (Raja et al., 2017). These organisms are the main components of natural cycles such as the carbon cycle, the nitrogen cycle, and the sulfur cycle (Cámara et al., 2011). There is no doubt that biotechnology provides a plethora of information

useful for setting up appropriate strategies that are safe for works of art, restorers. Considering the biodiversity of lichens and microorganisms in Persepolis and the high production potential of bioactive materials and the destructive chemical and physical effects of these organisms and the historical significance of Persepolis, the main purpose of this study was to investigate the microorganisms involved in biodegradation. The field survey was made from April 2017 to March 2019. The images were taken using a non-invasive method, without a flash. To study the microbial community, DNA was extracted using the OMEGA kit. PCR was performed utilizing specialized primers 27 F and 338R. All samples were then sent for sequencing by NGS. Some samples were prepared for studies related to the substrate and the effect of microbial secondary metabolites on them and studies for mineral bio colonization, were analyzed using XRD, ICP, and FTIR/ATR methods. some samples were prepared for culture using a general culture medium. The results of this study show that the diversity of biological factors involved in Persepolis biodeterioration is not limited to macroscopic factors that can be seen with the naked eye. The results of primary cultures indicated the presence of a variety of heterotrophic bacteria and pigment-producing bacteria. The culture results also confirmed the presence of microscopic algae, which, under microscopic observations, confirmed that these algae belong to the family *Trebouxiophyceae*. We also managed to grow some meristematic fungi in the culture medium, which are also known as black fungi due to the production of black pigments, which has led to visual pollution in the palaces of the world heritage of Persepolis by creating black spots. On the other hand, the presence of photosynthetic algae and fungi has provided the basis for the symbiosis and colonization of lichens, which cover a large part of the palaces of this historic monument. The results of XRD analysis were measured at 30% humidity at 40 Kv voltage and mA40 current and the results identified the type of the sample as calcite. The presence of calcite provides a calcareous substrate for the growth of microorganisms. The FTIR results confirmed the presence of carbonic acid due to the presence and activity of microorganisms in this calcareous environment, which in turn causes corrosion in the substrate. The Results of ICP analysis showed the presence of important elements for the bio colonization of microorganisms. One of the predominant element is the presence of phosphorus with a concentration of 227.9ppm, which is involved in many microbial biochemical cycles. Sulfur, which plays a key role in the metabolism of sulfate-reducing bacteria, was also reported as 438.7 ppm.

Keyword: Biodeterioration, Biotechnology, Microorganisms, Cultural Heritage, Persepolis

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Abstract

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works of art, restorers .Considering the biodiversity of lichens and microorganisms in Persepolis and the high production potential of bioactive materials and the destructive chemical and physical effects of these organisms and the historical significance of Persepolis, the main purpose of this study was to investigate the microorganisms involved in biodegradation. The field survey was made from April 2017 to March 2019. The images were taken using a non-invasive method, without a flash. To study the microbial community, DNA was extracted using the OMEGA kit. PCR was performed utilizing specialized primers 27 F and 338R. All samples were then sent for sequencing by NGS. Some samples were prepared for studies related to the substrate and the effect of microbial secondary metabolites on them and studies for mineral bio colonization, were analyzed using XRD, ICP, and FTIR/ATR methods. some samples were prepared for culture using a general culture medium. The results of this study show that the diversity of biological factors involved in Persepolis biodeterioration is not limited to macroscopic factors that can be seen with the naked eye. The results of primary cultures indicated the presence of a variety of heterotrophic bacteria and pigment-producing bacteria. The culture results also confirmed the presence of microscopic algae, which, under microscopic observations, confirmed that these algae belong to the family *Trebouxiophyceae*. We also managed to grow some meristematic fungi in the culture medium, which are also known as black fungi due to the production of black pigments, which has led to visual pollution in the palaces of the world heritage of Persepolis by creating black spots. On the other hand, the presence of photosynthetic algae and fungi has provided the basis for the symbiosis and colonization of lichens, which cover a large part of the palaces of this historic monument. The results of XRD analysis were measured at 30% humidity at 40 Kv voltage and mA40 current and the results identified the type of the sample as calcite. The presence of calcite provides a calcareous substrate for the growth of microorganisms. The FTIR results confirmed the presence of carbonic acid due to the presence and activity of microorganisms in this calcareous environment, which in turn causes corrosion in the substrate. The Results of ICP analysis showed the presence of important elements for the bio colonization of microorganisms. One of the predominant element is the presence of phosphorus with a concentration of 227.9ppm, which is involved in many microbial biochemical cycles. Sulfur, which plays a key role in the metabolism of sulfate-reducing bacteria, was also reported as 438.7 ppm.

Keyword: Biodeterioration, Biotechnology, Microorganisms, Cultural Heritage, Persepolis.

Isolation and selection of local carotenoid-producer yeasts

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Abstract

Carotenoids are the most-known pigments in nature, synthesized by plants, algae, and some fungi and bacteria. Many of these compounds are precursors of vitamin A which eliminates free radicals and prevents cancer as well. Carotenoids are utilized in numerous industries like pharmaceuticals, cosmetics, food, and feed industries. Nowadays due to the concerns about chemical pigments, the biotechnological production of carotenoids by microorganisms such as yeasts has attracted much attention. In this study, 77 samples were collected from different regions and colorful yeasts were isolated. Isolated yeasts were studied for various features (e.g. the ability of pigmentation in liquid and solid media, starch consumption, growth in nutrient-poor media, etc.). Based on the results, the best yeasts were selected for further studies in the future.

Keyword: Yeasts, Carotenoids, Isolation, Selection.

Synthesis and characterization of GAAMCs nanobiocomposite hydrogel: antibacterial activity

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Abstract

Designing novel nanobiomaterials for tissue engineering purposes is obviously necessary considering the ever-increasing need for suitable biocompatibility. In this study, a biopolymeric network poly (Acrylamide-co- 2-acrylamido-2-methylpropane sulfonic acid)/Chitosan hydrogel incorporated with graphene oxide (GAAMCs) was synthesized through free radical polymerization method. The GAAMCs nanobiocomposite hydrogel and its swelling behavior were characterized by FTIR and equilibrium swelling ratio, respectively. In addition, antibacterial properties of GAAMCs were investigated against *Staphylococcus aureus* and *Escherichia coli*. The results revealed that the zone of inhibition was more in *S. aureus* i.e. 15 mm as compared to *E.Coli* i.e., 8 mm. Chitosan-based hydrogel-GO nanocomposites demonstrated antibacterial activity against both Gram-negative and Gram-positive bacteria. These properties verified the GAAMCs hydrogel for the used as antibacterial material in the biomedical field.

Keyword: Biopolymeric network, Nanobiocomposite hydrogel, Antibacterial, Gram-negative bacteria, Gram-positive bacteria.

Study Quantity of naphthalene degrading yeasts in the Persian Gulf

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Abstract

Yeasts are ubiquitous in distribution, and their populations depend on the type and concentration of organic matter; The distribution of species, as well as the number and metabolic characteristics, are controlled by existing environmental conditions. PAH compounds are one of the toxic environmental pollutants that have accumulated due to various human activities in the environment. Naphthalene is a polycyclic aromatic hydrocarbon (PAH) that is of environmental concern due to its carcinogenicity and the properties of persistent organic pollutants. This study aimed to isolate and quantitatively evaluate naphthalene degrading yeasts from the Persian Gulf. Water, sediment, and living samples were collected from crude oil-contaminated areas in the Persian Gulf; They were then cultured in 100 ccs of Bushnell Haas broth containing 200 ppm naphthalene with 10 µg of cefazolin and 10 µl of gentamicin for two weeks to prevent the growth of gram-positive and gram-negative bacteria. In this study, 13 species of marine yeast that were capable of degrading naphthalene were isolated, the results of which are shown in graphs.

Keyword: Biodegradation-Oil Pollution-Marine yeasts-Naphthalene-Marine environment.

Evaluation of the effect of Nano iron oxide/dextran/PEG conjugated with Ceftizoxim on the expression of efflux pump genes *AdeB* and *QacED1* in strains of *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is an important cause of nosocomial infections, especially in the intensive care unit (ICU), which is resistant to a wide range of antibiotics, including cephalosporins. One of the mechanisms of antibiotic resistance is increased expression of *QacED1* and *AdeB* efflux pump genes. Therefore, the aim of this study is to evaluate the expression level of *AdeB* and *QacED1* genes in *A.baumannii* strains under the influence of Fe_3O_4/dextran/PEG nanoparticles conjugated with Ceftizoxim. Iron oxide nanoparticles were synthesized by co-precipitation method and coated with dextran and PEG to reduce toxicity and increase stability. Ceftizoxime antibiotic was conjugated with nanoparticles to increase antibacterial properties. The minimum inhibitory and lethal concentrations of *A.baumannii* strains were determined by micro dilution method. Expression levels of *QacED1* and *AdeB* genes were measured by Real time-PCR. The results of FESEM and XRD showed that Fe_3O_4/dextran/PEG nanoparticles were well synthesized and also the Ceftizoxim was properly bonded. The minimum inhibitory and lethal concentrations were determined at concentrations of 12.5 and 25 µg/ml, respectively. Decreased expression of *QacED1* and *AdeB* genes compared to the control strain at three concentrations of 12.5, 3.125 and 0.78 µg/ml showed a significant difference.

The results of this study indicate a decrease in the resistance of *A.baumannii* strains to the antibiotics conjugated with Fe_3O_4/dextran/PEG nanoparticles. The results also

showed that there is a significant difference between the expressions of genes in the efflux pump compared to the control strain of *A.baumannii*, so that the effect of Fe_3O_4/dextran/PEG-ceftizoxime nanoparticles on reducing *AdeB* gene expression is much greater than the expression of *QacED1* gene. Therefore, identifying the mechanisms of antibiotic resistance in *A.baumannii* and using nanotechnology in the control and treatment of nosocomial infections can be useful.

Keyword: *Acinetobacter baumannii*, Iron Oxide Nanoparticles, *QacED1*, *AdeB*, Real time-PCR..

Effect of pH on copper recovery using bacterial leaching in chloride medium

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Abstract

Nowadays, following the reduction of high-grade copper reserves and the many disadvantages and processing problems of pyrometallurgy method of copper sulfide concentrate, hydrometallurgy and biohydrometallurgy methods are being designed and used. Biohydrometallurgy is considered as a suitable method for extracting metals from low-grade ores and sulfide concentrates due to various reasons such as copper grade reduction in ore, less energy consumption and less pollution to the environment. The resistance of sulfide minerals to dissolution in sulfuric acid causes the addition of various chemical compounds to facilitate the biooxidation of these minerals. In many cases, supplying low-salinity water is difficult for industrial processes, and usually the water used contains large amounts of various salts, including chloride. In this study, the bioleaching process of copper sulfide concentrate of Khatun Abad in various pH and in the presence of chloride ion was investigated. Experiments were performed on a laboratory scale with shaky containers using the bacterium *Sulfolobus acidocaldarius*. The effect of pH parameter was investigated at constant pHs of 1.5 and 2 and chloride ion concentrations of 0.5 and 1 mol/L NaCl with a constant 1% solid and temperature of 60°C. In this experiment, changes in dissolved copper concentrations were measured during the bioleaching process. The results showed that about 100% of copper was extracted within 21 days at a constant pH of 1.5, and a concentration of 0.5 mol/L NaCl.

Keyword: Copper, Sulfuric acid.

Effect of pH on copper recovery using bacterial leaching in chloride medium

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Keyword: Copper, Sulfuric acid.

Culture and isolation of *Chelonodon patoca* gastrointestinal microorganisms for the production of tetrodotoxin with medicinal applications

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Abstract

Chelonodon patoca, is known as the predominant species of pufferfishes in the Persian Gulf. Due to the coexistence with various microorganisms these fish have important bioactive compounds such as tetrodotoxin (TTX), one of the strongest and most valuable neurotoxins. It is known that the primary source of TTX is yet an unknown biosynthetic pathway in symbiotic microorganisms living with these fish. The present study examines the possibility of culturing and isolating the microorganisms of the gastrointestinal tract of pufferfish *Chelonodon patoca*, provides a basis for further research on the bioactive compounds, especially TTX. For this purpose, three pufferfish were caught from the waters of Qeshm Island, Persian Gulf, Iran. The washed contents of the fish gastrointestinal tract were prepared in five serial dilutions and inoculated in two solid culture media of Marine agar and Actinomycete isolation agar and incubated at 30°C in the dark. Colonies formed over time were isolated by the streak method over several passages based on morphological characters. Additionally, several marine fungi were isolated and grown using a new medium with salinity similar to that of Persian Gulf water (40 ppt). Finally, a total of 137 bacterial and 13 fungal strains were isolated and their stocks were named and stored for further experiments.

Keyword: *Chelonodon patoca*, Symbiotic microorganisms, bacterial culture, fungal culture.

Investigation and comparison of volatile organic compounds profiles in recombinant and wild strains of Trichoderma harzianum with HS-GC-SPME technique

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Abstract

Trichoderma species are known as probiotic microorganisms with biocontrol and biofertilizer activity. They are producers of secondary metabolites like volatile organic compounds (VOCs) with antifungal, antibacterial, and growth promoter properties. *Trichoderma* VOCs can induce resistance to plant pathogens leading to improved plant growth and health. In this study, we compared the volatile organic compounds produced of *Trichoderma harzianum* recombinant strains (T13 and T15), containing chimeric chit42 with Chitin Binding Domain (ChBD) and wild-type (Tw) strain by headspace gas chromatography-mass spectrometry (GC-SPME). VOCs profile of strains revealed a total of 11, 57, and 29 metabolites from the Tw, T15, and T13 respectively. Most of the VOCs produced from T13 and T15 had growth enhancement and biocontrol activity, respectively, on the plant. Also, the diversity of VOCs from *Trichoderma* recombinant strains was higher than the Tw strain. These compounds might work synergistically to promote growth, and enhance biocontrol and antifungal activity, and thus, recombinant strains with higher diversity of VOCs might be more effective than Tw.

Keyword: *Trichoderma* recombinant strains, Volatile organic compounds, HS-GC-SPME, biocontrol, biofertilizer.

Molecular Identification and Evaluation of *Streptomyces* species efficacy on the Growth Indices of Cucumber (*Cucumis sativus* L.) under Biotic Stress Induced by *Botrytis cinerea*

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Abstract

Introduction : Cucumber with the scientific name of *Cucumis sativus* is of special importance in terms of export in Iran. B. cinerea is a plant pathogen with a wide range of hosts, including strawberries and cucumbers. Biological control seems to be a promising option and on the other hand *actinomycetes* are one of the valuable factors in biological control. In order to control the disease biologically, from a number of soil Actinomycetes isolated from Kahnooj city, three isolates MKH1, MKH7, MKH25 that had effective antifungal activity against the pathogen were selected. To better understand the control mechanism performed by these isolates. The results showed that control of fungal damage in the desired traits by these isolates. Three isolates MKH1, MKH7, MKH25 isolated from soil were evaluated to evaluate the antagonistic activity against *B. cinerea* in cucumber. Among them, MKH7 isolate in vitro and in the greenhouse after statistical analysis had an inhibitory effect on cucumber gray mold. The isolate was identified by sequence analysis of small ribosomal RNA subunit (16S rRNA) and based on the results of this isolate had the highest overlap with *Streptomyces pratensis* strain BTU19 (93.6%). We hope that based on the presented research, the selected *Streptomyces* strain will be investigated in further studies for its potential for commercialization of biofertilizers, improvement of growth indices and yield of agricultural products. Special attention should be taken for development of biofertilizers and biopesticides in the form of purposeful research and commercialization of such products which will lead to the adjustment

of chemical fertilizers and pesticides usage and environmental protection.

Keyword: *Botrytis cinerea, Cucumis sativus*, *Streptomyces*, Biocontrol, Plant Growth Promoting.

Production of biodegradable polylactic acid film containing *Lippia citriodora* nanoemulsion to investigate the biological properties

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Abstract

There are a variety of synthetic and natural antimicrobial compounds for use in antimicrobial packaging, which due to the side effects of synthetic compounds and also the general popularity of natural compounds, the use of antimicrobials with natural origin is increasing day by day. In this regard, the aim of this study was to design and produce biodegradable active films based on polylactic acid containing *Lippia citriodora* nanoemulsion by molding method and to investigate the antibacterial effects of films against two common foodborne pathogens to increase the shelf life of rainbow trout. Based on the dynamic light Scattering test, the particle size of the nanoemulsion prepared by ultrasound was 22.4 nm. MIC and MBC tests were performed by microdilution method and confirmed the antibacterial activity of Lippia citriodora nanoemulsion. Considering the count of Staphylococcus aureus and Escherichia coli bacteria on days 0, 3 and 7, rainbow trout fillets wrapped in polylactic acid films containing nanoemulsion showed good quality for consumption. As a result, this type of film can effectively increase the shelf life of rainbow trout fillets preserved at refrigerated temperature.

Keyword: Polylactic Acid, *Lippia citriodora*, Nanoemulsion, Rainbow trout, Shelf Life.

Semi-industrial optimization of L-asparaginase production from Candida utilis

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Abstract

L-asparaginase enzyme is known to be a fundament treatment of acute lymphoblastic leukemia, and also the efficient factor in decreasing of acrylamide formation in food industry. Generally, prokaryotic source of L-asparaginase enzymes is used as a medicinal purpose in ALL patients. The main obstacle in ALL treatment with bacterial L-asparaginase is undesirable side effects including immunological hypersensitivity reactions. Therefore, other source of L-asparaginase is subject of new research. Candida Utilis is capable of producing L-asparaginase as a eukaryotic source. To enhance production of L-asparaginase by Candida Utilis first sequencing batch reactor (SBR) were used to produce higher concentration of L-asparaginase. Consequences are as follows, firstly production of L-asparaginase from Candida Utilis at SBR was leaded in increasing of L-asparaginase activity from 4.8 IU/ml to 15.31IU/ml in the first and ninth batch. Secondly, period of batches was decreased during SBR from 22h to 4h, respectively. Ultimately cell protein concentration was increased from 1117mg/l to 2120mg/l. As well achieving to the species of Candida Utilis being morphologically different from primary cells had been another significant advantage of SBR while no genetic modification was performed. Next optimization of culture conditions for L-asparaginase production by submerged fermentation of Candida Utilis was studied which I modeled in RSM in order to reduce the number of experiments. The experimental L-asparaginase activity of 36 IU/ml was obtained at the optimum conditions of molasses and corn steep liquor, pepton as carbon and nitrogen source.

Keyword: L-asparaginase, Acute lymphoblastic leukemia, Sequencing Batch Reactor, Candida Utilis, optimization.

Evaluation of protective effect of encapsulated essential oil of *Satureja hortensis* on the qualitative characteristics and control of fire blight

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Abstract

Fire blight has caused great damage to quince, apples and pears globally. So far, no clear-cut method of treatment for this disease has been proposed. Nowadays, medicinal plant bioactive compounds are suggested as a safe compound for combating plant diseases due to their safety for humans and the environment. Therefore, this experiment was performed to investigate the potential of edible coating based on *Satureja hortensis* essential oil microcapsules to protect quince, apples and pears fruits against *Erwinia amylovora* infection. The results showed that the *S. hortensis* essential oil encapsulated in the wall material with 89.03% encapsulation efficiency and with an average particle size of 3.5µm. The p-cymene and δ-terpinene were the major bioactive compounds present in the microcapsules. Fruits of quince, apples and pears were treated with one, two and three gram per liter concentrations of microcapsules. The edible coting not only prevented the proliferation of *E. amylovora* and controlling spoilage, but also improved the quality properties of quince, apples and pears including antioxidant properties, phenolic compounds and sugar content.

Therefore, using edible coting based on microcapsules-loaded *S. hortensis* essential oil could minimize the damage and spoilage caused by fire blight disease agent in the apple, pear and quince fruits during the harvest and storage period.

Keyword: Targeted Release, Encapsulation, Phenolic Compounds, Biopesticides, Plant Bioactive Compounds.

A Review of the Potential use of Plant Bioactive Compounds in Controlling Plant Pathogen Factors- Fire Blight

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Abstract

The fire blight, caused by the bacterium *Erwinia amylovora*, is one of the most important destructive diseases of pome fruit trees and flowering plants. In this study, a review of the potential application of plant bioactive compounds against *E. amylovora* was performed. Among the effective compounds of the plants studied for controlling this disease, the most effective in the laboratory, greenhouse and garden tests reported to be Thymol and Carvacrol compounds which are presented in thyme oil. Thymol and Carvacrol are phenolic compounds potent in fire blight control through inhibition of free radicals, enhancement in antioxidant enzymes production, increase in production of Phytoalexins and stimulation of the production of pathogen-related proteins. Nowadays, employing new technologies in the field of bioactive compounds delivery and encapsulation not only preserve the plant bioactive compounds against evaporation, sublimation, oxidation, but also increase absorption efficiency, and improve the effectiveness of these compounds in plant disease control.

Keyword: Phenolic Compounds, Thymol, Microencapsulation, Biological Control, Pear.

Investigation of performance and optimization of bio-cementation process for improvement of granular soil used in road construction operations

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Abstract

The focus of biotechnology topics is on the development of biological materials and construction based on biological improvement of soil, biological mortar and biological mulch. There are many bacteria that can produce calcium carbonate percipitates (MICP). The basis of the method of using biotechnology in construction is based on the biological function of bacteria, their adaptability to the environment and the production of bio-cement, which is due to enzymatic hydrolysis of urea and production of ammonium ions in the soil environment. In this research, the relative moisture and soil compaction and the type and amount of bacterial suspension on grain soil resistance have been investigated. This approach is important because the previous studies were performed under desirable conditions and with pressure injection of bacteria or cementitious liquid that is not applicable in field conditions. The results showed that the use of 3 soil layers for a 15 cm soil operation layer and concentrated bacterial suspension to achieve a compressive strength of 2.1 kg/cm^2 based on the existing standards in road construction operations provided a favorable result of biological improvement. This study can pave the way for the practical applications of biocementation in a large scale.

Keyword: Microbial-induced calcite precipitation, Biological soil improvement, Relative compaction, Biocementation.

Increase of alcohol production by optimizing aerobic bioreactor culture medium using produced vinasse from alcohol distillation unit waste

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Abstract

The aim of this study was to increase the amount of yeast in aerobic fermenters and find its effect on the production of alcohol in aerobic fermenters, reduce production costs and solve the effluent problem of Bidestan alcohol factory by replacing current nitrogen sources (such as CSL and urea di-ammonium phosphate) with vinasse produced in the factory production line. For this purpose, the effect of four factors, the ratio of glucose syrup to molasses, ammonium sulfate, urea and vinasse at two different levels in 31 experiments based on the of response surface methodology (RSM) with central composite design algorithm (CCD) on the amount of live yeast was investigated. All experiments were performed twice in a system consisting of 10 parallel bioreactors with a working volume of 1 liter with aeration built in this study. Statistical analysis of the results showed that 1) vinasse can be a suitable alternative to other current nitrogen sources of the plant, 2) the ratio of glucose syrup to molasses does not have much effect on the result and therefore the use of both carbon sources of glucose syrup and molasses in any ratio can lead to 3) In the optimal state of the factors, the ratio of glucose to molasses 2, vinasse and urea reached 80 and 0.4 (g / l), respectively, the cell content reached 70-70 10×10^6 /ml. In this case, it is possible to reach a cell count of 70-80 million per ml. Using this medium in anaerobic and aerobic conditions with Brix 22, 72% alcohol was produced in 72 hours, while

in the production line of Bidestan company, with the previous culture medium, an average of 45 million cells per milliliter and 8% alcohol was produced.

Keyword: Bakery yeast, Alcohol, Vinasse, Experimental design, Glucose syrup, response surface statistical design (RSM), central composite design algorithm (CCD).

Coating granular urea fertilizer with starch-based polymer latex nano-composite

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Abstract

overuse of chemical fertilizers pollutes the environment and imposes heavy costs on farmers. The aim of this study was coating granular urea fertilizer using biodegradable polymer latex based on starch poly (styrene-co-butyl acrylate) to tackle the environmental issues derived from the use of fertilizers overuse and Slow fertilizers with non-degradable plastic coating. Different formulations of slow-release fertilizers containing different amounts of NCNPs were synthesized, and their urea release rates were investigated. SEM images confirmed the random and uniform distribution of biochar nanoparticles in the polymer matrix. Moreover, results showed the release time was prolonged with increasing the amount of NCNPs because the favorable interfacial polymer-filler interactions resulted in slower nitrogen diffusion and consequently slower release rate. Comparison of urea release rate from different formulations showed that synthesized polymer latex was more efficient at controlling urea release than commercial latex. In addition, urea release increased with increasing temperature. The study of the effect of temperature on urea release showed that with increasing temperature, urea release rate increases.

Keyword: Slow release, Starch, Natural char nanoparticles, latex.

The potential of microalgae for CO_2 sequestration

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Abstract

Fossil fuels, which are recognized as unsustainable sources of energy, are continuously consumed and decreased with increasing fuel demands. Microalgae have great potential as renewable fuel sources because they possess rapid growth rate and the ability to store high-quality lipids and carbohydrates inside their cells for biofuel production. Microalgae can be cultivated on opened or closed systems and require nutrients and CO_2 that may be supplied from wastewater and fossil fuel combustion. In addition, CO_2 removal via photosynthesis to directly fix carbon into microalgae has also attracted the attention of researchers. The conversion of CO_2 into chemical and fuel (energy) products without pollution via this approach is a promising way to not only reduce CO_2 emissions but also generate more economic value. The harvested microalgal biomass can be converted into biofuel products, such as biohydrogen, biodiesel, biomethanol, bioethanol, biobutanol and biohydrocarbons. Thus, microalgal cultivation can contribute to CO_2 fixation and can be a source of biofuels. This article reviews the literature on microalgae that were cultivated using captured CO_2, technologies related to the production of biofuels from microalgae and the possible commercialization of microalgae-based biofuels to demonstrate the potential of microalgae.

Keyword: CO_2 removal, microalgae, Fossil fuels, photosynthesis.

Fortification of *Spirulina platensis* with micronutrients of iron and zinc

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Abstract

In this study, the effects of various iron and zinc concentrations on *Spirulina platensis* growth, and the capability of this cyanobacterium for the accumulation of these metals were investigated. Data depict that growth was significantly affected by the concentration of selected metals in the culture medium. The *Spirulina platensis* biomass of each concentration reached the maximum at the end of the cultivation. However, high levels could inhibit the growth of microalgae. Maximum growth was exhibited by *Spirulina platensis* at 0.1 g/L iron and 2 mg/L zinc. The maximum bioaccumulation value was observed in 0.3 g/L iron and 8 mg/L zinc concentrations. Overall, this study indicated that *Spirulina platensis* can uptake and accumulate iron and zinc metals in its cells and it can be suggested for involvement in further functional food developments.

Keyword: *Spirulina platensis*, Iron and zinc ions, Growth parameters, Bioaccumulation**.**

Nostoc calcicola ISC 89 is the most effective species for phenanthrene biodegradation in contaminated soils

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Abstract

The accumulation of polycyclic aromatic hydrocarbons (PAHs) can cause adverse effects to the environment and human health. This research was carried out to investigate the phenanthrene (PHE) biodegradation by five microalgal species, namely *Scenedesmus* sp. ISC 94, *Chlorella* sp. ISC 23, *Nostoc calcicola* ISC 89, *Anabaena* sp. ISC 88, and *Leptolyngbya fragilis* ISC 108. Screening of microalgal species for the degradation of PHE was done based on GC analysis and growth parameters under control condition (without PHE) and at 0.1% PHE. The results showed that the tolerance of *N. calcicola* ISC 89 to PHE was more significant than other species under the mixotrophic condition. Therefore, this study suggests that *N. calcicola* ISC 89 could be an ideal candidate for its use in the bioremediation of PAHs contaminated areas that has significant economic and ecological importance.

Keyword: Biodegradation, Microalgae, Phenanthrene, Mixotrophic condition.

Co-production of cellulase-xylanase enzymes by different species of *Trichoderma* fungi using corn bran waste of high fructose corn syrup factories

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Abstract

In this study, six different strain of *Trichoderma* (*T. aureoviride* NAS106, *T. afroharzianum* NAS107, *T. ghanense* NAS108, *T. pleuroticola* NAS109, *T. atroviride* NAS112 and *T. lixii* NAS114) were used for the production of extracellular enzymes with Corn bran waste as a substrate. submerge fermentation conditions are performed at 28 °C and stirring speed of 150 rpm for 72 hours. The amount of extracellular protein, cellulase (exo-glucanase, endo-glucanase, β-glucosidase and total cellulase) and xylanase enzymes activity were assayed in the supernatant of fermentation medium. The purity and composition of enzyme-rich proteins were also evaluated using polyacrylamide gel electrophoresis (SDS-PAGE) test. The results showed that the highest amount of extracellular protein was observed in T. afroharzianum NAS107. Activity of endoglucanase, exoglucanase, β-glucosidase and total cellulase enzymes of *T. afroharzianum* NAS107 and *T. lixii* NAS114 showed the highest levels of enzymatic activity among other strains. The difference in the molecular weight of the enzyme bands showed that the exuglucanase (CBH I, cell 7A), endoglucanase (EG III, Cel 12A) and the xylanase enzyme (Xyl I) hydrolyzed the corn husk, synergistically. These results indicate that, *T. afroharzianum* NAS107 has a higher potential than other studied species for the production of extracellular enzymes from corn husk as a suitable substrate for co-production of cellulase-xylanase enzymes.

Keyword: *Trichoderma*, corn bran, cellulose, Xylanase, SDS-PAGE**.**

hran and Their Function in Biodegradation of Diazinon

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Abstract

A Pesticides not even harmful for human although are noxious for water reservoir and soil and air quality. Contamination of surface water and underground water it's a serious threat for the ecosystem around them.Organophosphorous and Organochlorine pesticides are cause of health damage like tumor, Irritability, and such Seizure. In this study, in order to take advantage of the capabilities of treating bacterial for removal of Diazinon from aqueous solutions, Bacteria in the municipal sewage sludge of Tehran West Town Wastewater Treatment Plant were identified and diazinon removal was measured. Five bacterial genus were identified in urban sewage sludge, the most frequent of which belonged to Aeromonas. After the sludge compatibility with Diazinon insecticide, the 30% reactor with a concentration of 176 mg/L was selected and the results were recorded over a period of 14 days. And diazinon was removed by a designed reactor. Therefore, it seems that the use of environmental bacteria in wastewater can be effective in bioremediation of diazinon.

Keyword: Biodegradation, Municipal wastewater, Bacterial identification, Diazinon.

Optimization of xanthan gum production by sugarcane molasses broth using Plackett-Burman design

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Abstract

Xanthan is a water soluble Exopolysaccharide produced by *Xanthomonas* species. This polysaccharide has much common application and normally produced in submerged fermentation by using different carbon sources. The proposal of the present study was to investigate and optimize the possibility of xanthan gum production by *Xanthomonas campestris DSMZ 19000* in batch experiments on sugarcane molasses broth. Sequential methodology based on the application of two types of experimental designs was used to optimize the fermentation conditions for xanthan production from *X. campestris DSMZ 19000* in shaking flask cultures using sugarcane molasses as the sole substrate. Using Plackett-Burman design, sugarcane molasses and KH-2PO4 were identified as significant variables which highly influenced xanthan gum production and these variables were subsequently optimized using a steepest ascent design. The steepest ascent method was demonstrated effectively and efficiently to approach the neighborhood of the optimum. The optimum medium composition was found to be (g/l): Sugarcane molasses, 100; citric acid, 1; KH2PO4, 10; MgCl2, 0.3; CaCl2, 0.006; NH4Cl, 0.2; FeCl3, 0.006. Xanthan production increased markedly from 7.83 to 15.03 g/l, when *DSMZ 19000* strain was cultivated in the optimal me-

dium, compared to the preoptimized condition. The xanthan gum produced by this method was confirmed by comparing the infrared spectrum of commercial xanthan gum with the infrared spectrum of the xanthan gum produced using this method. The infrared spectra were very similar, which confirmed the identity the xanthan gum produced using our method.

Keyword: Xanthan, Xanthomonas campestris, molasses, Plackett-Burman design.

Identification of clay loam soil bacteria and their function in biodegradation of Pyrene

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Abstract

Oil pollutants are not only toxic to humans, but also a threat to the health of water and soil. Pollution of the soil is a serious threat to the ecosystem around them. Oil pollutants cause carcinogenic, mutagenic and toxicity in the environment. In this study, bacterial identification was done to use for determining the bacterial biodegradability and bioremediation of the provided contaminated clay loam soil with hydrocarbon pyrene from the Iranian Research Institute of Plant Protection. The soil was poured into 27.77 grams in 500 ml water bottles. Then, it was smeared pyrene. The results showed 5 bacterial genera in the clay loam soil. The dominant bacterial genus in the soil was related to the Pseudomonas genus. The removal of pyrene results was recorded at four concentrations of 50, 100, and 200, 400 ppm at 5, 1, 5, 10, 15, and 20 days. Therefore, it seems that indigenous bacteria can be effective for bioremediation of pyrene in this type of soil.

Keyword: Biodegradation, Contaminated soil, Bacterial identification, Pyrene.

Increased Astaxanthin Accumulation in *Xanthophyllomyces dendrorhous* as a Response to Oxidative Stress

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Abstract

Astaxanthin as a microbial carotenoid in *Xanthophyllomyces dendrorhous*, has potential health-promoting effects in human. Carotenoinds are amongst the antioxidant agents and microorganisms with capability to synthesis these compounds are able to better survive and tolerate the oxidative environments. To obtain a *X. dendrorhous* strain with higher yield of astaxanthin, a combination of an oxidative agent with a carotenoid inhibition compound was used in this study (Novoveská, 2019). The culture media of *X. dendrorhous* was initially grown on yeast mold (YM) agar and the media was exposed to hydrogen peroxide as a mutagen and oxidative stress agent along with diphenylamine (DPA) as an carotenoid inhibitor. The survival rate was evaluated in the presence of reactive oxygen species (ROS) along with carotenoid inhibitors as an inducing carotenogenesis genes measuring the number of colonies recovered on YM agar with and without treatment of hydrogen peroxide. The colonies with red color were further studied. The selected strain, Or6 was chosen for quantitative and qualitative evaluations. Extraction of carotenoids for quantitative and qualitative evaluation were performed by spectrophotometric determination with UV-vis absorption spectra recorded at 480 nm, Thin-layer chromatography and also High-performance liquid chromatography method equipped with a UV detector. The initial screening of the yeast colonies treated with hydrogen peroxide on YM agar revealed that some colonies with the red color hue in the present of DPA as a carotenoid inhibitor. The obtained colonies and mutants had higher production of pigment and astaxanthin as main carotenoid evaluated by spectrophotometric, TLC and HPLC determination.

Keyword: Astaxanthin, *Xanthophyllomyces dendrorhous,* Carotenoid inhibitor, Oxidative agent.

Hyper-tolerated Yeast Mutant of *Xanthophyllomyces dendrorhous* against Hydrogen Peroxide via Evolutionary Engineering

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Abstract

Carotenoids are the second most abundant pigments in nature. Since synthetic carotenoids have deteriorating effects, there is a great demand for natural carotenoids. One of the most important microbial sources for astaxanthin is the yeast *Xanthophyllomyces dendrorhous.* The important role of astaxanthin in various industries highlights the essential need for further studies in this regard. However, the amount of yield is low and it is necessary for strain improvement (Zhuang, 2021). In this study an evolutionary engineering approach was conducted for improving the yeast strain. Firstly, the O_6-methyl-guanine (MNNG) as mutagen agent was applied to obtain a population of yeasts for further selection via an oxidative pressure. The culture media of yeast mold (YM) broth were prepared with to diphenylamine as a carotenoid inhibitor and hydrogen peroxide in proper concentration. The selection was then carried out based on the amount of the color of the pigmented colonies. This oxidative stress and carotenoid synthetize inhibition repeated through a design evolutionary engineering process. The spectrophotometry, thin layer chromatography and high-performance liquid chromatography were finally used for data evaluations. The screening of pigmented colonies showed that the mutation procedure had been efficient enough to give a mix population for further selection of higher astaxanthin producing strains. The carotenoid inhibitor agent also was properly selected the colonies with higher amount of pigments. The quantity and quality of the selected colonies showed that the mutant MuL has higher carotenoid production against hydrogen peroxide as oxidative pressure.

Keyword: Evolutionary Engineering, *Xanthophyllomyces dendrorhous*, Astaxanthin, Hydrogen Peroxide.

Optimization of corn starch enzymatic hydrolysis process using alpha-amylase and glucoamylase enzymes

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Abstract

Corn syrup (dextrose syrup) is a natural and liquid sweetener produced by the hydrolysis of corn starch. This syrup has less sweetness than sugar and has a high viscosity. Interest in consuming this sweetener has increased in various industries, due to the limited cultivation of sugarcane and sugar beet in the world, high fluctuations in sugar prices, as well as its disadvantages. Enzymatic hydrolysis of starch is much more effective than acidic hydrolysis, because both the product is produced with better quality and there is no need to remove the unwanted salts (resulting from acid neutralization in acidic hydrolysis). In addition, the enzymatic hydrolysis process is performed over a wider range of pH and at lower temperatures than the acidic hydrolysis method. Therefore, it is economically viable and has a higher efficiency. The rate of starch hydrolysis is affected by several factors such as temperature, pH, mixing intensity, process time, enzyme content, viscosity and specific additives. In this study, the aim was to optimize the enzymatic hydrolysis process of corn starch to dextrose syrup. Therefore, by designing the experiment by Taguchi method (L9), the hydrolysis activity of alpha-amylase and glucoamylase was investigated separately and finally by designing the response surface methodology (RSM), the simultaneous activity of two enzymes was optimized. Based on the results, using alpha-amylase and glucoamylase enzymes in the amount of 0.3 and 0.5µl of enzymes solution per gram of starch, respectively, at a temperature of 60 °C, pH = 4.5 and a concentration of 25% w/v of starch, more than 97% of starch is converted to glucose.

Keyword: Glucoamylase, Corn syrup, Hydrolysis.

Molecular Detection of Biomarkers in *Hypervirulent Klebsiella Pneumoniae* Isolated from clinical Cases in Tehran, Iran

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Abstract

As the multi-drug resistant (MDR) *and hypervirulent pathotypes of Klebsiella pneumoniae* (HvKp) cause the majority of community-acquired and life-threatening infections, hence in this study we aimed to investigate the prevalence of the hypervirulent and MDR pathotypes of *K. pneumoniae* among our enrolled clinical samples. In this investigation which was achieved from 2017 to 2018, 100 strains of K. pneumoniae were isolated from clinical specimens in Tehran, Iran. Different standard phenotypic tests including biochemical and microbiological, antibiotic susceptibly assay, genotypic tests including PCR related virulence genes in isolated *K. pneumoniae* pathotypes. *HvKp* was defined by the presence of some combination of *hly*, *iutA*, *macA*, and *oxA51*, genes shown to accurately identify hvKp. Out of 100 strains of *K. pneumonia*, 49 to gentamicin, 42 to tetracycline, 37 to amikacin, , 19 to ciprofloxacin, 15 to meropenem, 13 to clositin, and 12 equally to cefotaxime and imipenem. Besides, all of the isolated strains were armed by the virulence genes of *iutA* and *macA*. The PCR results showed the presence of *hly* and *oxA51* genes in 16 isolates (16%) and 12 isolates (12%), respectively. Eighty-one percent of these isolates were MDR. A significant number of isolated pathotypes were highly resistant against the used antibiotics and a high number of isolated pathotypes were armed by virulence genes. A close relationship between the virulent phenotypes and resistance genes was recognized. Our finding highlight a combination of resistant and virulent phenotypes could lead to a crisis for treatment of infections caused by *K. pneumoniae.*

Keyword: *Klebsiella pneumoniae*, hypervirulence genes, antibiotic resistance, PCR .

Identification of core defense transcriptional responses against various pathogens using meta-analysis

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Abstract

Plants defend themselves using multiple defense mechanisms to prevent biotic stresses. Despite significant advances in understanding complex plant-pathogen interaction, further elucidation is required to identify intricate molecules, signaling pathways and strategies induced by host plants against infectious agents. New meta-genomic techniques, nevertheless, have proven useful in providing an overall image of plant-pathogen interactions. In this study, techniques of meta-analysis and systems-biology analysis were employed to search for general molecular plant defense responses among different transcriptomic data reported from different pathogen attacks in Arabidopsis thaliana. Data from eight studies were subjected to meta-analysis and revealed a total of 3694 differentially expressed genes (DEGs) in a comparative manner whereby healthy and infected plants were considered. Using network analysis, we highlight the importance of WRKY40, WRKY46 and STZ and in suggesting that they serve as major points in protein-protein interactions. This is especially true regarding networks of composite-metabolic responses by pathogens. In summary, this research provides a new approach that illuminates how different mechanisms of transcriptome responses can be activated in plants under biotic stress condition.

Keyword: Plant-pathogen interaction, Transcriptomic responses, RNA-seq, Systems-biology.

Identification of core defense transcriptional responses against various pathogens using meta-analysis

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Abstract

Plants defend themselves using multiple defense mechanisms to prevent biotic stresses. Despite significant advances in understanding complex plant-pathogen interaction, further elucidation is required to identify intricate molecules, signaling pathways and strategies induced by host plants against infectious agents. New meta-genomic techniques, nevertheless, have proven useful in providing an overall image of plant-pathogen interactions. In this study, techniques of meta-analysis and systems-biology analysis were employed to search for general molecular plant defense responses among different transcriptomic data reported from different pathogen attacks in Arabidopsis thaliana. Data from eight studies were subjected to meta-analysis and revealed a total of 3694 differentially expressed genes (DEGs) in a comparative manner whereby healthy and infected plants were considered. Using network analysis, we highlight the importance of WRKY40, WRKY46 and STZ and in suggesting that they serve as major points in protein-protein interactions. This is especially true regarding networks of composite-metabolic responses by pathogens. In summary, this research provides a new approach that illuminates how different mechanisms of transcriptome responses can be activated in plants under biotic stress condition.

Keyword: Plant-pathogen interaction, Transcriptomic responses, RNA-seq, Systems-biology.

Effect of influential and controlling factors in the process of activated sludge settling of wastewater treatment plants

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Abstract

Due to the lack of water resources in the country and increased demand for water reserves, water treatment and reuse of water resources have become a significant concern for human societies. Exercises to treat wastewater have led to the development of treatment methods and the progress of this field. Treatment methods are classified into three categories: physical, chemical, and biological. The activated sludge process is one of the widely used biological technologies for wastewater treatment. In this process, the growth and proliferation of microorganisms lead to the elimination of pollutants to meet the industrial effluent standards. The successful operation of the activated sludge process mainly relies on efficient biological conversions in bioreactors and normal sludge separation in secondary clarifiers. In the activated sludge process, the main problems include the lack of proper sedimentation of the sludge, the bulking and swelling of the sludge in the secondary clarifier. One of the main reasons for the swelling sludge phenomenon is the proliferation of filamentous bacteria. Common methods to prevent this phenomenon include ozonation, chlorination, and control of operating parameters such as dissolved oxygen, pH, and temperature. This study aims to investigate this phenomenon and the factors affecting it with the approach of reducing adverse effects on biological processes and increasing sludge retention time (SRT).

Keyword: Activated sludge, settling, sludge bulking, filamentous sludge bulking, secondary clarifier.

Extraction of pine nut extract and evaluating the antioxidant activity of it

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Abstract

Today, the use of natural antioxidants around the world has attracted a lot of attention. These compounds can be obtained from fruits, vegetables, spices, edible seeds, etc. Natural plant antioxidants are mainly polyphenols (phenolic acids, flavonoids, anthocyanins and lignans), carotenoids (xanthophylls and carotenes) and vitamins (vitamins E and C) have a wide range of biological effects such as anti-inflammatory, anti-bacterial, anti-viral, anti-aging and anti-cancer properties. In this study, pine seed extract was extracted by two solvents of water and ethanol in different ratios (zero, 93.75%, 70.31%, 46.87%, 23.43% ethanol) and the antioxidant properties of the extracts. The result was examined. The results showed that pine nuts had high antioxidant properties and maximum antioxidant activity were obtained using a solvent ratio of 70.31% ethanol and 29.69% water. Due to its high antimicrobial and antioxidant properties, pine nuts can be widely used in the food industry, including the production of functional food products.

Keyword: pine nut, antioxidants, free radical.

Kinetic of browning reaction in low-lactose milk and the influencing factors

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Abstract

The maillard reaction is a non-enzymatic browning reaction between reducing sugars and amino acids and is usually performed in heat-processed foods. This reaction plays a prominent role in the non-enzymatic browning of food and can lead to a decrease in nutritional value, product quality and also a shelf life, although some of maillard compounds have antimicrobial and antioxidant properties. Low lactose milk is one of the products sensitive to maillard reaction and the resulting quality loss because to produce this product, lactose sugar is hydrolyzed to glucose and galactose. These two monosaccharides are more reactive to maillard reaction than lactose. This increases the likelihood of browning of low-lactose products and reduces its marketability, so increasing awareness of the mechanism of maillard reaction and controlling it to maintain quality and increase the shelf life of food is of particular importance. This study provides an overview of the kinetics of browning in low lactose milk and the factors affecting it.

Keyword: Browning reaction, Melanoidin, Maillard reaction, Low lactose milk.

The study of ability of two species of *Amaranthus retrofelxuse* , *Closeia argenta* for absorbtion Nickel and cadmium of Leachate Landfill

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Abstract

Phytoremediation is an effective , economical and biocompatiable method for remediation of can taminated soils .In the study , in order to evaluate the extent of environmental contamination of heavy metals,at first the cahrizak landfill , Located in south of Tehran ,was selected in order to determine the Level of Polution caused by heavy metals ,the extent of their translocation from the roots aerial parts (shoots) in the flora of Arad koh rejoin and Identify the indigenous plant species capable of absorbing heavy metals.Cadmium (cd) and (Ni) , classified as Heavy metals ,enter the soil from various sourse ,especially with the application of phosphate fertilizers contaminig high amounts of cadmium ,the used of industrial wastewaters in irrigation and Landfill causing environmental pollution. The aim of the study of Heavy metal concentration in cluding obsorbed Nickel and cadmium in the root and shoots of two species of plants. The yield of 2 species of plants *Amaranthus retrofelxuse, Closiea argenta* in a factorial ,completely randomized desion with four replication in 5 weeks irrigation landfill leachate in the amount of (0,50,100,150,200) with was studied. After the end of the perioid growth of plants ,like height , number of Leaves,Length and with of Leavs ,wegith of shoots dry and wet and measurement of root ,and at the most ,measurement of the amount of cadmium and nickel in the grinded species by the atomic absorbtion device. In the end , amount of absorbed cadmium and Nickel available existing in shoots and the roots plants *Amaranthus* was measured and cadmium and Nickel absorbed in the soil and the ability of phy-

toremidiation studied.

Keyword: ability of plant, Phytoextraction , Phytoremediation, Phytostabilization, Leachate Landfill.

Viability assessment of HT-29 cells after treatment with *Streptomyces* extracts from Persian Gulf

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Abstract

Colon cancer is the fifth most common malignancy among Iranian men and third among Iranian women, respectively. Although various chemotherapeutic regimens are available for colon cancer patients, unfavorable side effects and acquired resistance limit their application. To investigate new approaches for colon cancer treatment, we evaluated anticancer potential of *Streptomyces* extracted from Persian Gulf *in vitro*. After isolation and antibacterial screening of *Streptomyces* extracts, two strains were selected (AC7 and AC24). For *in vitro* toxicity assay, HT-29 cells, as well as normal fibroblasts, were treated with 60, 120 and 240 µg/ml AC7 and AC24 extracts for 24 h. Then, viability was evaluated by alamarBlue assay and morphological alterations were recorded. Results revealed that viability of HT-29 cells and fibroblasts dramatically decreased upon treatment with both extracts, while toxicity of AC7 extract was more than AC24 extract. Based on obtained findings, AC7 and AC24 extracts induced their effects in a cell type- and dose-dependent manner. In conclusion, our findings indicated cytotoxicity of AC7 and AC24 strains from Persian Gulf for the first time, although more research is required to determine their mechanism of action.

Keyword: *Streptomyces* extracts, Colon cancer cells, *in vitro* viability assay, Persian Gulf.

Comparing cytotoxicity of *Streptomyces* extracts on the viability of LoVo cells in normoxic and hypoxic conditions

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Abstract

Streptomycetes are gram-positive prokaryotic organisms that are known for production of many bioactive secondary metabolites like antibiotics and anticancer agents. Colon cancer is among the top 5 most common cancers worldwide, with high mortality rate. Hypoxia is a common phenomenon in most solid tumors that negatively affect clinical outcomes during the treatment of cancer. In the current study, we compared cytotoxicity of *Streptomyces* extracts on the viability of human colon cancer cells in normoxic and hypoxic conditions. To do so, *Streptomyces* were isolated from water samples and based on the production of secondary metabolites and antibacterial activities, two strains (AC7 and AC24) were selected. Then, submerged fermentation was applied to produce cytotoxic agents by AC7 and AC24. Afterwards, LoVo cells were treated with 60, 120 and 240 µg/ml AC7 and AC24 extracts in normoxic and hypoxic conditions and their viability was evaluated by resazurin assay after 24 h. Results indicated that both extracts reduced cell viability in normoxic condition in a dose-dependent manner. Treatment of cells with AC7 and AC24 extracts in hypoxic condition also decreased viability, and worth to note, toxicity of AC7 extract was more than AC24 extract. To sum up, current findings revealed cytotoxicity of AC7 and AC24 extracts on colon cancer cells, and future research on other cell lines would expand our knowledge regarding toxicity potential of *Streptomyces* extracts.

Keyword: Colon cancer, *Streptomyces* extracts, Hypoxia, Cytotoxicity.

Optimization of biodecolorization of Acid Red 88 dye

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Abstract

Azo dyes are the largest group of dyes used in the textile industry. Azo dyes are one of the main constituents of colored wastewater due to their many applications in the textile industry. However, some azo dyes or their decomposing compounds are toxic, carcinogenic, and mutagenic. Due to their toxicity, these dyes decompose slowly when they enter the environment and cause irreparable damage to the environment. Therefore, the removal of color contaminants, especially from textile effluents, is very important. Various microorganisms, including bacteria, have been used to microbial degradation of these dyes. In this study, a bacterial strain called strain N was isolated and selected from Kashan textile effluent. This gram-positive strain had a very high ability to decolorize Acid Red 88. Carbon sources such as fructose, xylose, lactose, and sucrose effectively optimize the decolorization conditions of Acid Red 88 dye. The results of UV-Vis analysis showed that the decolorization was due to microbial decomposition. Strain N decolorized Acid Red 88 (100%) after 120 h at 50 ppm of xylose carbon source.

Keyword: Decolorization, Acid Red 88, Optimization, Textile effluent.

Extracellular biosynthesis of silver nanoparticles using by Actinomycetes isolated from of tomato root

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Abstract

In recent years nanotechnology has been emerging as a rapidly growing field with numerous applications in science. Biosynthesis of metal nanoparticles is an exciting recent addition to the large repertoire of nanoparticles synthesis methods and now, nanoparticle have entered a commercial exploration period. In this study we report the extracellular green synthesis of silver nanoparticles (AgNPs) by using a *Streptomyces* sp (isolate SM88). Absorption UV-visible light spectroscopy is applied to follow up with the reaction process. Our measurements indicate that extracellular synthesis of AgNPs by SM88,the utilization of silver as a disinfesting agent is not new, and silver compounds were shown to be effective against both aerobic and anaerobic bacteria by precipitating bacterial cellular proteins and by blocking the microbial respiratory chain system.

Keyword: AgNPs, *Streptomyces*, Nanotechnology, biosynthesis.

Evaluation of secondary and tertiary structure stability of laccase in the presence of urea and mannitol

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Abstract

Laccases, the copper-containing oxidoreductases, have received much attention of researchers during the last decades due to their capability to oxidize both phenolic and non-phenolic compounds. This makes these biocatalysts very useful for their application in several biotechnological processes such as bio-fuel, bio-sensor, fiber board synthesis, bioremediation, textile industry, food, cosmetics, and many more. However, application of the enzyme in various industries and chemical processes has been limited by the lack of enzyme stability in the urea-rich system. It is generally believed that urea promotes protein unfolding in an indirect manner by altering water structure and dynamics. Today osmolytes are widely used to modulate the stability of native or folded conformations of proteins and nucleic acids. These molecules stabilize proteins, not interacting with them directly but altering the properties of the surrounding water and hence protein-water interactions. Among these, mannitol, a 6-carbon polyol, is the most prevalent molecule used by nature to protect organisms against the stresses of high osmotic pressure and freezing. It has also been found to be an effective stabilizer of proteins against heat- and chemical-induced denaturation (i.e. urea and guanidine-HCl). Circular dichroism was used to investigate the effects of mannitol and urea on secondary structure of laccase. The obtained result from fluorescence spectroscopy was also applied to evaluate the tertiary structure of the enzyme in the presence of urea and the osmolyte. In order to estimate catalytic activity and velocity of the enzyme in solutions containing urea and the osmolyte, kinetic studies were performed and the results were compared with the native enzyme. Laccase showed a slight decrease in α-helical content after exposure to urea, while the presence of mannitol conserved the secondary structural of the enzyme in the solution containing urea. Increase in the fluorescence intensity of laccase after ex-

posure to urea can be attributed to the unfolding of laccase molecules. The presence of mannitol in the environment preserved the natural structure of the enzyme molecule and thus reduced the fluorescence intensity spectrum. The urea also reduced the maximal velocity of laccase from 398.2 to 76.5 μmol mg^{-1} min^{-1}, whereas the maximal enzyme velocity rate was calculated 150.9 μmol mg^{-1} min^{-1} in the presence of mannitol. Therefore, mannitol seems to be a promising stabilizer for laccase in urea-rich systems such as cosmetic formulations and industrial wastewaters.

Keyword: Laccase, Mannitol, Urea**,** Protein stability.

Evaluation of the function of uricase stabilized on the graphene oxide surface

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Abstract

Enzymes are used as biocatalysts for analytical purposes in diagnostics and preparative purposes in large-scale industrial processes. Despite perfect catalytic properties of enzymes, their industrial applications are limited due to the drawbacks regarding the lack of long-term stability under process conditions. The most important mode of enzyme inactivation is thermal inactivation. Enzyme immobilization, as a novel approach, can improve the half-life, stability, catalytic activity, and reusability of enzymes. Graphene-based nanomaterials have gained high research interest in different fields related to proteins and thus are rapidly becoming the most widely investigated carbon-based materials. Their exceptional physiochemical properties such as electrical, optical, thermal and mechanical strength enable graphene to render graphene-based nanostructured materials, i.e., graphene oxide (GO) suitable for applications in different fields such as electroanalytical chemistry, electrochemical sensors and immobilization of biomolecules and enzymes. Uricase (UOX) was stabilized at high temperatures with immobilization on GO surface. The thermodynamic parameters and optimum temperature of free and immobilized enzymes were examined in this study. Also, an attempt was made to investigate the effect of GO on the adsorption and conformation of uricase using molecular dynamics (MD) simulations. In comparison with free enzyme, the immobilized enzyme displayed an improved stability at high temperatures and, therefore, the immobilized enzyme is suitable for use in the industry because most reactions in the industry happen at high

temperatures.

Keyword: Uricase, Graphene oxide, Thermal stabilization, Molecular dynamics.

Extraction and purification of pharmaceutical docosahexaenoic acid from *Aurantiochytrium* omega-3 oil

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Abstract

Omega-3 oils contain significant amounts of docosahexaenoic acid (DHA). Docosahexaenoic acid is an unsaturated fatty acid with several double bonds. This fatty acid is essential for the health of the nervous system, cardiovascular system and fetal brain development. DHA is now produced by the microbial strains of auranthocytrium. In the above study, after production of omega-3 oil *Aurantiochytrium*, omega-3 oil was extracted by Bligh & Dyer method. Purification of docosahexaenoic fatty acid was performed using refrigeration and urea complexation method. Finally, docosahexaenoic fatty acid was analyzed by gas chromatography (GC) and obtained as purity of 66.1%.

Keyword: Docosahexanoic acid, *Aurantiocytrium*, Extraction, Purification, Urea complex.

Evaluation of the effect of inositol on the stability of recombinant urate oxidase enzyme

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Abstract

Osmolytes are known to affect protein stability by its effect on the structure of water, not by direct interaction with the protein groups. Osmolytes include polyhydric alcohols, sugars, polyols, amino acids and methylamines. In this work, the effect of inositol (cyclitols) was investigated on the structural stability of the urate oxidase (UOX) enzyme. Initially, response surface methodology (RSM) was applied to optimize different process variables for the stabilization of UOX using inositol. The stabilization of UOX with inositol was examined under various experimental conditions, including inositol concentration, pH, temperature, and incubation time. The effect of the processing parameters was tested using the RSM method and a central composite design (CCD) model. According to the ANOVA results, there was a close correlation between the predicted and experimental values of the response parameter. The optimum values of inositol concentration, pH, temperature, and incubation time were found to be 21 mM, 8.5, 27.5 °C, and 5 min, respectively, for achieving the maximum activity. Then, the kinetic and thermodynamic parameters of UOX in the absence and presence of inositol were examined. The UOX half-life at 40 °C was 91.20 min, while, in the presence of inositol, the enzyme had a longer half-life (130.78 min) at this temperature.

Keyword: Urate oxidase, Inositol, Response surface methodology.

Removal of heavy metals by novel bacterial strain isolated from Choghart iron mine

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Abstract

The activities of humans to improve their lives will cause changes in societies and industries. Water is one of the most important natural resources in the world. Water pollution with heavy metals threatens human health and can cause various diseases. Chemical removal of heavy metals is expensive and time consuming and pollutes the environment. Today, bioabsorption is one of the solutions that has received much attention. Removal by microorganisms is cheap and environmentally friendly. This study aimed to investigate the removal of heavy metals by bacterial isolated from the Choghart iron mine in Yazd. The selected bacterial strain belonged to *Bacillus genus*. They removed 100% of heavy metals at 25 ° C and 150 rpm after 24 h.

Keyword: Water pollution, Heavy metals, Bacteria, Bioabsorption.

Isolating and Studying of Indigenous Oil degrading Bacterial Isolate with almost Potential from Oil Refinery Soil

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AbstractThe aim of this research is isolating and studying oil hydrocarbons degrading bacteria from oil polluted soil for soil bioremediation. Soil samples consisting of indigenous oil biodegrading bacteria were collected from Tehran Oil Refinery. The streak culture is conducted in simple culture media such as nutrient agar and specified culture which is also called R2A for increasing the growth of biodegrading bacteria. Among 8 isolates 4 isolates showed beta hemolysis. All isolates were inoculated on blood agar medium but isolates which produce biosurfactant with beta hemolysis were purified. Then, biodegradation indigenous microorganisms (IMO) were placed in to Broth culture and one percent crude oil as sole source of carbon in order to investigate the extent of bacteria biodegradation. Biodegradation of crude oil by s-1c isolate was measured by spectrophotometer in 420 & 600 nm. The isolate with 71% of biodegradation were selected.

Keyword: oil hydrocarbons, biodegrading bacteria, biodegrading, biosurfactant, spectrophotometer.

Immobilization of enzymes on metal-based supports

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Abstract

Enzymes are vital bio-macromolecules that catalyze biochemical reactions in all living organisms . They are, as biocatalysts, efficiently applied in a wide range of biological and chemical processes. By reducing the chemical reaction procedures, enzymes accelerate biochemical processes and make them more environmental-friendly. Despite the important role and high impact of enzymes in biological and chemical reactions, their low resistance to environmental stresses leads to decrease of their activity and stability. Enzyme immobilization is one of the most effective technologies for enhancing enzyme activity, stability, and reusability. To this date, different supports for enzyme immobilization are recognized and discovered that can be classified into two major groups including organic and inorganic materials. Immobilization of enzyme onto inorganic material increases their feasibility for wide ranges of applications due to enhancement of enzymatic activity and stability. Advantages of protein immobilization onto inorganic supports are stabilization of the immobilized enzyme, presence of functional groups, and high potential for creating useful chemical groups on the surface of the applied materials. Physical properties of inorganic supports such as particle size, pore size, and the active surface area directly affect the enzyme activity Among inorganic supports, metals are attracted much attentions attributable to their unique properties such as their high electrical conductivity, low toxicity, good physicochemical and magnetic properties, and facile fabrication. Metal oxides such as aluminum oxide, cerium oxide, iron oxide, and titanium oxide have been utilized as enzyme carriers. Alumina, so-called aluminum oxide, has been used for immobilization of laccase, lipase and many other enzymes due to its porous and crystalline structure . Cerium oxide possesses high isoelectric point that is suitable for immobilization of enzymes (e.g. diamine oxidase) with low isoelectric point. Iron oxide with excellent magnetic features commonly utilized in combination

with other organic and inorganic materials to synthesized various types of enzyme carriers. The magnetic property of iron oxide allows the immobilized enzyme to be easily removed from the reaction mixture and reused for further reactions. Titanium oxide enhances the stability of immobilized enzyme because to its abundant hydroxyl functional groups. Moreover, the mentioned support is able to protect immobilized enzyme by photomasking ultraviolet light. Novel metal-organic structures such as hybrid nanoflowers (HNFs) and metal organic frameworks (MOFs) have attracted great attentions owing to their high stability and wide surface area. MOFs comprise of metal ions and organic linkers leading to controllable porous and crystalline structures. HNFs composed of enzymes as organic components and metal ions as inorganic agents, enhance enzyme activity and stability toward a wide range of temperatures and pH values. Copper and cobalt HNFs have been utilized for immobilization of hydrogen peroxidase and nitrile hydrolase, respectively. Immobilization of peroxidase on Zn-MOF with porous and crystalline structure for removal phenolic compounds, has led to tremendous increase in the activity compared to the free enzyme. Gold nanoparticles are widely used for enzyme immobilization in preparation of biosensors due to their high electron transfer ability, low toxicity, and good biocompatibility.The purpose of this study is to investigate the properties of metal-based supports for enzyme immobilization. Also, the recently-discovered metal-based supports for immobilization uses are introduced. Metal-based materials have been utilized for enzyme immobilization for many years. The possibility to fabricate metal-based supports with different sizes and effective functional groups, make them remarkable for enzyme immobilization. Enzymes immobilized onto metal-based supports exhibits significant stability toward physical and chemical stresses. These supports by providing high surface area increase both enzyme loading and suitable interaction of biocatalysts with substrates. The use of magnetic metal particles in combination with other materials has been extensively utilized caused by their feasible separation from reaction medium. Metal nanoparticles have important role in design of biosensors owing to their high electron conductivity. Through their outstanding features, metal-based supports are considered suitable and cost-effective materials for enzyme immobilization.

Keyword: Enzyme, Immobilization, Support, Metals.

Investigating biogas production from co-digestion of protein/carbohydrate contents of food waste

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Abstract

In recent decades, following the increase in population growth and the expansion of urban lifestyles, the consumption of fossil fuels and the production of waste has been on the rise, and the management of these resources has been identified as a vital challenge for developing countries. Today, the use of renewable energy, especially biofuels, has not only reduced the speed of use of fossil resources, but also introduced a suitable and environmentally friendly alternative by managing waste and converting it into biofuels. Anaerobic digestion is a process in which various microorganisms produce biogas as a final product of the process using complex biological pathways. Since the metabolic pathways of this process have been studied for many years, now finding the optimal conditions according to the nature of the substrate used and studying the symbiotic relationships between microorganisms is the main goal of research in this field. In this study, the potential of biogas production from plant and animal wastes was studied with focus on the co-digestion with sewage sludge in different inoculum to substrate ratio (ISR) and in mesophilic batch reactors during 60 days. Among different states, ISR2 with a mixture ratio of 70/30 (carbohydrate/protein contents) with the production of 12552 ml biogas and 9423 ml methane was determined as the optimal condition.

Keyword: anaerobic digestion, biogas, bio-methane, organic waste, inoculum substrate ratio.

Isolation, Screening and potential probiotic characterization of isolated lactic acid bacteria

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Abstract

Probiotics are living microorganisms that provide beneficial properties to the host. Due to their health properties, they are widely used in the pharmaceutical and food industries. Lactic acid bacteria (LABs) are the largest group of probiotics. These bacteria must tolerate to harsh gastrointestinal conditions, until they reach the target point, and then they can offer their physiological activities and beneficial capabilities. The aim of this study was to identify and isolated native strains with probiotic properties and investigation of their probiotic properties to evaluate the feasibility of their use in the production of probiotic products. Probiotic characteristics of isolated bacteria were determined based on acid tolerance, bile tolerance, and tolerance to digestive enzymes pepsin and trypsin tests. Of the 180 isolates studied, 23 microorganisms were resistant to bile, 5 isolates were resistant to acid, pepsin and trypsin. The results of 16S rRNA analysis showed that all five resistant isolates belonged to different *Lactobacillus* species. The lactic acid bacteria isolated in this study had probiotic properties.

Keyword: Probiotics, microorganisms, Lactic acid bacteria, pharmaceutical industry, *Lactobacillus*.

Increase the production of dietary fiber based on bacterial cellulose with application in food industry by optimizing the culture medium

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Abstract

Microbial cellulose is a homopolysaccharide biopolymer that is synthesized by some microorganisms, including bacteria, algae, etc. This material has unique mechanical and structural properties as well as high purity compared to plant cellulose and due to these properties, it has many applications, especially in medical-related applications. Cellulose has nutritional value and also contains large amounts of fiber and is free of fat and cholesterol. In addition, cellulose is a special food to be added to beverages and desserts, which is obtained under the brand name Nata Di Coco in East Asian countries by fermenting sugars, especially coconut water and coconut milk, by the bacterial strain of komagataeibacter xylinus. To optimize the production of bacterial cellulose, factors such as culture medium, microorganism, physical conditions, type of process and type of bioreactor must be optimized. In Iran, due to the lack of abundance and high price of coconut water and coconut milk, we produced cellulose in an alternative medium containing corn extract syrup (CSL) and glucose syrup by *Komagataeibacter xylinus* strain of the same quality.

Keyword: Bacterial Cellulose, *Komagataeibacter xylinus*, Corn Steep liquor, Dextrose, Optimization.

Production and optimization of pullulan from sugarcane residues by yeast-like fungus *Aureobasidium pullulans*

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Abstract

In this study, production of pullulan from sugarcane bagasse and molasses by *Aureobasidium pullulans* IBRC-M 30351 in batch culture was investigated. The experiments were carried out at three different values of initial pH (5, 6.5 and 8) and two different inoculum sizes (10 and 15% (v/v)). The results showed that the highest pullulan contents were produced at pH of 6.5, in both bagasse and molasses medium. In molasses medium, the highest pullulan concentration of 25.48 g/l was achieved at an inoculum amount of 10% (v/v), while the optimum initial inoculum ratio was found as 15% (v/v) for bagasse medium with the maximum pullulan production of 23.92 g/l. The present peaks in the FTIR spectrum validated that the obtained polysaccharide was composed of pullulan. These observations demonstrate that the sugarcane residues have the potential to be a promising alternative source for efficient pullulan production by *Aureobasidium pullulans.*

Keyword: *Aureobasidium pullulans*, Extracellular polysaccharide, Optimization, Pullulan, Sugarcane residues.

Biosynthesis of manganese nanoparticles mediated by bacteria using the extracellular method

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Abstract

In recent years, nanostructures have received much attention from researchers in the field of science and technological applications and are in continuous development. Nanotechnology due to the multifunctional properties of nanomaterials, has wide applications in different fields such as food industry, space, electronics and optics, wastewater treatment, as well as in the fields of biology and medicine. Among the various nanoparticles, nanomanganese is important because of its abundance, low cost, availability, multiple ion capacities, and various compounds. Besides, manganese nanoparticles have different oxides with various crystal shapes and sizes and so varied applications. Bacterial production of nanoparticles is an environmentally friendly and inexpensive green synthesis method. Biological synthesis of manganese nanoparticles mediated by bacteria has been very limited worldwide, and in this study, we examined the production of manganese nanoparticles by bacteria isolated from the soil of Zagros Mountains. After examining 28 bacterial strains and testing their tolerance and survival in 25 mM manganese salt, 4 bacteria were selected and after testing different production methods, bacterium 13 (B13) could synthesize a blackish brown precipitate in the extracellular method. Characterization tests confirmed that this bacterium has the ability to produce manganese nanoparticles.

Keyword: Biosynthesis, nanoparticles, manganese, bacteria, extracellular method.

Biogas production by using codigestion method of sugarcane bagasse with marine algae

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Abstract

Waste generation is one of the most important environmental challenges due to the increasing energy consumption in the world. Microbial anaerobic digestion is an economic and environmentally friendly methods along with the production of methane and fertilizer. Digestion of suitable substrates with the right mixing ratio removes barriers to digestion alone. The aim of this study was to investigate the effect of codigestion sugarcane bagasse with Caspian seaweed on biogas production. To measure the production of biogas in the digester, different treatments including sugarcane, algae and their mixtures were prepared in two ways: untreated and treated with one molar sodium hydroxide. In each anaerobic digester, a volume of 20 ml of inoculum (cow manure) and two grams of substrate were added and the final volume of digesters was increased to 80 ml with water. The digestion bioreactor was incubated for 30 days at 37 ° C. The volume of biogas produced in the digestion was measured at 5-day intervals. The results of biogas production showed that the highest volume of gas production was in the digester containing untreated sugarcane in the first 5 days with daily value of 22.57% (v / v). The amount of biogas production in the digester containing sugarcane treated with sodium hydroxide with one molar was appropriate so that from day 21 to 25, the amount of gas produced per day was 4.765%(v / v). Also, the results of biogas production in sugarcane and algae codigestion was averaged 3.184% (v / v) per day, which compared to the average daily gas production over a 30-day period, was more than both digesters containing untreated and treated sugarcane. The results of the present study showed that the digestion of sugarcane with algae increases the production of biogas. Also, alkaline pretreatment increased and continued gas production. Increasing the efficiency of biogas produc-

tion by co-digestion method requires further studies.

Keyword: Biogas, Codigestion, Waste, Sugarcane, Algae.

Electricity generation in a microbial fuel cell from marine products industry effluent using *Shewanella* ME1

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Abstract

Microbial fuel cell (MFC) is a promising technology with wide applications of the environment compatible such as wastewater treatment and electricity simultaneous generation by microorganisms. The aim of this study was to investigate the production of electricity by *Shewanella* ME1 from some marine products industry effluent in a two-chamber MFC with mediator iron and mediator less with aeration at the cathode chamber. *Shewanella* was inoculated into LB broth and then 1% of the fresh culture of *Shewanella* was inoculated into the anode chamber containing effluent. The cathode chamber containing the phosphate buffer was continuously aerated 100 $mL.min^{-1}$ but the anode chamber was kept under anaerobic condition. Electricity generation was measured in a microbial fuel cell for 48 hours at a temperature of 30 ° C. The results of electricity measurement in marine products effluent was showed an open circuit voltage, maximum power density , current density respectively mediator less microbial fuel cell 624,6mV, 31,212$mW.m^{-2}$,174,953 $mA.m^{-2}$,and also with mediator microbial fuel cells 345,8mV, 38,464$mW.m^{-2}$,235,54 $mA.m^{-2}$ and was measured marine products effluent removal percentage in two MFC respectively7,31 % .Open circuit voltage, maximum power density ,current density and removal percentage substrate in MFC containing artificial lactose effluent2.226($gr.l^{-1}$)in mediator less MFC respectively 465.8 mV, 33.156$mW.m^{-2}$,180.391 $mA.m^{2-}$and 17% and also with mediator MFC was measured 333mV,36.419$mW.m^{-2}$,188.996$mA.m^{-2}$ and 47%. The results of the current study showed that the highest electricity in marine products effluent with iron mediator and aeration at the cathode.

Keyword: microbial fuel cell, electricity generation, marine products effluent, *Shewanella.*

Polyhydroxyalkanoate production using *vibrio.sp*

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Abstract

Due to the increasing amount of plastic waste and the resulting environmental problems, researchers have long sought to replace oil-based plastics with bioplastics. One of the most important materials is polyhydroxyalkanoate polymer, which is often produced by different bacteria and its suitable properties have made this polymer useful in various fields in addition to the production of bioplastics. In this research, polyhydroxyalkanoate is produced using *Vibrio* bacteria. After the production stage, the polymer is extracted using SDS and chloroform and in the end the production efficiency using this method in *Vibrio* bacteria was about 30%. XRD and FTIR were used to examine the polymer and both methods showed the similarity of the polymer extracted from *Vibrio* in this study and the standard polyhydroxyalkanoate. The antioxidant properties of the polymer were also investigated in this study. Finally, it can be concluded that *Vibrio* bacteria has an acceptable efficiency for the production of polyhydroxyalkanoate, and considering the advantages of this bacteria, it can be used to produce the said polymer more easily and cheaper.

Keyword: Polyhydroxyalkanoate, bioplastic, biopolymer, *Vibrio*, antioxidant.

Isolation and characterization of biosurfactant producing bacteria from the sediments of oil contaminated sites

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Abstract

Biosurfactant is one of the most valuable compound produced by microorganisms with wide applications to enhancing oil recovery and cleaning up oil contaminated sites. The aims of current study were isolation and characterization of biosurfactant producing bacteria from sediments of oil pollutant sites. For isolation of biosurfactant producing bacteria, sediment samples collected from oil pollutant areas. After bacterial enrichment in minimal salt medium containing oil, pure bacterial culture was prepared in nutrient agar. For screening of biosurfactant producing isolates, some specified tests including hemolysis on blood agar, oil emulsification, collapses of oil drop and oil dispersion performed. Out of 17 bacterial isolates, 5 isolates MAK1, MAK8, MAK10, MAK12 and MAK13 with the good ability to produce biosurfactant were selected. All isolates had the ability to β hemolysis of red blood cells and 85-98% oil emulsification. In addition, the results demonstrated that all isolates was able to collapse oil drop and oil dispersion. The results of current this study revealed that the isolates with the good ability to produce biosurfactant may a suitable candidate for study of oil enhancing recovery and clearing up oil-polluted areas.

Keyword: Biosurfactant, bacteria, oil emulsification.

Evaluation of applications of alkaline protease enzyme extracted from extremophilic bacteria of hot springs in biotechnology

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Abstract

Hot springs are considered as one of the habitats of thermophilic microorganisms as a source for direct separation of heat-resistant enzymes. Living microorganisms in hot springs are resistant not only to high temperatures, but also to the pH of the environment and to the presence of certain chemical compounds. Microorganisms have recently been introduced as a rich source for the synthesis and separation of industrial enzymes and are the most important and ideal source for the production of various enzymes with a wide range of applications. About 31% of industrial enzymes today are produced by microorganisms. The advantages of enzymes of microorganisms include the possibility of physiological and physicochemical control, enzyme diversity and high production amounts. Proteases are one of the most important industrial enzymes, accounting for approximately 60% of world enzyme sales. Of these, alkaline proteases are the most widely used in industry. Various microorganisms are able to produce the alkali protease enzyme. Extracellular proteases have many applications in various industries. Alkaline proteases created by Bacillus species are of special importance due to their thermal stability and stability at different pHs. Alkaline pro-

teases are among the most widely used industrial enzymes that have applications. They are widely used in biotechnology, including detergent additives, wastewater treatment, in the food industry, leather making, silver recycling from X-ray film and pharmaceutical films, etc.

Keyword: Hot springs, Extremophilic bacteria, Alkaline protease, Biotechnology.

In Vitro Cell Viability Investigation of Human Dermal Fibroblast Cells under the Effect of Active and Inactive *P. acidilactici* Bacteria

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Abstract

Although the benefits are undeniable, the increased application of ionizing radiation in the medical field results in greater potential risk for the patients. Through decades, a broad spectrum of compounds and their derivatives were tested for protection properties, but an ideal radioprotector is still unmet. Probiotic bacteria were examined as potential radioprotective agents. The aim of this study is to evaluate the safety of different numbers and concentrations of active and inactive probiotic *Pediococcus acidilactici* respectively in cell viability assays for testing *P. acidilactici* radioprotective properties. The experiments were established using the MTT colorimetric assay for assessing normal human dermal fibroblast viability through metabolic activity. After 24hour incubation of the cells with active and inactive *P. acidilactici*, no significant viability changes were observed. Instead, it was found that high number of active *P. acidilactici* and high concentration of heat killed *P. acidilactici* increased cells' growth by 2 and 4 folds respectively. In conclusion, the results suggest that the application of high numbers of active *P. acidilactici* as well as high concentrations of inactive form is considered nontoxic.

Keyword: Probiotics, *Pediococcus Acidilactici*, viability assay, Radioprotection.

Phenol bioremediation in the presence of alginate coated oxygen releasing compounds

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Abstract

Phenol is an important pollutant in the effluent of various industries and due to its high toxicity and solubility, therefore entry of this compound into the body causes skin, digestive, neurological problems. One of the important methods of phenol removal from effluent is biodegradation method using phenolic degrading microbial agents. However, since the biological removal of phenol from groundwater is difficult due to limited oxygen dissolution and lack of proper growth of microbial population in these areas, in this study we tried to isolate phenol-degrading bacteria on the phenolic base environment and also use the sample Activated sludge and compost as microbial sources along with the use of calcium peroxide nanoparticles stabilized in the alginate bed as a source of oxygen release to remove phenols from the contaminated well water flow in column reactors. Also, the results obtained from column reactors showed that columns containing activated sludge and capsules containing calcium peroxide nanoparticles have the highest capacity in removing phenols from contaminated water flow, so that the concentration of phenols in water flow of column containing sludge was decreased from 100 mg/l to zero after 40 days while in the column containing natural micro flora it was reduced to 60 mg/l.

Keyword: Bioremediation, Phenol, Calcium peroxide, Column reactor.

Antifungal activity of the essential oils of *Thymus eriocalyx* and *Thymus daenensis* species on two phytopathogenic fungi

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Abstract

Fungi are an important cause of food spoilage and the production of toxic secondary metabolites called mycotoxins. Side effects of using chemical preservatives and commercial antimicrobial drugs have led to the use of natural compounds, especially essential oils to prevent the growth of fungi and the production of toxin. Essential oils are biocides that also have anti-inflammatory, antioxidant and anti-cancer properties. In this study, the effect of essential oils of two *Thymus eriocalyx* and *Thymus daenensis* species on the growth of two species of fungi *Fusarium oxysporum* and *Aspergillus niger* were investigated. The essential oils of both species were extracted by hydro-distillation method and then analyzed by GC and GC/MS apparatuses. The antifungal effect of essential oils of two plants was investigated by mixing with culture medium method. The results showed that all concentrations used had a significant inhibitory effect on the growth of two species of fungi. The essential oil of *T. eriocalyx* lacked MIC and MFC for *F. oxysporum* fungus and MFC of essential oil of this species was obtained for *A. niger* fungus at concentration of 1600 ppm and the essential oil of this species had no MIC for this species of fungus. MFC of essential oil of *T. daenensis* species for *F. oxysporum* fungus was obtained at concentration of

800 ppm and the essential oil of this species had not MIC for this species of fungus. Also, MIC and MFC of essential oil of this species for *A. niger* fungus were obtained at concentrations of 400 ppm and 800 ppm, respectively. The results of this research is indicative of the inhibitory effect of essential oils of two plants on the growth of two species of fungi, which follows a dose-dependent pattern.

Keyword: *Thymus eriocalyx*, *Thymus daenensis*, Essential oil, Inhibitory activity, Fungicidal activity.

Isolation and evaluation of anti-insect of a scorpion toxin α-NaScTxs peptide from Iranian Mesobuthus eupeus

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Abstract

Scorpion neurotoxins often can be highly specific and targeting one species or groups of species. This allows production of specific insecticides. Scorpion sodium channel toxins (NaTxs) are a family of scorpion toxins that can targeting different insects. Voltage-dependent sodium channels (VGSCs) are large integral membrane proteins that are important for initiating and disseminating action potential in evoked cells. NaTxs exert their effect by altering the activity of VGSC activity. In order to investigate and isolate meuTx17 toxin from Iranian species of *Mesobotus eupeus* scorpion, RNA was extracted from Telson area containing venom glands and cDNA synthesis was performed by RT-PCR. Primers were designed and in the PCR reaction, the toxin coding fragment was amplified with 198 bp sequences. A comparative study of the peptide sequence of meuTx17 toxin in NCBI gene bank showed 90% similarity with Makatoxin-2 and Makatoxin-3 from *Mesobotus martensi* scorpion. MeuTx17 toxin weighs 9279 Daltons, contains 85 amino acids and 4 disulfide bridges. Structural analysis of the peptide showed that this peptide has an alpha helix structure and a beta chain. Due to the similarity of nucleotides and amino acids sequences, this toxin is belonged to the alpha toxins affecting the sodium channel. These toxins can affect the sodium channels of insects and block or slow down the inactivation of sodium channels by binding to site 3 of the VGSCs (voltage-dependent sodium channels), leading to death of insects. The present study shows that meuTx17 toxin can be investigated as an anti-insect toxin in the production of bio-insecticides.

Keyword: Scorpion,Anti insect toxin,Sodium channel,meuTx17.

Investigation the extraction methods of plant and bacterial nanocelluloses with their application in paper industries

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Abstract

nowadays the reduction of fossil fuels leads us to use of renewable materials as an inevitable matter. Cellulose is the most abundant biomass in nature and use it effectively and appropriately as an industrial precursor in industries such as paper and pulp has shown high potential. Cellulose can be obtained from plant and bacterial sources and due to their hierarchical structure extraction of nanocrystals and cellulose nanofibers has been accessible. in This research we investigate various aspects of extracting cellulose nanocrystals and cellulose nanofibers from extensive plant and bacterial sources .finally, the use of plant and bacterial cellulose ability in pulp and paper industries as antimicrobials, fillers, reinforcement, etc. will be studied.

Keywords: Plant cellulose, bacterial cellulose, nanofiber, nanocrystals, paper industry

Identification of core defense transcriptional responses against various pathogens using meta-analysis

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Abstract

Plants defend themselves using multiple defense mechanisms to prevent biotic stresses. Despite significant advances in understanding complex plant-pathogen interaction, further elucidation is required to identify intricate molecules, signaling pathways and strategies induced by host plants against infectious agents. New meta-genomic techniques, nevertheless, have proven useful in providing an overall image of plant-pathogen interactions. In this study, techniques of meta-analysis and systems-biology analysis were employed to search for general molecular plant defense responses among different transcriptomic data reported from different pathogen attacks in Arabidopsis thaliana. Data from eight studies were subjected to meta-analysis and revealed a total of 3694 differentially expressed genes (DEGs) in a comparative manner whereby healthy and infected plants were considered. Using network analysis, we highlight the importance of WRKY40, WRKY46 and STZ and in suggesting that they serve as major points in protein-protein interactions. This is especially true regarding networks of composite-metabolic responses by pathogens. In summary, this research provides a new approach that illuminates how different mechanisms of transcriptome responses can be activated in plants under biotic stress condition.

Keywords: Plant-pathogen interaction, Transcriptomic responses, RNA-seq, Systems-biology

Factors affecting the growth of Lactobacillus plantarum on non-alcoholic malt waste in solid state fermentation

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Abstract

In recent decades, the use of solid state fermentation (SSF) has received much attention due to its ability to produce value-added products using inexpensive by-products of industry and agriculture. However limited researches have been conducted on the cultivation of bacteria, especially probiotic bacteria, on SSF been done. Since our country is one of the largest barley producing countries in the world and its waste volume is very significant, especially in the non-alcoholic malt beverage industry, so in the present study, the waste of non-alcoholic malt beverage factory (Behnoosh Iran factory) as the raw material was used for fermentation and identification of factors affecting the growth of *Lactobacillus plantarum*. In this study, the effective factors on the growth of the probiotic specious *Lactobacillus planetarium* MT.ZH593 in the substrate of waste malt beverage (malt pulp) by SSF using partial factorial screening design with Design Expert software (Version 11.2.1.0) is identified. To conduct this research, seven factors in two levels were evaluated. These factors were included the incubation temperature (35 C°, 40 C°), pH (5.5,7), particle size (Sieve mesh 15,40), moisture content (65%, 75%), amount of inoculation (106 CFU/ml, 108 CFU/ml), tween 80 (1,0), buffer (1,0). This study demonstrated that buffer, moisture content, tween 80 and particle size had the most significant effect on the growth of *Lactobacillus planetarium* MT.ZH5 in the substrate.

Keywords: Growth conditions, Probiotics, wastes of non-alcoholic malt beverage, Solid state fermentation.

Construction of recombinant *Streptomyces rimosus* glucose isomerase protein in *Escherichia coli* BL21 strain

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Abstract

Xylose isomerases (EC 5.3.1.5) are intracellular enzymes that catalyze the reversible isomerization of D-xylose to D-xylulose in the natural environment. They are also called glucose isomerase because of their ability to convert D-glucose to D-fructose in vitro. Glucose isomerase enzyme is a very important enzyme that is used in various industries including food and pharmaceutical industries to produce fructose syrup as a substitute for sucrose and sweetener, and in the alcohol industry in the production of ethanol and in the production of biofuels and biogas. Nowadays, the microbial production of this enzyme with high efficiency has received much attention in the world. In recent years, biological approaches to the use of glucose isomerase enzymes resistant to acidic environments and high temperatures in industrial processes have become important. In the present study, *Streptomyces rimosus* isolate (ATCC 10970) was selected as the bacterium producing glucose isomerase. The expression vector was pET26 b, and finally the recombinant vector entered the *E. coli* BL21 bacterial strain and expressed the glucose isomerase protein.

Keywords:Glucose Isomerase Enzyme, *Streptomyces rimosus,* Cloning, XylA, Expressed vector

Investigation antibacterial properties of copper ferrite and polypyrrole nanoparticles

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Abstract

The purpose of this study is to fabricate and study copper ferrite and polypropylene nanoparticles. In addition, the antibacterial properties of these two nanoparticles were investigated. Copper ferrite was polymerized by co-precipitation method and polypyrrole by chemical oxidation method and their properties were investigated by FTIR and XRD analyzes. The antibacterial properties of nanoparticles against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli were evaluated using disk diffusion methods, which showed the best performance with a 7.3 mm bacterial growth inhibition zone.

Indentification of Taxus endophtic fungi in Arasbaran region with molecular markers

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Abstract

Taxol is a complex diterpene structure that often extracted from the yew plant, which is currently used as the most important natural anti-cancer compound with different mechanism compared to other medicines. Great efforts are currently made for the full and semi-synthesis of Taxol as well as its extraction in cell cultures each of which has specific problems. The identification of Taxol- producing symbiotic fungi has been targeted due to its importance in the treatment of various cancers as well as its low production in plant tissues. Yew plant samples are collected from 10 regions in Arasbaran and surface disinfection is performed using surface sodium hypochlorite and it is cultured on PDA medium. One month later when the fungal colonies are observed, they are purified in PDA medium and are cultured in PDB medium and DNA is extracted. After DNA extraction, the polymerase chain reaction is performed using specific ITS1 and ITS4 primers to identify their species and genus and specific *ts*, *dbat* and bapt genes primers are used to identify Taxol-producing endophytic fungi. Results showed that its fragment is amplified in five isolates out of seven and sequencing of the fragments indicated that the F1 isolate of the yew plant so called *Guignardia philoprina* had a similarity of 98. 83%. In addition, F2 sample isolated from the yew plant (*Platychora ulmi*) had a similarity of 98. 83%. The studies have shown that F3 sample isolated from the yew plant (*Ochrocladosporium elatum*) has a similarity of 97. 04%. F5 sample isolated from the yew plant so called as *Auobsidium pullulans* was 94. 88% similar. Finally, the results indicated that F7 sample isolated from the yew plant is known *Alternaria tenuissima*, Additional studies using *ts*, *dbat*, *bapt* genes indicated that none of the isolates are Taxol- producing ones.

Keywords: Endophytic fungi, Molecular markers, Taxol, Taxus.

Simultaneous Effluent Removal and Electricity Generation by the Use of Microbial Desalination Cell

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Abstract

Improving wastewater treatment processes and desalination of saline waters are the two major strategies to access the fresh water. Microbial desalinations cells (MDCs) have the ability of simultaneous desalination of water, wastewater treatment and energy production. In this technology, biodegradation of organic wastes and the release of electrons cause the electricity production. Desalination occurs due to the ion exchange between desalination chamber and anode/cathode chambers for maintaining the ionic balance. In this study, a microbial desalination cell with synthetic wastewater containing glucose as carbon source and electron supplier in anode chamber, inoculated with anaerobic sludge of a wastewater treatment plant, was used for investigating the desalination of synthetic salt water and electricity generation. The open circuit voltage of 698±10 mV and the closed circuit voltage of 615±13 mV across an external 500 Ω resistor occurred in this treatment. Desalination of salt water was 52±1% in 3d. The initial COD of anolyte was1.5 g/l which reduced 78 2%± at the end of the process. The results of this study showed the synergistic effects of voltage production, desalination and COD removal.

Keywords: Microbial Desalination Cell, Desalination, COD, Electricity generation

Effect of *Lactobacillus plantarum* on genes involved in oocyte maturation in DFO poisoned rats

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Abstract

Introduction: Probiotics are living microorganisms that are widely used, both as medicine and as food supplements, and if used in sufficient quantities and in the right place, they have beneficial effects on human health. The aim of this study was investigating the effect of probiotics on the process of follicular growth in rats fed on heated oil.

Materials and Methods: Twenty adult 8-week-old female rats were randomly divided into 4 groups 5 each: control, DFO treatment, DFO treatment group receiving Lactobacillus and the healthy group received Lactobacillus.

Results: After applying treatments, the expression of BMP15 and GDF9 genes in ovarian tissue was assessed by real-time PCR method. Results were analyzed by ANOVA and Tukey test. The results showed that long-term consumption of DFO in female rat led to impaired expression of genes involved in follicogenesis and oocyte maturation. After for one month treatment of DFO-treated mice with *Lactobacillus planetarum,* the probiotic can reduce the observed detrimental effects of the DFO and expression of BMP15 and GDF9 genes involved in oocyte maturation increased compared to the untreated toxic group.

Conclusion: Consumption of the *Lactobacillus plantarum* can reduce the effects of DFO on the growth and maturation of rat oocyte

Keywords: Probiotics Lactobacillus plantarum, DFO, GDF9, BMP15, Rat

Identification, Investigation and Experimental Study on Factors Affecting Hexavalent Chromium Removal by Chromium Resistant Bacteria Isolated from Paint Factory Effluent

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Abstract

In recent years, the production of effluents containing toxic substances such as heavy metals has increased sharply. Chromium is one of the heavy metals that is highly toxic and pollutes both the terrestrial and aquatic ecosystems and poses many dangers to living organisms. Chromium is available in various oxidation forms. The most common chromium are trivalent chromium and hexavalent chromium, and hexavalent chromium is much more toxic and dangerous. Using biological methods is an economical and environmentally friendly way to treat wastewater containing hexavalent chromium. In this method, biomass or microorganisms can help purify and cleaning wastewater by removing hexavalent chromium or reducing it to a less toxic form, trivalent chromium. Bacteria have been considered for biological operations due to their characteristics such as their abundance and ability to grow under controlled conditions. Different factors affect the growth and function of bacteria. Factors such as temperature, pH, distance, carbon source concentration, initial metal concentration and culture medium. In this study, the effect of these factors on the reduction of hexavalent chromium in wastewater by bacteria isolated from the chromium-containing effluent in different conditions was investigated and the most important and effective factors were identified using Minitab.

Keywords: Bioremoval, hexavalent chromium, bacteria, microorganism

Evaluation of in vitro Antimicrobial, Antidiabetic and Antioxidant Potential of Alyssum homalocarpum and Green Synthesis of the Silver Nanoparticles

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Abstract

In the present work synthesis of silver nanoparticles using Alyssum homalocarpum (Fisch. & C.A.Mey.) Boiss. extract has been considered. The methanolic extract of A. homalocarpum was concentrated and analyzed using Gas Chromatogra. Also silver nanoparticles were synthesized by the bio-reduction of silver nitrate solution (1 mm) using the methanol extract. Scanning electron microscopy (SEM), and Fourier transforms infrared spectroscopy (FT-IR), have been used to determine physicochemical properties of silver nanoparticles. α- Glucosidase inhibition assay, α-amylase inhibition activity, and IC50 test have been performed and the results reported. Folin- Ciocalteu reagent and aluminium chloride colorimetric methods have been used to estimate total phenolic and flavonoid content of the extract. Six bacteria and four fungi were used to measure antimicrobial of extract. 9,12,15-Octadecatrien-1-ol, n-Hexadecanoic acid, 2-Pyrazoline, 2,4-Decadienal, and 9,12- Octadecadienoic acid as most important compounds have been determined. The extract showed strong α- glucosidase inhibitory activity (18.01 µg/mL) and also DPPH radical scavenging (IC50: 64 µg/mL). The maximum antibacterial activity was investigated against Salmonella typhi (30.9 mm).

Keywords: Antibacterial, Enzyme, Nanoparticles, Synthesis

Isolation, screening, and identification of biosurfactant-producing native probiotic strain

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Abstract

Introduction: Probiotics are living microorganisms that provide beneficial effects while colonizing the host. Lactic acid bacteria species and Bifidobacterium are among the best-known probiotics. One of the compounds produced by probiotic bacteria are biosurfactants (BSs). BSs capable to emulsify and decrease surface tension and interfacial tension. Due to their interesting properties such as higher biodegradability, lower toxicity and higher activity at extreme conditions, BSs have become the interests of researchers as promising alternative of a number of synthetic surfactants. In this study, isolation of probiotic bacteria from local dairy products was carried out in order to search for biosurfactant producing bacteria.

Methods: The 70 dairy samples were collected aseptically of the various environments of Iran. The bacteria were isolated by serial dilution method. The bacterial isolates were purified by repeated subculturing on MRS medium at 37°C. Production of BSs of the isolates was done by fermentation in flasks containing MRS broth for 48 h at 37°C. Primary screening was performed by using oil spreading assay in order to find promising producers.

Results: Eighty bacteria were isolated from 70 dairy samples. Results showed 25% of isolates were positive for the oil-spreading assay. Three of isolates showed the highest oil spread in activity whit more than 6 cm clearing zone diameter. Based on the results of probiotic tests and BS production, F20S2 isolate was identified by 16S rRNA method as *Lactobacillus brevis* and introduced as valuable candidate for producing biosurfactant.

Keywords: Biosurfactant; Probiotic; Oil Spreading assay

Isolation and characterization of wastewater bacteriophages on clinical *Enterococcus* spp.

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Abstract

In recent decades, enterococcal resistance to common antimicrobials has greatly increased. Furthermore, these antimicrobials include several side effects for the patients. Alternatively, researchers have investigated novel treatments to solve these problems, including bacteriophages. Therefore, the major purpose of this study was to isolate and identify bacteriophages on clinical multiple-resistant enterococci. Results showed that the three isolated bacteriophages were effective on clinical *Enterococcus faecium* as well as *Streptococcus dysgalactiae* ATCC 27957. In general, the bacteriophages belonged to *Siphoviridae, Myoviridae* and *Inoviridae* families of the *Caudovirales* order. In conclusion, bacteriophages widely invade bacterial hosts and hence can be suggested as viable alternatives to the current antimicrobials. However, further *in vivo* studies are necessary to verify the maximum effectiveness of the enterococcal bacteriophages.

Keywords: *Enterococcus* spp., Bacteriophages, Clinical samples, Wastewaters

Investigation of the effect of chromium on some morphological traits of garden cress in in vitro culture

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Abstract

Chromium is a heavy soil pollutant that has toxic effects on plants, even in very small amounts, and is carcinogenic to humans. In order to investigate the effect of chromium element (0-100-200-300-400-500 mg/l) in MS culture medium on growth and germination of garden cress (percentage and germination rate, root and shoot length, Wet and dry weight of the plant was performed experimentally in a completely randomized design with three replications. After disinfection, seeds were grown in MS medium containing different concentrations of chromium. The results showed that there was no significant difference between the treatments in terms of germination percentage, but increasing the concentration of chromium reduced the germination rate of garden cress so that at a concentration above 200 mg /l this plant could not grow. Stem length was more sensitive to increasing chromium concentration than root while root length showed a significant decrease in chromium-containing medium compared to the control. The fresh and dry weight of the plant in the control medium showed a significant difference compared to the chromium-containing medium, and these traits decreased with increasing chromium concentration. Due to the fact that this plant can grow up to 200 mg per liter of chromium, this plant can be used as a phytoremediation.

Keywords: Watercress, Heavy Metal, Chromium Element, Germination

Antibacterial and antifungal activity of boiling water extract of Walnut green husk

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Abstract

Introduction: Walnut (Juglans regia) is one of the popular nuts largely consumed. Different parts of this valuable plant have applications in the cosmetic, medical, and industries.

Walnut green husk is one of the main agro-waste products generated in the walnut harvest that could be used as a natural source of bioactive compounds and antimicrobial agents.

Purpose: The aim of this study is the evaluation of the anti-microbial activity of boiling water extract from the walnut green husk.

Methods/ Material: Walnut green husk was boiled in distilled water for 20 min. After filtration, the extract was dried. The antimicrobial effects of various concentrations (3.2-100 mg/ml) of the extract were investigated, using the well diffusion method on 6 different standard microorganisms.

Results and Discussion: In Gram-positive strains the highest zone of inhibition of extracts was observed in *S. aureus* and *M. luteus* with 21, 19.5 at 100 mg/ml respectively. In *B. subtilis* there wasn't any inhibition zone. In Gram-negative strains the highest zone of inhibition was observed in *P. aeruginosa* with 15 mm at 100 mg/ml. In E. coli was not observed inhibition zone. The highest antifungal activity was observed in *C. albicans* 19 mm at 100 mg/ml.

In conclusion, our results showed boiling water extract from Walnut green husk can introduce as a natural source of antibacterial agents.

Keywords: Walnut green husk, Anti-bacterial activity, Antifungal activity.

In silico investigation of alpha, beta and gamma carbonic anhydrases as catalysts of CO_2 biomineralization processes

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Abstract:

Global warming is a worldwide concern, being mainly caused by CO_2 emission. Thus, carbon capturing and storage options have been explored to reduce this greenhouse gas. Biomineralization, a process where CO_2 is reacted with mineral ions to produce mineral carbonates, has been identified as a good sequestration method. Carbonic anhydrases (CAs) are suitable catalysts for this reaction. Thermostability is a desirable characteristic for carbon sequestration at industrial sites, where there is mass production of industrial flue gas at high temperatures, containing large concentrations of CO_2. Therefore, the main focus of this study was to identify and assess CA proteins from three different classes via integrated computational approaches for thermostability properties. Proteins from each class were separately subjected to multiple sequence alignments followed by sequence analysis which included phylogenetic tree calculations, motif analysis and the prediction of signal peptides. Multimeric structure calculations were performed for each class in their respective biological assemblies. Protein-protein interface analysis was performed on all three classes, and hotspot residues which contribute the most to interface stability, present in conserved motifs, were identified. Molecular dynamics simulations were performed at temperatures 300 K, 363 K, 393 K and 423 K, and subsequent analyses were undertaken. We argue that our results contribute to the knowledge of functionality of the three classes of CAs investigated as well as to biotechnology applications by revealing potential thermostable CO_2 sequestration agents which could potentially be used in industry (PMID: 33799806 and PMID: 33138066).

Keywords: Carbonic anhydrases, CO_2 sequestration, motif analysis, phylogenetic tree calculations, molecular dynamics.

Two-liquid phase trickling bioreactors for sustainable waste-gas treatment

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Abstract

Many industries have been constantly striving to reduce their emissions of odorous compounds from various plant operations that usually contain a single or a mixture of volatile organic compounds (VOCs). Trickling bioreactors (TBRs) have received much attention for the removal of VOCs from gas-phase streams. In TBRs, the continuous trickling liquid recirculates through the packed bed to feed the immobilized microorganism and to facilitate the control of pressure drop, pH, temperature, and composition of nutrient medium.

However, some of VOCs are categorized as hydrophobic compounds which restrict the pollutant mass transfer to the liquid and biofilm phases. One proposed solution to this problem is to add a non-aqueous phase liquid (NAP) to the nutrient medium in the bioreactor. This dispersed organic phase increases the absorption and the driving force for mass transfer of hydrophobic compounds into the liquid-phase. NAP addition also improves the bioavailability of carbon source and increase the stability of TBR against shock loading or sudden changes in the inlet VOC concentration and acts as a reservoir during starvation or shutdown periods.

During the biodegradation of mixtures of gas-phase pollutants, the influence of one pollutant on the degradation rate of other components, diffusion or kinetic limitations, absorption/adsorption, the shift in the microbial community structure, and providing a medium for the production of some value-added compounds are the other key issues that will be addressed in this presentation.

An introduction to full scale application of aerobic granular sludge process for municipal and industrial wastewater treatment

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Abstract

Aerobic granular sludge-based reactors represent an attractive alternative to conventional activated sludge systems due to their small footprint and their low energy consumption and excess sludge production. The granules developed in such systems have high biomass concentration, good settling properties, high COD removal efficiencies and eventually high phosphorus removal capacity. In addition, and depending on bulk oxygen concentrations and granule size, both nitrification and denitrification can occur in the granules. Therefore, sequencing batch reactors operated with granular sludge have the potential to achieve organic matter and nutrient removal in a single compact system.

In this presentation, design considerations and performance of different full-scale aerobic sludge plants used in urban and industrial wastewater treatment in Iran will be discussed. Aerobic granular sludge process enables extensive treatment in compact and uncomplicated designs. The amount of mechanical equipment is much less than in conventional processes. For example**, primary clarifiers, selector basins, separate anoxic and aerobic compartments, and secondary clarifiers,** return sludge pumping stations or moving decanters are not necessary. Therefore, Operation and maintenance costs are much lower. Energy savings due to reduced mechanical equipment and reduced air requirements are usually up to 50%. Due to the process of complete removal of nutrients in a single reactor, the footprint of aerobic granular sludge treatment plants is reduced by up to 25% compared to traditional plants.

Keywords: Aerobic granular sludge; Full scale; Granulation; Industrial wastewater; Nutrient removal; Sewage treatment

Simulation of Microbial Process using Molecular Dynamics

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Abstract

The molecular dynamics (MD) method is one of the most accurate simulations in bio physics that is used to simulate complex multi-particle systems. The results of simulation show the change in positions and velocities of the system particles versus time. Molecular dynamics simulations are widely used in almost all critical sciences, including microbial processes such as pollution removal and bio production process, medicine, and in targeted drug delivery. The effect of material size on microbial reactivity and bio thermodynamic parameters were considered via MD. Microbial specious capability in the presence of magnetic nanoparticles for removal of pollutions such as nitrate elimination and also, bio production process such as bio surfactant production were simulated via material studio software. Thermodynamic principles and proper equations were used via molecular dynamics simulation. The results of software predictions were demonstrated by radial distribution function (RDF), density, potential energy and temperature graphs. According to the graphs, the simultaneous in the presence of nanoparticle and microorganisms increased the removal efficiency and production rates.

Keywords: Microbial activity; Molecular dynamics; Simulation

Omics

Metabolomics
proteomics
genomics

Isolation and cloning of *Cauliflower Mosaic Virus* coat protein

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Abstract

Cauliflower Mosaic Virus (CaMV) is a member of the *Caulimovirus* genus of the Caulimoviridae family. It is known as the most important virus infecting plants of the Brassicaceae family. The virus genome is a double-stranded DNA with seven open reading frames (ORF) among them ORF IV encodes the viral coat protein (CP). Virus coat protein gene cloning is used for expression in the bacterial system and its recombinant protein production is important in various fields such as immunization, biochemical studies, biotechnology and therapies, and the production of artificial virus particles. In the present study, due to the importance of the virus coat protein gene, this gene was cloned in the bacterial expression vector, pGEX2TK. After DNA extraction from suspected virus-infected samples, PCR was performed with CP-specific primers and a fragment of about 1467 bp was observed. After purification and enzymatic digestion of PCR products and also the plasmid by *Bam*HI and *Sma*I, PCR products were7 ligated to plasmid and transferred to *E. coli* (TOP10) competent cells by electroporation. Recombinant colonies were selected by PCR on colony, and after plasmid extraction they sent for sequencing. The obtained sequences were in accordance with KF357590.1 accession number in the NCBI database. Studies on the expression and purification of this protein are underway.

Keyword: Coat protein, Cloning, *Cauliflower Mosaic Virus, E.Coli*.

Metabolomic Profiling of Resistance and Susceptible Peanut (*Arachis hypogaea* L.) Genotypes in Response to *Cercospora arachidicola* infection

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Abstract

Cercospora arachidicola reasons early leaf spot disease in the peanut plant, which results in excessive yield loss. Metabolomic profiling of *Arachis hypogaea* was performed to identify the pathogen-induced production of metabolites involved in the defense mechanism of peanut plants. In this study, two peanut genotypes, one susceptible (JL-24) and one resistant (GPBD-4) were inoculated with *Puccinia arachidis* fungal pathogen. The metabolic response was assessed on the control stage (0 day-without inoculation), 2 DAI (Day after inoculation), 4 DAI and 6 DAI by Gas Chromatography-Mass Spectrometry (GC-MS). About 75 metabolites were recognized by NIST library, comprising sugars, phenols, fatty acids, carboxylic acids and sugar alcohols. Sugars and fatty acids had been primary in leaf extracts in comparison to other metabolites. Concentration of various metabolites which include salicylic acid, mannitol, flavonoid, 9,12-octadecadienoic acid, linolenic acid and glucopyranoside were higher in resistant genotype than in susceptible genotype for the duration of infection. Systemic acquired resistance (SAR) and hypersensitive reaction (HR) components such as oxalic acid was elevated in resistant genotype during pathogen infection. Partial least square-discriminant analysis (PLS-DA) was applied to GC-MS data for revealing metabolites profile among resistant and susceptible genotype during infection. The phenol content and oxidative enzyme activity i.e. catalase, peroxidase and polyphenol oxidase were found to be very high at 4 DAI in resistant genotype (p-value <0.01). This metabolic approach provides information about bioactive plant metabolites and their application in crop protection and marker-assisted plant breeding.

Keyword: GC-MS; *Arachis hypogaea; Cercospora arachidicola;* Metabolomics, pathogen infection; Phenolics.

Effect of endophytic bacteria volatile metabolites on pathogenic factors of sugarbeet sof rot bacteria

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Abstract

Strains of *Enterobacter* sp. are one of the soft rot-causing bacteria that cause extensive damage to sugar beet roots in the field and post-harvest. Volatile metabolites are low molecular weight and low boiling point compounds that evaporate easily. Recent findings emphasized importance of volatile compounds in microbial interactions. effect of volatile metabolites that produced by four endophytic bacterial strains isolated from sugar beet, *Bacillus* sp.B186, *Bacillus* sp.H274, *Bacillus* sp.Andi542 and *Pseudomonas* sp. B451 (sampled from Khuzestan, Isfahan and Kermanshah provinces) were investigated on population, biofilm formation and pathogenicity of *Enterobacter* sp.Kh2, the soft rot agent of sugar beet (sampled from Khuzestan province). Results of volatile metabolites effects on the bacterial population was shown that *Bacillus* sp. B186, *Bacillus* sp. Andi542 and *Pseudomonas* sp. B451 had significant reduction on the bacterial population of *Enterobacter* sp.Kh2. And also, the Biofilm results were shown that three strains of endophytic bacteria, *Bacillus* sp.H274, *Bacillus* sp. Andi542 and *Pseudomonas* sp. B451 caused a significant reduction in biofilm formation of pathogen. The greatest reduction of biofilm formation was occurred by *Bacillus* sp. Andi542. Evaluation of the volatile metabolites effect of endophytic strains, on the pathogenicity, indicated the decreasing on the pathogenicity of pathogens by effect of all four endophytic strains in sugar beet tissue. *Bacillus* sp. H274, *Bacillus* sp. Andi542 and *Pseudomonas* sp. B451 have reduced pathogenicity by seventy-three percent.

Keyword: Volatile metabolite, Endophytic bacteria, *Enterobacter* sp. Soft rot, Sugar beet.

Identification of volatile metabolites of two endophytic bacteria as the sugar beet soft rots biocontrol agents

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Abstract

Numerous bacteria have been reported as the sugar beet root rot agents. Strains of *Enterobacter* sp. is one of the bacterial soft rot agents that causes a lot of damage to sugar beet roots in the field and after harvest. Endophytic bacteria control pathogens using various mechanisms such as volatile metabolites. Two biocontrol bacteria, *Pseudomonas* sp. B451 and *Bacillus* sp. H274, were reduced pathogenicity of *Enterobacter* Kh2 sp. about 70% by production volatile metabolites. Volatile metabolites of *Pseudomonas* sp. B451 and *Bacillus* sp. H274 was trapped in the presence of pathogen using activated charcoal and isolated using ethyl acetate. Identification of volatile metabolites was performed using GC-MS. 52 organic compounds in the volatile metabolites of *Pseudomonas* sp. B451 and 41 compounds in *Bacillus* sp. H274 was detected. Dodecane and Pentadecane, the major volatile compounds of *Pseudomonas* sp. B451, were identified whose antimicrobial properties have already been proven. The major volatile compounds of *Bacillus* sp. H274 were 2,4-Bis (1,1. dimethylethyl) phenol, p-Ethyltoluene, Hexadecane and Eicosane. And also, some volatile compounds with antibacterial properties such as Ethylbenzene, p-Xylene, Decane, Tridecane and Tetradecane were identified

Keyword: volatile metabolites, *Enterobacter* sp. Kh2, Biocontrol.

Isolation, sequencing and bioinformatics analysis of phenylalanine ammonialyase (ObPAL) gene promoter in basil

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Abstract

Basil (*Ocimum basilicum* L.) is a plant from the mint family that is used as a medicinal plant in traditional Iranian medicine. Essential oil of basil is a rich source of terpenoid and phenylpropanoid compounds and in the treatment of diseases such as diarrhea. Cough, intestinal parasites, and kidney and liver disorders are important. Valuable compounds of volatile phenylpropanoids such as methyl chavicol and methyl eugenol constitute the major volume of basil essential oil, which has recently received much attention due to its medicinal value and it has attracted pharmacological activities, especially as a migraine inhibitor. Phenylalanine ammonialyase (PAL) is the first enzyme and rate limiting of phenylpropanoid metabolism, which is one of the key enzymes in the biosynthesis of methyl chavicol and methyl euginol. The activity of this enzyme is regulated at the transcriptional level and varies widely depending on the stages of cell differentiation and exposure to various types of stress. In the present study, isolation, sequencing and study of functional motifs and location of transcription factors in the pPAL promoter were performed. The results of pPAL gene promoter analysis showed that important regulatory elements responsive to high temperature and drought stress, including MYB, MYC and HSE, were the most abundant in this sequence.

Keyword: PAL gene, promoter, *cis*- regulatory element, phenylpropanoid.

In Silico Investigation of the effect of lycopene on the expression of BRCA1 and BRCA2 inhibitor genes in prostate cancer

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Abstract

Cancer is a genetic disease that results from mutations in genes that control cell activity. Prostate cancer is one of the most common types of cancer found in men. Surgery, radiation therapy, hormone therapy and chemotherapy are used to treat this disease. These treatments have numerous side effects after treatment, including impotence and the high cost of treatment. In this study, lycopene was studied as a carotenoid compound synthesized in plants. Lycopene is used by plants and microorganisms to Absorb of light is made during photosynthesis. Lycopene is one of the effective antioxidants used to prevent the growth of cancerous glands. BRCA1 and BRCA2 proteins are tumor inhibitors. These two proteins are associated with a range of cellular processes such as DNA damage repair and repair, transcriptional regulation, and chromatin regeneration. Defects in BRCA1 and BRCA2 function lead to defects in DNA repair. This instability in the genome is associated with a variety of breast, ovarian, and prostate cancers.

In this project, In Silico method and bioinformatics tools, used to determine the effect of lycopene on the expression of BRCA1 and BRCA2 genes which are effective in prostate cancer inhibitory genes. For this study, gene expression data were obtained from the NCBI database of GEO section. These raw data were extracted using microarray method and published in the NCBI database, so these raw data were used in accordance with the purpose of this study. For Optimal analysis of these data, using the powerful Matlab software, the expression changes of the desired genes treated with lycopene were investigated. Also, for the communication of genes with each other and other effective genes, Cytoscape software has been used.

Bioinformatics study of the effect of lycopene on BRCA1 and BRCA2 genes has

shown that this compound has an increasing effect on the expression of these inhibitory proteins therefore treatment of patients with this compound, the expression of BRCA1 and BRCA2 genes has increased. Based on the analysis of microarray data, it was concluded that the incremental impact of lycopene on the two inhibitory genes BRCA1 and BRCA2 can be used as a preventer and even treatment of prostate cancer.

Keyword: Bioinformatics, gene expression, Lycopene, BRCA1, BRCA2, In Silico, Cytoscape, cancer.

Study of genomic structure and transcriptome of *Protopine 6-hydroxylase* gene isolated from *Chelidonium majus L.*

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Abstract

Benzyl isoquinolines (BIAs) are a large group of nitrogen-containing alkaloids, of which more than 2,500 structures have been identified. *Chelidonium majus* L. is one of the most important medicinal plants. Many benzyl-isoquinoline alkaloids are involved, and many genes are involved in this biosynthetic pathway. The aim of this study was to isolation, identify and study the structure of *Protopine 6-hydroxylase (P6H)* gene involved in the biosynthetic pathway of sanguinarine in *Chelidonium majus* L. In the present study, in order to determine the genetic structure of the gene, genomic DNA was successfully isolated and sequenced. The alignment results between the genomic DNA sequence and the CDS sequence in the NCBI database showed that the two fragments (genomic DNA sequence and CDS) of the P6H gene were 100% identical, indicating the absence of introns in the structure of this gene. In fact, the results indicated that the gene studied in this study contained only one exon. It should be noted that the identification and study of genes without introns can provide researchers with useful information for genomic and evolutionary studies.

Keyword: Isolation, *Chelidonium majus* L., Gene structure, Intronless genes.

NGS as a diagnostic tool in personalized medicine: A case-report of breast invasive ductal carcinoma

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Abstract

The emergence of personalized medicine based on molecular techniques, such as next-generation sequencing, has increased our understanding of drivers of complex diseases, including cancers. In many cases due to the complexity of cancer, it is difficult for human physicians and biologists to make decisions on the basis solely of clinical practice or laboratory evidence. Thus, the personalized medicine approach comes into play and provides large volumes and valuable data for experts. Further, data analysis with bioinformatic tools has opened a new horizon in the process of prognosis and screening of in risk individuals. It has caused significant recent advances in diagnostic technology and improved targeted treatments. In the present study, formalin-fixed paraffin-embedded tissue from an Iranian female patient with invasive breast carcinoma was investigated. In this way, after DNA extraction and purification, the whole exome was sequenced and the mutation data were analyzed. Obtained information could help to the enrichment of the Iranian genome databases. In the light of this research and by studying other Iranian samples, we can provide an optimized roadmap for precision oncologists to increase the life expectancy of breast cancer patients.

Keyword: Next-generation sequencing, Personalized medicine, Bioinformatics, Mutation data, Invasive breast carcinoma.

Genome diversity investigation of Iranian olive cultivars called Mari and Shengeh

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Abstract

Olive (*Olea europaea* subsp. *europaea*) is one of the most important crops of nutritional and economical value. Olive trees are reproduced by sexual crosses that drive the development of new varieties and many variations. The olive gene pool in Iran, as an olive-growing country, constitutes a potentially significant subset of the olive gene pool in the world. In this study, the genomes of Iranian olive cultivars called Mari and Shengeh have been studied. The Mari is one of the most critical Iranian olive cultivars, which contains a very high-quality oil due to more than 75% oleic acid in the composition of the produced oil. In contrast, the Shengeh is not of good quality due to the low ratio of oleic acid to linoleic acid in the oil composition. For studying genetic reasons of these differences, genome sequencing of these cultivars was performed by Illumina HiSeq 4000 2 × 150 method. The trimmed reads were mapped against the reference genome of Farga cultivar (OLEA9). The analysis of Single Nucleotide Polymorphisms in these genomes was shown more diversity (one SNP every 239 bps) in the Shengeh than in the Mari (one SNPs per 254 bps). In both, less than 1% of these varieties have a high-effect impact. In each of these two cultivars, about 0.48% and 0.45% of the diversities are stop-gained and stop-lost SNPs, respectively. The largest class was SNPs in the upstream and downstream region, accumulatively about 54% in each cultivar. In conclusion, the differences between the studied cultivars were shown the high similarity of number, region, and impact of variations in Iranian cultivars with each other in contrast with the European cultivar

Farga. These results were confirmed by comparing the genome of Iranian and Mediterranean olive cultivars by SSR marker, which proved the complete separation.

Keyword: Genome diversity, Olive, SNP.

MICA rs1051792 polymorphism and Cytomegalovirus Infection in Kidney Transplant Recipients

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Abstract

Cytomegalovirus (CMV) is a prevalent herpes virus that affects 40% to 70% of the population. Kidney transplant recipients (KTRs) are at increased risk for CMV infection/disease. The *MICA* gene is situated on chromosome 6 in the Class I Major Histocompatibility Complex gene region. The expression of MICA is triggered in pathological conditions including cellular stress, tumorigenesis or pathogenic infection. MICA acts as a ligand for the natural killer (NK) group 2-member D (NKG2D) receptor. The single nucleotide polymorphism (SNP) rs1051792 result in an exchange of valine with methionine at position 129 in the α2 domain of the MICA protein and divides the MICA alleles into two groups. Isoforms of MICA containing methionine at position 129 bind NKG2D with high affinity, while those with valine bind NKG2D with low affinity. The aim of this study is the assessment of the association between rs1051792 polymorphism susceptibility to CMV infection in KTRs in the northwestern population of Iran. This study involved 51 cytomegalovirus-infected kidney transplant recipients as patients and 50 kidney transplant recipients without cytomegalovirus infection as control subjects. Genotypes have been determined using the RFLP-PCR method and *Rsa*I restricting enzymatic activity. We observed no significant association between rs1051792 polymorphism and susceptibility to CMV infection ($p>0.05$). This study is not able to provide evidence of the association between rs1051792 polymorphism and susceptibility to CMV infection in KTRs in the northwest Iranian population.

Keyword: cytomegalovirus infection, kidney transplant, rs1051792, *MICA* gene, northwestern of Iran.

Comparison of omega-6 content in Persian Echium seed oil with continental and island species

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Abstract

Echium oil is of high-interest research, medicinal and nutritional importance due to its richness in omega 6 and 3 fatty acids. The seeds of these plants contain multiple polyunsaturated fatty acids. The most important of these are the Gamma-linolenic acid (GLA, 18: 3n-6) and Stearidonic acid (SDA, 18: 4n-3), which are synthesized by the Delta-6 desaturase (D6D) enzyme using the Linoleic acid (LA) and α-linolenic acid (ALA) substrates, respectively. At the molecular level, the availability of substrate and the specificity of the D6D enzyme is the cause of diversity that determines the ratio of omega 6 to 3 seed oils in different species of *Echium*. In this study, the amount of omega-6 fatty acids in persian *Echium* (*Echium amoenum*) compared with other corresponding species in Europe, North Africa, and the Canary Islands. The results showed that a great variety among the compared species has existed in terms of total omega-6, linoleic acid, and gamma-linolenic acid content percentage. The *Echium amoenum* seeds contain more linoleic acid than the average (٪٢٠,٤٥) while had the lowest amount of gamma-linolenic acid (3.73%) related to other species. Also, the D6D enzyme of *Echium amoenum* showed the lowest conversion rate (٪٤,٧) of LA substrate to GLA among the compared species.

Keyword: *Echium*, polyunsaturated fatty acids, Gamma-linolenic acid, omega-6 content, Delta-6 desaturase (D6D) enzyme.

Effect of Camphor on apoptotic factors against gentamicin-induced nephrotoxicity in rats

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Abstract

Camphor is a natural antioxidant with anti-inflammatory and tissue repair properties. Nephrotoxicity is the most important side effect of gentamicin use. Therefore, investigating the effect of natural antioxidants can resolve this complication. We aimed to assay the effect of camphor on apoptotic (BCL2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), caspase-3) factors against gentamicin- induced nephrotoxicity in rats. Thirty adult male Wistar rats were allocated to 5 groups. Positive control and treatment groups were given gentamicin to induce nephrotoxicity. Animal treatment groups were treated with camphor in olive oil for 12 days. Extraction of renal tissue of rats were taken after the twelfth day. Apoptosis factors were investigated by suitable methods. Camphor reduced the gene expression of Bax and caspase-3 and increased the gene expression of Bcl-2. We think camphor can be useful in the attenuation of gentamicin-induced nephrotoxicity based on expression levels of examined factors.

Keyword: Gentamicin, Nephrotoxicity, Camphor, Apoptotic factors.

Identification and Efficiency Comparison of Several Housekeeping Genes for Expression Studies in *Papaver bracteatum* Lindl.

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Abstract

Housekeeping (reference) genes are known as one of the essential components in gene expression analysis using Real-time PCR. In most gene expression studies reported in the Iranian poppy (*Papaver bracteatum*) medicinal plant, some reference genes with sequences which were previously identified in the common poppy (*P. somniferum*) have been used. In this study, five genes consisting of *18SrRNA*, *Act1*, *Act2*, *Ubiquitin* and *Elf1a*, which were known as the most widely reported reference genes in studies on different species of Papaveraceae family, were selected for sequencing. The species-specific sequences of the selected genes were obtained through primer designing and regular PCR using *P. bracteatum* extracted DNA followed by sequencing the amplified fragments. The obtained sequences, were used to design real-time PCR primers. The real-time PCR experiment was performed using 12 RNA samples extracted form various tissues of randomly selected Persian poppy plants. Based on the results of this study, the *18SrRNA* showed the lowest standard deviation in C_t values followed by *Act1*, *Act2* and *ubiquitin*, respectively. Thus, these genes are recommended for use as reliable reference genes in the expression studies on the Persian poppy.

Keyword: Papaveraceae, medicinal plant, gene expression, housekeeping gene, primer design.

Metagenomic survey of fungi community of tannery effluent contaminated soil

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Abstract

Tannery industry effluent discharges remarkable amounts of heavy metals to their surrounding environments. According to hazardous effects of these industrial wastes for the environmental health it is necessity to manage these effluents before discharging to the environments. Fungi bioremediation is a promising technology especially for biosorption based approaches due to their diverse metabolic activity, growth in harsh environmental conditions and high surface sorption capacity. In the current study the fungal resident in the tannery effluent contaminated soil, with the potentials in bioremediation, were identified by culture in-dependent high throughput DNA sequencing method. Environmental DNA was extracted from contaminated soil of charm-shahr, Mashhad. Illumina Miseq high throughput DNA sequencing was performed. The received sequences were analyzed by DADA2 pipelines after the quality control and trimmed with FastQC and Trimmomatic software, respectively. According to the results, dominant fungal taxa, in order of abundant, were as Aspergillus fumigatus, Aspergillus oryzae, schizosaccharomyces pombe, Candida glabrata, Lachancea thermotolerans, Debaryomycetaceae sp., Zymoseptoria sp., thermothelomyces sp., Neurospora sp., Colletotrichum sp. and Botrytis sp. The results confirm that tannery-effluent contaminated soil has high fugal diversity that could be considered as new sources for the isolation of fungi with high potentials in heavy metals bioremediation.

Keyword: Bioremediation, Metagenomic, Fungi, High throughput DNA sequencing, Heavy.

Genome analysis of an *Enterococcus faecium* prophage

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Abstract

Bacteriophages are viruses that prey on bacteria. These viruses greatly affect the evolution of bacterial species. Prophages can integrate their genomes into chromosomes of their hosts and hence transfer resistance genes to bacteria. Enterococci are commensal bacteria that show high resistance to common antibiotics, acquired or intrinsic. In this study, *Enterococcus faecium* EntfacYE was isolated from a clinical sample. The EntfacYE genome was analyzed and 88 prophage genes were identified. The prophage content included four housekeeping genes, 29 replication and regulation genes, 25 structure and packaging genes, and four lysis genes. Moreover, 26 genes were identified with unknown functions.

Keyword: *Enterococcus faecium*, prophage, genome analysis, rophage gene group analysis.

Screening of novel arginine deiminase enzyme via large scale analyses of Caspian Sea metagenomes

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Abstract

The enzymatic activity of arginine deiminase clears arginine from the blood serum. Hence it can be a candidate to use for treatment of hepatocyte, melanoma, and kidney tumor. Brackish environments such as Caspian Sea have salinity and osmolality similar to that of human blood serum and thus can be a good resource for screening new therapeutic enzymes of superior function. Traditional cultivation and expression screening methods are laborious and time consuming. Additionally, they suffer from the limitation that only 1% of prokaryotic diversity has been brought to culture so far. Novel in-silico methods to screen metagenomes allow us to access the uncultured prokaryotic majority.

In this study we compiled a database of arginine deiminase protein sequences to build a hidden markov model profile and then used this model to scan for this enzyme in the Caspian Sea metagenomes. A total of 2807243 open reading frames were screened. We identified 366 putative Arginine deiminase coding sequence. These putative sequences were further examined for specific catalytic domains of the enzyme and three-dimensional structure. among these, 26 verified arginine deaminase enzymes were identified in the Caspian Sea metagenome.

Keyword: Metagenome, Arginine deiminase, Data mining, Hidden Markov Model.

Metabolically engineered rice biomass and grain using genes associated with lipid pathway show high level of oil content

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Abstract

Increasing lipid content using metabolic engineering methods in seeds and other parts of plant, including, leaves and stem can be considered as an innovative platform for achieving more energy and biofuel in more green habits. Two key enzymes, including, diacylglycerol acyltransferase (DGAT) and phospholipid:diacylglycerol acyltransferase (PDAT) catalyze the final step of TAG assembly. WRINKLED1 (*WRI1*) is one of the important transcription factors which regulate the fatty acid biosynthesis network and TAG accumulation by balancing carbon flux between carbohydrates and lipids. In addition, oleosin encoding gene (OLE) can protect TAGs from degradation by packing into oil bodies. In the current study, four important genes involved in TAG assembly and protection (i.e., *AtDGAT1* and *AtPDAT, AtWRI1*, and *AtOle*) were overexpressed under a constitutive promoter. TAG content of transgenic seeds increased significantly ($P \leq 0.05$) by 26% in compared with those of control plants. Oleic, Linoleic (as unsaturated fatty acids), and palmitic acid (as a saturated fatty acid) contents were significantly increased by 28% (from 32 to 41), 22% (32 to 39), and 27% (11 to 14) in seeds of transgenic plants in compared with controls, respectively. Our results showed an increase in the total grain and leaf oil contents by 70 % (from 1.1 to 1.87 %) and 22.5 % (from 1.88 to 2.3 %) in the metabolically engineered lines, respectively. This is the first report of transformation in rice for enhancing oil content and energy density in its seeds and vegetative parts. Such metabolically engineered crops would be cultivated not only for food but also

for biofuel and energy production purposes.

Keyword: rice, oil, *Dgat1, Pdat, ole, Wir1*.

Association Analysis of expression patterns of WRKY2, 11 and 35 vs. metabolic changes in a susceptible cucumber (*Cucumis sativus*) cultivar

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Abstract

In this study, we studied the relation between the expression changes of some transcription factors (WRKY2, 11, 35) *vs.* content or activity of some secondary metabolites. The results showed that WRKY2 and WRKY35 act as negative regulator but WRKY11 acts as positive regulator (enhancer) for these metabolites. Therefore, with respect to role of these metabolites in plant growth and resistance, it can be concluded that WRKY2 and WRKY35 are transcription factors that are positively related to regulation of plant growth, and stress conditions reduce their content or activity. Conversely, the WRKY11 protein is likely to be positively correlated with plant ‘s stress metabolites.

Keyword: transcription factors, metabolites, regulator, WRKY, protein.

Design and synthesis of PD-L1 PD-1 inhibitory peptides and evaluation of their effect in the treatment of cancer by immunotherapy

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Abstract

Blocking the interaction of programmed cell death protein 1 (PD-1) and its ligand PD-L1 is known as a promising immunotherapy for treatment of a variety of tumors expressing PD-L1 on their cell surface. In the last decade, several antibodies against the PD-1/PD-L1 interaction have been approved, while there are very few reports of small-molecule inhibitors against PD1/PDL1 axis. Due to many advantages of cancer treatment with small molecules over antibodies, we developed several peptidic PD-L1 antagonists using computational peptide design methods, and evaluated them *in vitro* and *in vivo*. Importantly, among six peptides with best affinity to PD-L1, four peptides exhibited significant potency to block PD-1/PD-L1 axis at molecular level. Moreover, the PD-L1 expression in nine human colorectal cancer cell lines stimulated with interferon-γ was compared and LoVo cells with the highest expression were selected for further experiments. The peptides could also restore the function of activated Jurkat T cells which had been suppressed by stimulated LoVo cells. A blockade assay in tumor-bearing mice experiments indicated that peptides HS5 and HS6 consisting of a D-amino acid in their structures, could also effectively reduce tumor growth *in vivo,* without induction of any observable liver or renal toxicity, tissue damages and loss of body weight. As new designed peptides showed no toxicity against murine colon cancer cells *in vitro*, the observed anti-tumor results in mice are most probably due to disrupting the PD-1/PD-L1 interaction. Thus, peptides de-

scribed in this study can be considered as proper and safe low molecular weight candidates for immunotherapy or diagnosis of cancer.

Keyword: Anticancer, Immunotherapy, Peptide, Small molecule, PD-1/PD-L1.

Evaluating the presence of mono nucleotide microsatellites within the human genome

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Abstract

Repetitive sequences are one of the most common elements in genome of eukaryote organisms. They appear as DNA motifs repeating through the genome, some are tandemly repeated like tandem repeats and others are interspersed repeats such as long interspersed nuclear elements (LINEs). Replication slippage and other molecules such as transposable elements contribute to development of tandemly repeated sequences. These sequences which are called variable number of tandem repeats (VNTRs) are classified into minisatellites and microsatellites. In some cases, minisatellites are often referred to as VNTRs while microsatellites are called short tandem repeats (STRs) or simple sequence repeats (SSRs). Minisatellites and microsatellites are tandemly repeated sequences with a repeating unit length of about 13 to 60 nucleotides and 1 to 13 nucleotides, respectively. Microsatellites have many applications such as DNA profiling, linkage mapping, paternity testing and etc. Mononucleotide microsatellites are microsatellites with only one nucleotide as the repeating unit. These sequences are abundant in genome of organisms. Mononucleotide microsatellites could be used as genomic markers for various applications. After making random chromosomes based on the content of real human chromosomes. We analyzed the number of these repeated elements on original chromosomes and random ones. Our analysis on random chromosomes and original ones confirms the non-randomness of these sequences.

Keyword: SSR, microsatellites, Mononucleotide microsatellites, VNTRs, STRs.

The effect of recombinant Trichoderma harzianum strains on increasing bean growth (Phaseolus vulgaris L.) by altering the expression profile of genes involved in root growth

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Abstract

Fungi in the genus *Trichoderma* are frequently found in soils, and many strains have abilities to increase plant growth. The objective of the present study was to evaluate the plant growth- promoting activities of two recombinant *Trichoderma harzianum* strains (T13 and T15) which were containing chimeric chit 42 (with ChBD) and *Trichoderma* wild-type (Tw) on bean plants *in vivo* conditions. In order to achieve this goal, the growth indexes *in vivo* including root fresh and dry weight, root length, as well as the growth related genes expression (*NAC1, EXP1, DGL1*) were measured by using Real-time PCR. The results showed that the bean plants treated with T13 had a significantly increased size, root fresh and dry weight, root length compared to the control and those plants treated with *Trichoderma* wild type. Besides, the plants treated with T13, showed more expression levels of the plant growth-related genes. The results suggesting that T13 had better performance in terms of increasing nutrient uptake and root growth thereby enhancing the growth rate of the whole plant compared to the plants treated with *Trichoderma* wild type and control plants. This ability was attributed to its better colonization in the root surface that triggered plant metabolism by changing growth genes expression.

Keyword: *Trichoderma* recombinant strains, chimeric chit 42, growth-related genes, Real- time PCR.

Differential expression of diacylglycerol acyltransferase 1 (DGAT1) gene in two olive cultivars

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Abstract

The unsaturated fatty acids as the main component of olive oil (up to 80%) highly contribute to the health promoting effects of this oil producing fruit. Despite extensive culture and high consumption of olive, there is still many issues regarding oil biosynthesis that should be uncovered to improve qualitative and quantitative characteristics of olive oil. The molecular mechanisms of oil production are one of the most important factors that can highly contribute to improving oil components. The exact function of oil related gene and their contribution to the oil quality is somewhat blur and is considered as one of the main breeding practices in olive improvement programs. Diacylglycerol acyltransferase 1 (DGAT1), a member of linoleate desaturase, is one of the most important component of oil biosynthesis machinery which catalysis the last step in three acyl glycerol (TAG) synthesis. In order to shed more light on the molecular mechanisms of oil production in the olive fruit, quantitative real time PCR (q-PCR) was employed to investigate the expression pattern of DGAT1 gene during the main stages of oil production in two Iranian olive cultivars "Mari" and "Shenge". The results indicated that "Mari", the high quality oil cultivar, had higher expression at beginning stage than "Shenge" but it expression reduced to lower level at three next stages. However, the expression of DGAT1 in "Shenge" cultivar was the lowest at beginning stage and then increased at the next stage and remain constant till the fruit ripening. A 10 fold expression was recorded in "Mari" at 64 day after full bloom (DAFB), which suggest a crucial role for his gene in olive oil biosynthesis. In addition 64 DAFB was identified as the most critical stages in oil production in olive oil.

Keyword: Olive oil, olive cultivars of Iran, qPCR, oleat desaturase, DGAT 1.

Machine learning applications to design and optimize synthetic biological systems

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Abstract

Synthetic biology is the field of making biology engineerable, standardized and modularized. Synthetic biology aims at establishing design-build-test-learn cycles for the development of new-to-nature biological systems for medical, industrial and environmental applications. In this direction, this talk focuses on the application of machine learning applications to design biological sequences and optimize biological systems. A versatile and modular active learning workflow is introduced for experimentally guided optimization of a biological objective function that depends on multiple numerical and/or categorical features. Distinct applications of the workflow are showcased during the talk. Finally, a design-build-test-learn cycle powered by deep generative neural networks for the development of antimicrobial peptides is briefly presented.

Bioinformatics, Systems Biology & Synthetic Biology

Bioinformatics
Systems Biology
Synthetic Biology

NSP1 inhibition effects on Human 40s ribosomes in comparison to intermediate carrier animals

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Abstract

NSP1 protein is one of the first produced proteins of SARS-CoV-2, the virus which is responsible for Corona disease, sited at the beginning of 5́ end of virus gene number one. NSP1 binds to Human 40S ribosomal subunits has potential roles in host cells mRNA translation inhibition. to clarify subtle molecular reasons of carrier animal's pathogenicity, we used bioinformatics tools and 7k5i file structure acquired from PDB data bank to investigate interactions and internal bonds of 40S ribosomes in normal cell conditions. NSP1 protein bonds in complex with ribosomes of Human host cells in individuals affected by SARS-CoV-2 differ from normal states. The majority of NSP1 interactions involve 18SrRNA ribosomal subunit and one of the ribosomal proteins. These bonds are at A605and G600,601 nucleotide positions in 18SrRNA leading to both physically and spatially disrupting existing internal interactions in this subunit which eventually could cause the ribosome to lose its function. Additional to 18SrRNA, NSP1 also bonds with three ribosomal proteins: S2, S3, and S30.alignment comparison of these molecules in Humans and another mammalian in one hand and between Human and carrier animals such as camel and manis in another hand showed for example camel S2 protein is more like manis instead of being genetically close to human or cows. These crucial findings strongly support the important role of NSP1 in the translation inhibition of host cells ribosomes. by using these results and making a few structural changes at the carboxyl end of NSP1 protein suppression and restriction of cancerous cell growth can be gained.

Keyword: SARS-CoV-2, NSP1, Bioinformatics, 40s ribosomal subunits, Bats, Manis.

Bioinformatics analysis through the effect of rs55773150 on IL-6 gene and hsa-miR-26b microRNA in lung cancer

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Abstract

Lung cancer is the leading cause of cancer death in the world. Lung cancer is generally divided into small cell lung carcinoma (SCLC, approximately 15% of cases) and non-small cell lung carcinoma (NSCLC, approximately 85% of cases). SCLC is more aggressive than NSCLC and its prognosis is even worse, with overall 5-year survival of about 5%. A small number of patients realize their disease in the early stages of the disease and this makes the treatment process easier, but most patients find out later and the treatment process is more difficult for them and their survival rate is lower than the previous category. Chemotherapy (along with radiotherapy for limited disease) is the mainstay of treatment for small cell carcinoma, and chemotherapy can prolong survival in patients with stage IV NSCLC and SCLC. Today, bioinformatics is used for computer analysis of biological problems to increase the level of understanding of biological processes with mathematical and statistical algorithms. MicroRNAs are a small group of non-coding RNAs 25-18 nucleotides in length that can inhibit the translation of proteins. SNPs are the simplest, most useful, and most widely used method for determining markers in genetic studies. In this study, we identified genetic factors influencing lung cancer, including the association between mRNA-miR-LncRNA and SNP associated with this network. From the GEO analysis, 250 genetically modified altered genes are involved in the RNA transport signaling pathway, which examines the UBE2I gene as one of the most important genes in lung cancer. Based on these studies, it was found that hsa-miR-26b suppressed the IL-6 gene and the interactions between these components were affected by rs55773150. While this miR competes for adhesion to GS1.124K5.3 and LINC01347. In the end, these findings could lead to the suggestion of an effective biomarker or diagnostic model in the diagnosis and treatment of lung cancer.

Keyword: lung cancer, , mRNA , miR , LncRNA , SNP , GEO.

In silico study of the interaction of hyaluronidase enzyme with polyamidoamine dendrimer

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Abstract

Enzymes are used as biocatalysts for analytical purposes in diagnostics and preparative purposes in large-scale industrial processes. Despite the perfect catalytic properties of hyaluronidase (Hyal), its industrial applications are limited due to the drawbacks regarding the lack of long-term stability under process conditions. Enzyme interaction with polymers such as Polyamidoamines (PAMAMs) dendrimers is an interesting way for increasing enzyme stability. In the current work, *in silico* computational method (molecular docking) was used to query hyaluronidase–PAMAM interactions to find out the affinity of Hyal to PAMAM dendrimer. The hyaluronidase / PAMAM dendrimer interactions were followed by using the Autodock 4.2 program. The docking simulations results showed the hydrogen bond and van der Waals interactions were also found to play a key role in forming a favored complex. The suitable affinity of PAMAM dendrimer against Hyal was found from observed binding energy (-4.8 kcal/mol) and binding constant (10.98 × 105 M-1), and inhibition constant (9.1 mM) of the best-docking conformation. Thus, it can be concluded that PAMAM dendrimers can be potential support for the immobilization of the hyaluronidase enzyme.

Keyword: PAMAM dendrimer, Hyaluronidase, Molecular study, Autodock software .

Overexpression of MYCN through miR-101 rs1235943983 single-nucleotide variation promotes non-small cell lung cancer

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Abstract

MYCN is a member of the *MYC* family of oncogenes, which is detected to be overexpressed in non-small cell lung cancer (NSCLC). NSCLC is liable for more than 80 % of lung cancers and is mostly insensitive to chemotherapies. MicroRNAs (miRNAs) are small non-coding RNAs that participate in the central dogma of molecular biology by binding to the 3'untraslated region of mRNAs. Alterations in the miRNA coding genes (such as single nucleotide polymorphism (SNP)) are seen to affect the functional role of miRNAs. Herein, we investigated the effect of miR-101 rs1235943983 and rs1430733904 SNPs on the minimum free energy of this miRNA, and we postulate that these SNPs can affect NSCLC occurrence by affecting *MYCN* expression. "miRCancer" database was used to validate the expression profile of miR-101 in NSCLC. The sequence of the miRNA was obtained from "miRbase" database. "miRTargetLinkHuman" webserver was used to visualize the molecular interaction network of miR-101 and its targets. "ViennaRNA" web service was used to predict the impact of this variation on the stem-loop structure and to determine the minimum free energy (MFE) of rs1430733904-T allele, rs1430733904-A allele, and rs1235943983-C. While the MFE of the non-polymorphic sequence of mir-101 was determined -32.06 kcal/mol, the MFE for rs1430733904-A allele and rs1235943983-C allele was 37.35 kcal/mol and -27.86 kcal/mol, respectively. Here we report that *MYCN* is a target of miR-101. We also report that miR-101 rs1235943983 can elevate NSCLC predisposition by increasing the expression of *MYCN*.

Keyword: Non-small cell lung cancer; Single nucleotide polymorphism; microRNA.

Investigation of Ursodeoxycholic acid binding interactions with the catalytic portion of telomerase enzyme and human G-tetraplex DNA telomere

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Abstract

UDCA, as a secondary bile acid and a well-known drug in the treatment of several diseases of the hepatobiliary system, has a wide range of effects, including anticancer effects. The ability of this drug to enter the nucleus of cells and interact with molecules within it, such as a variety of transcription factors, has been reported in previous studies. Due to the biological importance of telomerase activity and its role in diseases such as many types of cancer; In the present study, the binding interactions of UDCA with the catalytic portion of telomerase enzyme and human telomeric G-tetraplex DNA were investigated using the molecular docking method. After receiving the structures of the ligand and target molecules and preparing them with the relevant software, molecular docking was performed using AutoDock Vina software. The results showed that UDCA binds to the catalytic portion of telomerase enzyme and human telomeric G-tetraplex DNA with binding energies of -9.1 and -9.6 kcal/mol, respectively. Based on these findings, some of the effects of UDCA, especially in relation to cancer, could be due to the drug's interactions with these target molecules. The findings of this study emphasize the importance of investigating the effects of UDCA interaction with telomerase and telomeric G-tetraplex DNA and can be used in the studies on the investigation of these effects as well as the effects of derivatives of this drug and improve its targeting.

Keyword: Ursodeoxycholic Acid, Telomerase, Telomeric G-tetraplex DNA, Molecular Docking.

Methylation Status of the Promoter Genes of hsa-miR-33b, hsa-miR-140-5p and hsa-miR-339 in Colorectal Cancer: A Bioinformatics Analysis

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Abstract

MicroRNAs are a group of small non-coding RNAs with a length of about 19-25 nucleotides and play an essential role in regulating gene expression at the post-transcriptional level. Evidence suggests that epigenetic changes, including DNA methylation, play a crucial role in cancer. It is now known that DNA methylation plays a very important role in various cellular processes such as embryonic growth and development, genomic imprinting, X chromosome inactivation, protection of genome stability, and diseases such as cancer. Compared to normal cells, cancer cells show two main types of changes in the methylation pattern: hypo-methylation and hyper-methylation. Research has shown cancer-related hypo-methylation is more likely to occur in repetitive sequences and is closely related to genomic instabilities and oncogene activation. Because promoter misplaced methylation occurs in the early stages of cancer, it can be a vital diagnostic biomarker in cancer. The prevalence of colorectal cancer (CRC) among all cancers is the fourth in men and the third in women. Bioinformatics plays an important role in predicting and analyze of methylation, which provides the safest and most effective strategy. In this study, we were able to analyze and predict vital microRNAs in colorectal cancer using several bioinformatics servers, including UCSC, methprimer, PROMO, and miRcancer. Based on the results and due to the reduced expression of hsa-miR-33b, hsa-miR-140-5p, and hsa-miR-339 microRNAs in colorectal cancer and other cancers, the possibility of hyper-methylation in the gene promoter of these microRNAs is expected as a novel

diagnostic biomarker in colorectal cancer in the future research.

Keyword: Methylation, MicroRNA, Colorectal cancer, Bioinformatics.

In silico analysis of R2R3-MYB transcription factor family in Sorghum bicolor

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Abstract

Plant tolerance to abiotic stresses is regulated by various responsive genes, including transcription factors (TF). One of these TFs is the *MYB* gene family. This gene family has widely spread in various plants. *MYB*-containing genes have greatly diversified in plants and is divided into four classes, 1R-, R2R3-, 3R- and 4R- MYB proteins. From these 4 classes of MYB transcription factors in plants, members of R2R3-MYB proteins are included in various activities such as response to abiotic stresses. So we have chosen R2R3-MYB proteins and their coding genes as our research goal. In the first step, 110 gene and 129 R2R3-MYB proteins were identified in *Sorghum bicolor*. The longest and shortest genes, the biggest and smallest proteins, and the most and least pI are respectively related to *SbMYB101*, *SbMYB 60*, SbMYB 44, SbMYB67.2(.3), SbMYB60, and SbMYB56. Also the most commonplace for 97% of R2R3-MYB proteins activity was predicted in the Nuclear. According to the results of this research, intron phase 0 was found in all of the R2R3-MYB genes (110). Additionally, intron phase 1 and 2 were respectively found in 74 (67%) and 78 (71%) of R2R3-MYB genes. Therefore *R2R3-MYB* family members have the diverse gene and protein structures.

Keyword: Bioinformatics, Transcription factor, Abiotic stress, Expn-Intron structure.

Identification of motifs in large intergenic region of *Oat dwarf virus* originated from Iran

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Abstract

Oat dwarf virus (ODV) is a plant DNA virus that causes crop damage by induction of stunting in some cereals including wheat, barley, and oat. ODV genome contains four coding regions that are responsible for the biosynthesis of proteins. Two non-coding regions are found on the genome including large and short intergenic regions (LIR and SIR, respectively) which are considered for acting as regulative elements in the gene expression and replication events. In order to investigate the ODW LIR sequence to find possible motifs involved in transcription/translation events, the LIR region of an Iranian isolate containing 400 nucleotides was extracted from the complete genome and analyzed using the MEME database. The results showed that 29 motifs are located on the ODV LIR region among which those functioning as common cis-acting elements in promoter and enhancer regions (CAAT-box) and core promoter element around -30 of transcription start (TATA-box) were the most frequent motifs. Also, several motifs with unrelated/unknown functions were identified which require more experiments to undertake their role in the virus life cycle.

Keyword: Gemini virus, Regulatory element, Promoter, Enhancer.

Promoter analysis of the RNA-dependent RNA polymerases genes involved in antiviral silencing in *Arabidopsis thaliana*

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Abstract

RNA silencing is considered as a potent defense mechanism against virus infections in plants. It has been shown that RNA-dependent RNA polymerase (RDR) proteins are key components of the RNA silencing pathway in plants. In this regard, RDR1, RDR2, and RDR6 have been revealed to play roles in small interfering RNA (siRNA) biogenesis and antiviral silencing highlighting their role in plant antiviral defense. In the present study, promoter analysis of RDR1, RDR2, and RDR6 genes of Arabidopsis thaliana was conducted by bioinformatics approaches. In silico promoter analysis of 1500 bp promoter regions of the genes identified various cis-acting regulatory elements and their respective transcription factors (TFs). DOFCOREZM, ARR1AT, GT1 CONSENSUS, CACTFTPPCA1 and TATA-box showed the highest numbers of regulatory elements of RDR1 promoter. Also, the RDR2 promoter contained the greatest numbers of regulatory elements including CACTFTPPCA1, ARR1AT, DOFCOREZM, and CAATBOX1. In addition, the RDR6 promoter was enriched with the highest numbers of regulatory elements including DOFCOREZM, ARR1AT, CACTFTPPCA1, CAATBOX1, GATABOX, and WRKY71OS. Regarding TFs, BZIP, DOF, AP2; ERF, and GATA; tify were the most TF numbers binding to the RDR1 promoter. Moreover, DOF, Myb/SANT, AP2; ERF, GATA; tify, and WRKY showed the greatest numbers of TFs binding to RDR2 promoter. Also, GATA; tify, bZIP, bHLH, AP2; ERF, WRKY, and DOF were the most enriched TFs binding to the RDR6 promoter. These results identified the highest TF numbers and their binding sites on the promoters of RDR genes involved in antiviral defense which might play important roles in gene regulation and defense responses to viral infections.

Keyword: Antiviral silencing, *Arabidopsis thaliana*, Regulatory elements, RNA-dependent RNA polymerase, Transcription factors.

In silico analysis of the small HSP gene family in Arabidopsis

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Abstract

Plants experience a variety of abiotic stresses that cause numerous physiological disorders and reduce their performance. The environmental stimuli are detected by plants through signaling pathways, using their defense systems to generate appropriate cellular responses to these stresses, thereby increasing their chances of survival. Nowadays, various studies are conducted on the role of molecular chaperones as effective defense proteins in stresses on plants. In this study, the family of small heat shock proteins (sHSP) were identified, and their function against abiotic stresses in Arabidopsis was investigated. The results show that the longest and shortest lengths of genes are respectively 617-1759 Related to At5g47600 and At1g25560 genes. The largest and smallest proteins are 361 and 131 aa. The highest and lowest pI with 9.3 and 4.4 are correlated with At1g25560 and At2g19310 genes. According to the results obtained from CELLO and DeepLoc sites, the prediction of subcellular localization for these proteins are cytoplasm and mitochondria. *sHSP* gene family members generally show decreasing or increasing changes under different abiotic stresses, but always show increasing expression during first 3 hours of heat stress.

Keyword: Arabidopsis, Bioinformatics, Gene expression, heat map, Abiotic stresses, sHSPs Singapore.

The role of miR-146b and miR-214 in regulation of HSC activation via autophagy

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, ranging from simple steatosis to advanced fibrosis. Liver fibrosis results from excessive accumulation of extracellular matrix (ECM), mainly collagen fibers. The activation of hepatic stellate cells (HSCs) is the crucial process responsible for the exceeding production of ECM and cytokines such as TGF-β4. Recent studies have shown that miR-146b and miR-214 were up-regulated in activated HSCs (aHSCs). Here, we aim to investigate the potential role of these miRNAs during the HSCs activation. we considered the miR-146b and miR-214 target genes interactions (MTIs) and selected those connected to the HSCs activation process. Using Cytoscape, the protein-protein interaction (PPI) network was constructed based on common target genes of miR-146b, miR-214, and those involved in HSCs activation. Then, we extracted the first cluster of the PPI network by MCODE and performed the pathway and gene ontology (GO) analyses. Our study revealed that SQSTM1 is in common with MTIs and HSCs activation associated-genes. The PPI network identified that SQSTM1, ATG7, BECN1, MAP1LC3B, ATG5, PINK1, GABARAPL1, MAP1LC3A, ATG12, and GABARAPL2 are the top 10 nodes with the highest interaction levels. Pathway and GO analyses of the SQSTM1 network showed that autophagy is the most significant mechanism among other signaling pathways, including mTOR, Ghrelin, RIG-I/MDA5, and NF-kappaB. In conclusion, this study suggests the potential role of miR-146b and miR-214 in autophagy regulation by SQSTM1 network in the HSCs activation and liver fibrosis progression.

Keyword: liver fibrosis, hepatic stellate cells, miR-146b, miR-214, autophagy.

Bioinformatics analysis of Heat Shock Transcription Factor genes regulation by miRNAs in Canola

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Abstract

Drought stress is one of the most important environmental factors that reduce Canola production in worldwide. Heat Shock Transcription Factors (HSFs) play a viral regulatory role in response to drought stress in plants. There is very little information on the regulation of HSFs by miRNAs in Canola. Therefore, in this study, with the help of bioinformatics methods, the most important members of the HSF gene family were analyzed for binding to 92 identified miRNAs in Canola. Molecular function, biological process, cellular component, protein class, and protein-protein interaction for target genes were also investigated. 27 miRNAs were detected for 5 members of the HSF gene family. The results showed that most of the HSF genes are targeted by bna-miRNA395. In addition, bna-miRNA403 and bna-miRNA6028 are also involved in the regulation of HSF genes, which may indicate their importance in HSF genes regulating in response to drought stress in Canola. Identification of HSFs function and regulation showed that HSFs are regulated by drought-responsive miRNAs and play an important role in responding to drought stress in canola.

Keyword: Drought stress, Protein-protein interaction, psRNATarget.

In silico analysis of N-glycosylation mutation in Gla Domain of Human Coagulation Factor IX in Hemophilia B patients

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Abstract

Hemophilia B or Christmas disease is a kind of bleeding disorder caused by a deficiency or malfunctioning of human clotting factor IX (hFIX) in plasma. A variety of mutations including deletions, insertions, point mutations, duplications, and complex mutations have been reported in hemophilia B patients. Missense mutations, the most common type of mutation in hemophilia B, may create a new N-glycosylation site (Asn-Xaa-Ser/Thr) in the hFIX amino acid sequence through single amino acid substitution. The pathogenic mechanism of N-glycosylation mutations in hemophilia B patients has not been studied yet. Our survey among Hemophilia B reported mutations, detected only one missense mutation resulted in a new N-glycosylation site in the Gla domain of the hFIX. Molecular dynamics simulation was applied to study the effects of the missense mutation on the three-dimensional structure of human coagulation factor IX. MD simulation of hFIX-wt and mutant hFIX demonstrated that the mutation slightly influenced the dynamic behavior of the mutant hFIX. RMSD analysis indicated that both peptides were converged during MD simulation,

but mutant hFIX displayed less fluctuation and more stability than the native one. These slight conformational changes due to mutation may influence the binding of the Gla domain with phospholipid bilayer which is necessary for coagulation activity of factor IX.

Keyword: Hemophilia B, N-glycosylation mutation, Gla domain, Human clotting factor IX.

Subject: Evaluation of effective expression networks in primary open angle glaucoma

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Abstract

The present study is related to the study of effective co-expression networks in primary open-angle glaucoma. For this purpose, gene expression data related to retinal trabecular tissue were used. The data after normalization were analyzed using R software and WGCNA package and gene expression networks were constructed and determined the main clusters and relationships between genes and hub genes were identified. Then, using the hub genes of each module and interpreting each gene, the relationships between these genes and glaucoma were investigated. In this study, seven important modules have been identified and two of the most important are mentioned in the article.

Keyword: Primary wide-angle glaucoma, Gene expression networks (GCN), Biological system, WGCNA package, Retinal trabecular tissue.

Identification of lncRNAs associated with genes involved in therapeutic response to azacitidine in AML patients

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Abstract

Acute myeloid leukemia (AML) is one of the blood and bone marrow-related malignancies, as well as is one of the most common leukemias with uncontrolled cell proliferation which is known to be life-threatening. The major molecular defects of AML are highly heterogeneous, hereupon it indicates the demand for more precise investigations on the disease and more successful therapeutic responses. One of the regulatory RNAs that play significant roles in the cell is the long non-coding RNA or lncRNA. lncRNAs have been considered as one of the most important therapeutic targets and effective biomarkers for the treatment and diagnosis of cancer. Azacitidine (AZA) is one of DNA hypomethylating compounds and pyrimidine nucleoside analogs that are used as the treatment of AML and myelodysplastic syndrome (MDS) with high risk. Although a large number of patients respond to chemotherapy, resistance to treatment is common. To identify lncRNAs that are associated with genes involved in the therapeutic response to azacitidine, we analyzed gene expression profiles of bone marrow samples using the GEO database, between a group of drug-resistant and a group of drug-sensitive patients who received AZA, which led to the identification of genes involved in therapeutic response to AZA by using LncRNA2Target v2.0 database, two lncRNAs (lincIRX5, MINCR) and also between the healthy group and the group of patients who received AZA, four lncRNAs (lincIRX5, EPB41L4A-AS2, SAMSON, THBS4-003) have been identified as lncRNAs that are associated with genes involved with the therapeutic response to azacitidine.

Keyword: AML, lncRNA, azacitidine, therapeutic response, drug resistant.

A review of the impact of artificial biology on agriculture and nutrition

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Abstract

Global food production to meet the needs of society will increase by about 70% by 2050. Current agricultural technologies cannot advance at this speed and, on the other hand, do not have complete environmental sustainability. As a result, we need innovative solutions to increase the productivity and quality of food products. The emerging field of artificial biology implements engineering principles in biological systems and has now revolutionized basic and applied research. In this study, a variety of applications of artificial biology on plant growth and quality have been reviewed. Today, scientific peaks are focused on the development of artificial pathways for carbon preservation in vitro, under natural conditions in the plant (In planta) to improve crop performance. Finally, we will focus on engineering approaches to increasing the nutritional value of products, as well as the use of photoautotrophic organisms as autonomous plants for the production of biomedical drugs and other commercially desirable compounds.

Keyword: Biology system, Artificial biology, Biomedicines, Carbon fixation, Biotechnology.

Bioinformatics analysis of DBAT and BAPT genes promoter in yew (*Taxus baccata*)

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Abstract

Paclitaxel is a widely used anti-cancer diterpenoid produced as a result of the secondary metabolism of yew trees (Taxus spp.). Only a limited amount of Taxol and other biosynthetic pathway metabolites (toxoids) can be obtained from current sources. Increasing the expression of key genes in the biosynthetic pathway using promoter engineering is a useful approach to improve this valuable substance production. In this study, bioinformatics analysis of the promoter of DBAT and BAPT as two key genes in the paclitaxel biosynthetic pathway was performed for general motifs. TATA box, CAAT box and GATA box, as well as specific motifs including GTGA, GAGAC, AGAAA, GCC, W-box, GT-1, G-box, and E-box using Place and Plantpan databases. Results showed differences in numbers as well as the positions of general TATA, CAAT and GATA motifs on promoter sequences. No significant difference was observed between the BAPT and DBAT promoters in terms of the number of TATA and GATA motifs. The number of CAAT motifs in the BAPT promoter was detected two folds compared to the DBAT promoter. Also, the number of motifs responding to chemical eliminators in the BAPT promoter was higher than DBAT According to the results the motif responding to salicylic acid played a more effective role. On the other hand, the DBAT promoter showed a large number of general motifs, especially the CAAT motif, which could be effective in the highest expression efficiency of this gene. Factors responding to elicitors and pathogens were also identified in this promoter. Also, this promoter has agents that respond to chemical elicitors, which can provide more expression of genes under its control due to the high number of agents that respond to salicylic acid when using chemical elicitors. In general, the results of the present study showed that in general, the results of the present study showed that the BAPT promoter is more susceptible in response

to salicylic acid than the DBAT promoter and can provide more expression of controlled genes. Also, the DBAT promoter is compared to the BAPT promoter in terms of response. Therefore, depending on the use of which exciters, these two promoters can be used to increase the expression under the influence of the mentioned elicitors.

Keyword: Paclitaxel, General and specific motifs, Secondary metabolites, Biosynthetic pathway.

Genome-wide analysis of ClpB/HSP100 gene family in soybean (Glycine max)

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Abstract

HSP100 is a large family of heat shock proteins (HSPs). ClpB/HSP100 protein act as a chaperone, mediating disaggregation of denatured proteins. ClpB/HSP100 proteins were up-regulated under heat stress and developmental cues. In this study, the genome-wide analysis revealed forty members of *the GmHSP100* gene family. Using some bioinformatics tools, their genetic structures, evolutionary relationships, physicochemical properties and protected motifs were investigated. Subcellular localization analysis showed that these genes were localized in different subcellular compartments. A phylogenetic analysis of the *HSP100* genes in soybean, rice and Arabidopsis species revealed that these genes could be divided into three major groups based on their subcellular localization. The findings of this study will be a useful resource for future studies to discover the function of *GmHSP100* genes and will help to understand the evolutionary history of *HSP100* genes in different species.

Keyword: Chaperone, Gene family, Heat shock protein, Motif, Phylogenetic analysis.

Transcriptomics Meta-analysis of Fatty Acids Biosynthesis Stages in Olive

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Abstract

Olive oil is the best and healthiest liquid vegetable oil, which has many nutritional, therapeutic and health properties due to its large amounts of unsaturated fatty acids. Because of the health benefits of olive oil, its global consumption continues to increase. For this reason, several studies have been conducted to increase oil production and various cultivars have been introduced. Due to the economic importance of olive oil and the need to export it to other countries, the quantity and quality of oil should be improved in parallel according to international standards. In international trade, the fatty acid composition is the most important factor determining the price of oil. The main reason for the difference in fatty acid composition and oil content in cultivars is due to their genetic differences. According to the diversity between genotypes in fatty acid composition, the current study seeks to identify genes and affecting the production of quality oil by transcriptional meta-analysis of fatty acid biosynthesis stages. In order to identify the genes involved in fatty acid biosynthesis, the raw sequencing data of seven olive transcriptomics experiments were re-analyzed. To identify the number of specific and common expressed genes, the developmental stages were compared at 0.01%. The results show that 54 genes (with Log FC +1) and 219 genes (with Log FC -1) are commonly observed in all comparisons. Meta-analysis of transcriptomic data can increase the speed of production of applied information related to the quality of olive oil and identify new cultivars.

Keyword: Olive, Olive oil, Fatty acids, Meta-analysis, Oil biosynthesis.

Identification and structural analysis of RAV transcription factors in four species of the Chenopodiaceae plant family

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Abstract

RAV transcription factors are regulatory proteins that play roles in plant response to hormones and abiotic stresses. RAV proteins contain one B3 and one AP2 domain and are classified as members of the B3 protein family or a subfamily of the AP2 / EREBP transcription factor family. There is some information about the structure and function of RAV proteins in a number of plant species, but little is known about them in species of the chenopodiaceae plant family. In this study, identification and structural analysis of RAV transcription factors were performed in Arabidopsis as well as the four economically important chenopodiaceae species including Amaranth, Sugarbeet, Quinoa and Spinach using bioinformatics approaches. 16 RAV genes including 6 genes in Arabidopsis, 2 genes in amaranth, 3 genes in quinoa, 2 genes in spinach, and 3 genes in sugarbeet were identified. The deduced amino acid sequences of these RAV genes were phylogenetically classified into three clades. The results revealed that the events resulting in the existence of different copies of the RAV genes partly occurred before the divergence of the species studied. All the RAV genes shared five conserved protein motifs. On the other hand, 4 and 2 protein motifs were unique for the amino acid sequences of the genes that fell into the clades 1 and 3 of the phylogenetic tree, respectively. Promoter analysis detected the cis-regulatory elements involved in response to abiotic stresses and abscisic acid within the promoters of some of the RAV genes indicating that they may play roles in stress-responsive biochemical pathways.

Keyword: Quinoa, Sugarbeet, Spinach, Amaranth, RAV transcription factors.

Goals and Challenges of Building a Biofoundry

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Abstract

Innovation in science will be critical to solving important challenges such as climate change and the destruction of nature, safety and health, and confronting epidemic diseases, economic problems and inequality at all times. Synthetic biology aims to control biological processes with its objectives in order to serve as a platform technology in a wide range of important economic sectors. With the help of biofoundry, these approaches in bioengineering practice will be promising new opportunities for production. A biofoundry provides an automation and integrated infrastructure for the rapid design, manufacturing and testing of genetically engineered organisms with the objective of exploring and studying applications and biotechnology research. Scientists are able to conduct artificial biology and field alignment experiments on high throughput scales and develop solution space that can be investigated for any particular problem or question. However, creating a biofoundry is a challenging task due to the numerous technical and operational issues that need to be considered. A global biofoundry association is recommended to coordinate activities around the world and engage extensively with existing facilities and community groups.

Keyword: Biofoundry, Synthetic biology, Global Biofoundries Alliance (GBA).

Long non-coding RNAs as potential prognostic biomarkers in gastric cancer

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Abstract

Gastric cancer (GC) is the fourth cause of cancer-related death in the world. Long non-coding RNAs (lncRNAs) can make us aware of the prognosis of cancers such as GC. The aim of this study was the investigation of the correlation between differentially expressed lncRNAs (DElncRNAs) in gastric cancer tissues compared to paracancerous normal tissues (from The Cancer Genome Atlas (TCGA) database), with overall survival. RNAseq data of gastric cancer were downloaded from the TCGA database using the TCGAbiolinks package in RStudio software. Spearman correlation of samples was done and DESeq2 package was utilized for analyzing and getting differentially expressed RNAs (DERNAs) according to adjusted p-value < 0.001 and $|logFC| > 4$, in cancerous compared to paracancerous normal gastric samples. DERNAs were annotated by biomaRt package and their correlation with survival was analyzed by the survival package in RStudio software. According to the mentioned cut-off, 99 DElncRNAs were identified in cancerous compared to paracancerous normal gastric samples. Among DElncRNAs, 18 lncRNAs had significant correlation with survival (p-value < 0.05 and Hazard Ratio (HR) > 1) and six of them inc *AC093895.1*, *LINC02864*, *LINC01194*, *LINC00392*, *AC011352.1* and *AC090809.1* had HR > 1.5. *These lncRNAs can be utilized as potential prognostic biomarkers in gastric cancer in the future.* Further investigations are needed to unravel the precise correlation of these lncRNAs with the prognosis of GC.

Keyword: Gastric cancer, lncRNAs, survival, DElncRNAs, RNAseq.

Identification of some important transcription factors in barley transcriptome data involved in insect pathogens

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Abstract

Aphids are a type of biotic stress and phloem-feeding insects that cause yield loss on a wide range of crops, including cereals such as barley. In this study, integrative meta-analysis was used to identify the significant transcriptomic mechanisms that are important for response to stresses of aphid species *Rhopalosiphum padi*, *Myzus persicae* and *Myzus cerasi*. The results of the meta-analysis showed that there are a total of 95 differentially expressed genes (DEGs) between normal and stress conditions that some of these DEGs were assigned to transcription factors (TFs). The results also showed that a total of 18 TFs were identified which belonged to eight conserved families that among them, C2C2-GATA/ LSD, VOZ, AUX/IAA, NAC families were the most abundant groups. In addition, we searched in upstream regions of DEGs for DNA motifs that finally, were identified 11 conserved sequence motifs. Functional analysis of these motifs with the GOMO and Tomtom tools revealed that these are involved in protein or amino acid phosphorylation, transmembrane receptor protein tyrosine kinase signaling pathway and regulation of transcription. The findings could help to better understand the mechanisms of response to aphid species stress and introduce candidate genes that may be a benefit for barley plant breeding programs.

Keyword: Meta-analysis, Transcription factors, Insect pathogens, Barley, Transcriptome data.

Identification of key genes in response to aphid using co-expression analysis of barley transcriptome data

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Abstract

The aphid *Rhopalosiphum padi* L. is a main pest on cereals causing plant growth reduction without specific leaf symptoms. Breeding of barley (*Hordeum vulgare* L.) for resistance to *R. padi* indicate that there are several resistance genes, reducing aphid growth. In this study, a system-biology analysis was performed to identify the underlying transcriptomic mechanisms that are critical for response to these pests. Weighted gene co-expression network analysis (WGCNA), enables the characterization of modules of co-expressed genes that may share biological functional linkages. Based on the results, WGCNA uncovered seven distinct co-expression modules. All module groups were significantly associated with genes involved in response to pests. The network analysis also determined hub genes such as *SCY1 and PDF1*, or genes with unknown functions which may be involved in regulating pathogen responses. The discoveries could help to understand the mechanisms of response to insect invasion and to detect candidate genes that may be effective to barley plant breeding programs.

Keyword: Co-expression, Transcriptome data, Aphid, barley, WGCNA.

Bioinformatic Design and Analysis of Chimeric Immunogen of eae and fliC gene Against E.coli O157:H7

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Abstract

Escherichia coli O157: H7 serotype is one of the main causes of food poisoning and diseases such as hemorrhagic colitis and hemolytic uremic syndrome. Human infections caused by this bacterium can be due to the consumption of contaminated water and food. The translated protein of *eae* gene (Intimin), binds to the target cell by its specific receptor called Tir. Also, the flagellin encoded by *fliC* plays an important role in pathogenesis, and can be used as a potent antigen in chimeric vaccine design. To increase the probability of expression of Intimin-H7 cassette, gene codons and various parameters that affect in expression were optimized, and thermodynamic analysis of mRNA structure was performed to increase the stability. The tertiary protein structure was also predicted, and the quality of the structures was assessed. Also, linear and conformational epitopes were determined. Protein with the sequence of HI showed the highest antigenicity index. Codon Adaptation Index of chimer increased to 0.97. The third predicted structure based on the RaptorX server showed good quality. The thermodynamic analysis of the mRNA structure showed that the predicted structure is stable. Conformational and linear epitopes were observed in all three domains of the chimeric protein. The results showed that the protein obtained from this engineered structure can be used as a suitable immunogen against EHEC bacteria.

Keyword: EHEC ,chimer gene bioinformatics design, Intimin, O157: H7.

Identification of drought stress responsive genes in Arabidopsis by analysis of microarray data

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Abstract

Drought stress is one of the most important environmental stresses in the worldwide. The regulatory network involved in plants' response to drought stress is complex. Therefore, identification of differential gene expression under drought conditions is crucial. In this study, microarray data of drought stress and well-water samples of Arabidopsis were analyzed to identify the common root and shoot genes in drought. Molecular function, biological process, cellular components, protein classification and protein-protein interaction analysis were performed using bioinformatics tools. The results showed that 51 genes were upregulated and 8 genes were downregulated in both shoot and root tissues. Gene ontology and protein network analysis showed that *LTI78*, *LTI65, HAI1, HAI2, LEA4-5*, *LEA7*, *F16B3.11* genes are hub genes in drought conditions. It seems that these genes can play a very important role in drought response mechanisms and they can be considered in breeding programs and genetic engineering for the production of drought-tolerant plants.

Keyword: Cytoscape, GEO dataset, PANTHER, Arabidopsis, bioinformatics.

Evolution and co-expression analysis of *HSP70* heat shock protein in Eukaryotes and Prokaryotes

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Abstract

Understanding the evolutionary relationship between eukaryotic and prokaryotic cells attracts high attention in biology. The current hypothesis is that eukaryotes are derived from archaea. The HSP70 protein is one of the most protected proteins that is used to examine the evolutionary relationship between different organisms. The purpose of this study was to determine the evolutionary relationship of HSP70 proteins in eukaryotes and gram-positive and gram-negative prokaryotes through phylogeny and synteny analysis as well as co-expression network of its coding gene with other genes. *HSP70* homologus genes of different prokaryotic and eukaryotic species were obtained from Genebank. Its evolutionary relationship was studied in different organisms by using the phylogenetic tree constructed using the maximum parsimony method in MEGA software. The gene ontology was determined by the SWISSPROT site and synteny analysis and co-expression network were done in the STRING site. Multiple sequence alignment (MSA) results showed that the HSP70 was more protected in bacterial species than eukaryotic species. The phylogenetic analysis of the HSP70 protein mainly separated the gram-negative and gram-positive bacteria in different clusters. In addition, the results of the phylogenetic analysis showed that, despite some differences, there is a great similarity between different species of eukaryotic and prokaryotic cells. Moreover, the ontology analysis of this gene in two eukaryotic and prokaryotic agents suggested a common function of this protein in prokaryotes and eukaryotes. The analysis of neighborhood relationship in *HSP70* in the prokaryotic agent and the Co-expression analysis of this gene in the eukaryotic agent demonstrated that in the prokaryotes, this gene is co-located with the genes associated with heat stress, and in Arabidopsis, the heat shock genes are expressed simultaneously with the *HSP70* gene that is probably due to the similarity of their

promoter region. The results demonstrated high protection of *the HSP70* gene between eukaryotes and prokaryotes and this gene usually co-expressed with other heat shock genes. The high similarity between HSP70 proteins in eukaryotes and prokaryotes indicates their potential use in novel drug discovery.

Keyword: Bioinformatics, ontology, Phylogenetic, synteny, *HSP70.*

Differential expression of tomato transcriptome in response to drought stress in susceptible and tolerant cultivars

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Abstract

Abiotic stresses have dramatic effects on crops production. Drought is one of the most important limiting environmental factors that affect yield quantity worldwide. Plants respond to drought stress by altering the expression of numerous genes involved in different biological and physiological processes. Understanding the molecular mechanism of response to drought stress is the key to develop drought-tolerant crops. In this study, genes and transcription factors that respond to drought stress in two tolerant and susceptible tomato cultivars were evaluated. The microarray data in two normal and drought stress conditions were analyzed. The results in the drought susceptible cultivar demonstrated that 790 and 535 genes were upregulated and downregulated in response to drought stress, respectively. In the drought-tolerant cultivar, 661 and 572 genes were upregulated and downregulated, respectively. The gene ontology analysis revealed that differential expressed genes (DEG) were mainly involved in cellular compounds as well as biological processes. Transcription factors that were effective in the regulation of genes such as MYB, WRKY, NAC, bZIP as well as proteins interaction were analyzed.

Keyword: Microarray, Gene regulation, Transcription factors, Protein.

Evaluation of genes responding to wheat drought conditions by data mining technique

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Abstract

Wheat is more important than other cereals in terms of area under cultivation and annual production, and the main part of agricultural land in dry and semi-dry regions of Iran is wheat, which is exposed to adverse environmental conditions such as salinity and drought. Although all biotic and abiotic stresses are important factors in reducing crop production, but drought stress is seriously the factor limiting crop production in arid and semi-arid regions. Data mining is a relatively new scientific major that has been formed from statistics, machine learning, computer science, especially database management researches and is using increasingly in analysis. Accessing a huge of data and information's datasets about gene expression in drought has led to use the new technologies, like data mining for the general analysis of wheat genes. Therefore, the study of gene expression under drought stress that is still unknown and the general function of genes is one of the goals of this research. 521 and 522 probes were evaluated as primary data in drought and control conditions groups by using weighting algorithms and a decision tree. The set of probes that had the highest regulation of gene expression in drought stress based on algorithms were selected and categorized. As a result, we obtained a set of genes that play a role in drought stress.

Keyword: Wheat, drought, data mining, algorithm.

Cell-in-cell structures are involved in the competition between cells in breast cancer

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Abstract

Breast cancer is the most common cancer in women worldwide, and discovering the biomarkers of this disease became so vital nowadays and Cell in Cell structure could be one of them, and it may be used as an available proxy for tumor malignancy. (CICs) are unusual in that keep morphologically healthy cells within another cell. They are found in various human cancers and result from active cell-cell interaction, and it has different kinds. In this study, we analyzed the microarray data from GEO (GSE103865) to genetically evaluate CICs' incidence in samples obtained from breast cancer patients to understand the relationship between the rate of CIC and the prognosis of breast cancer. The preprocessing was performed using R software. The DAVID website was used to analyze gene ontology (GO) and Gene and Genome (KEGG) pathways. The protein-protein interactions (PPIs) of the obtained DEGs were assessed using the STRING website, and hub modules in Cytoscape and cytoHubba were screened. According to the results from analyzing the 20 hub genes, we understood that overexpression of our Top genes is effective in focal adhesion, ECM-receptor interaction, platelet activation and PI3K-Akt signaling pathway, which shows that changes in these pathways could be the reason the overexpression of CICs in breast cancer. These data and research by many others have uncovered various genes involved in CIC formation and have started to give us an idea of why they are formed and how they could contribute to breast cancer.

Keyword: Cell in cell structure, Breast cancer, CICs, Microarray, Bioinformatics.

High expression of CDK1 and NDC80 predicts poor prognosis of bladder cancer

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Abstract

Bladder cancer is the 10th most common cancer worldwide, and its prevalence is increasing, especially in developing countries. In the present study, we employed gene expression profiles from the GSE163209 data set in the GEO database to identify potential molecular and genetic markers in BC patients. The data set comprised 217 samples, with 113 stage Ta tumor tissue samples and 104 stage T1 tumor tissue samples. The top 766 genes were chosen. P.value<0.0001 and |logFC|=1 was used to change the cutoff criteria for defining DEGs. Moreover, the MCODE plugin and cytoHubba plugin were employed to produce a module and detect 20 hub genes in these DEGs. We used GO and KEGG pathway enrichment analyses to get a better understanding of these DEGs. The KEGG pathway enrichment results indicated that the top genes were mainly involved: Systemic lupus erythematosus, Alcoholism, and Viral carcinogenesis. SLE activation in the renal glomeruli could explain the connection between this disease's route and bladder cancer, and according to our results and previous researches, heavy alcohol intake can increase the risk of BC in males and particular populations. According to our hub genes, we can consider CDK1 and NDC80 as bladder cancer biomarkers. Not much research has been done on the effect of this gene on bladder cancer.

Keyword: bladder cancer, microarray, CDK1, NDC80, bioinformatics.

Bioinformatics tools in Stem Cells researches

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Abstract

Stem cells are undifferentiated cells that have the ability to differentiate into specialized cells. They are of great importance because of their potential in tissue engineering and regenerative medicine. In the beginning, stem cell biology was a purely experimental subject, but continuous advances in the development of experimental approaches have changed this field. Recent high-power techniques generate large amounts of data on stem cells. Generating this vast amount of multidimensional data requires recording, accessing, integrating, and analyzing this data to provide deeper insights into the underlying mechanisms that underpin the basic task of bioinformatics. As a result, a new subfield called “Stem Cell Bioinformatics” has emerged, with the development of databases, algorithms/tools, analysis systems, and visualization and modeling of various aspects of stem cells with molecular data. It deals with high efficiency. This article is a review of bioinformatics frameworks developed in recent years that facilitate the analysis and application of stem cells in medical research. Advances in the bioinformatics approach in stem cell research enhance our ability to define the molecular properties of stem cells and accelerate the application of these stem cells in regenerative medicine.

Keyword: Stem Cells, Bioinformatics tools, Regeneretive medicine**,** Stem Cell Bioinformatics**,** medical research.

Variations in the expression pattern of genes responsive to the Rice Stripe Virus in rice plant

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Abstract

Rice Stripe Virus is a member of the genus Tenuivirus that is very common in rice fields in Japan, China and Korea. To control viruses; the exact pathways of the plant's response to the virus infection must first be identified. For this purpose, the identification of genes responsive to RSV in rice plants, ontological analysis of differential genes and the gene network of transcription factors were investigated. In the present experiment, the data of Rice Stripe Virus-infected plants, and their healthy counterparts from the Array Express database were examined using FlexArray software and differential genes in response to virus infection were identified after normalization by RMA method. Gene ontology analysis using AgriGO website revealed that the function of genes was divided into three categories: biological process, molecular function and cellular components, in which the most genes involved are in metabolic process (33.67%), binding (35.66%), and cellular processes (74.78%), respectively. Moreover, transcription factors included 28 families and among these families, the rate of WRKY (26.58%) was the highest. The gene network between the transcription factors was plotted on the String V10 website and the relationship between them was characterized. Gene expression and transcription factors are cross-linked, so if researchers want to produce rice plant that is resistant to the RSV, all of these factors must be considered.

Keyword: AgriGO, FlexArray, Ontology, Rice, Virus.

Comparative transcriptome analysis suggests a role for NAC transcription factors in the resistance to blue mold in apple

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Abstract

RPenicillium expansum is among one of the most destructive pathogens that cause post-harvest blue mold in apples. The comprehensively mining the transcriptome data of resistant and susceptible cultivars is reliable to recognize the critical expressed genes and the mechanism underlying the disease resistance. So, this study was conducted to explore the key genes and transcriptional profile of molecular response of apple to P. *expansum* pathogen via microarray data analysis followed by transcription factors (TFs) identification. The GEO repository made the gene expression microarray data available for the two resistant (R) cultivars and two susceptible (S) cultivars. A tissue cylinder was sampled for each side of the inoculated and control fruits at one-week post-inoculation, and RNA was extracted. Data normalization was done for each cultivar and the analysis was done using the Limma package. In resistant cultivars, there were 2269 DEGs consisting of 2084 significantly upregulated DEGs, and 185 downregulated DEGs at post-inoculation, while the corresponding numbers of upregulated and downregulated genes in susceptible cultivars were 2907 and 892, respectively. Afterward, taking into account the key roles of transcription factors as master regulators of a wide spectrum of downstream stress-response genes, the genes that encode them were examined. As the findings showed, there was a similarity between eight DEGs and TFs, belonging to NAC (4 genes), MYB-related, LOB, MYB, and bHLH families. By these results, analysis of comparative transcriptome and expression profiling of resistant and susceptible banana cultivars during infection by Fusarium oxysporum indicated that TF families such as NAC, MYB, and bHLH were mostly up-regulated in response to pathogen stress.

Keyword: *Penicillium expansum*, Regulatory genes, Transcriptome, GEO, microarray data.

Investigation of Mink Protein Function and Pathogenicity of S38G Polymorhism:An *in silico* Analysis Study

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Abstract

KCNE gene family includes 5 genes KCNE1, KCNE2, KCNE3, KCNE4, KCNE5 that encode beta subunits of potassium voltage channel in different tissues. The product of the KCNE1 gene is called the Mink protein and forms the beta regulatory subunit of the KvLQT1 potassium channel in intercalated disks of the heart. These channels are most important in membrane depolarization and atrial and ventricular action potential. In each individual, the activity and expression of ion channels create a balance between depolarization and repolarization of the action potential in myocytes. Mutations in these genes are responsible for many atrial-ventricular arrhythmias that can be familial in origin. Mink protein, which is mainly expressed in the heart, is a membrane protein and plays a modulating role in the passage of ions through the potassium channel. The S38G polymorphism, also known as A112G and rs1805127, is one of the known KCNE1 polymorphisms in the coding region. Studies have shown that this polymorphism can affect mRNA expression. The aim of this study was to investigate S38G polymorphism by several bioinformatics tools such as I-Mutant 2.0, PolyPhen, SNP & GO, PROVEAN, Panther, SIFT, Mutation Assessor, Scan for Motif, STRING and PhD-SNP. Our findings show that the mutant state of S38G polymorphism can reduce mRNA expression on the one hand and reduce the stability of Mink protein on the other. Based on these findings, further clinical study to link this polymorphism with some cardiovascular diseases, including LQT syndrome, Jervell and Lange-Nielson syndrome, Romana-Ward syndrome, Atrial fibrillation, non-valvular atrial fibrillation is recommended for other researchers.

Keyword: *KCNE1* gene, Mink protein, Polymorphism, Bioanformatics, *In silico.*

Evaluation of gut microbiome biodiversity in COVID-19 disease

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Abstract

In 2019, a virus from the coronavirus family called SARS-CoV-2 infected populations throughout the world. Coronavirus disease (COVID-19), a disease induced by this virus, attacks vital organs in the body, such as the respiratory system. One of the other targets of the invasion is the gastrointestinal tract. The gut microbiome, a crucial component of the gastrointestinal tract, can be imbalanced by viral affliction. Recent studies have confirmed alteration in the gut microbiome caused by the COVID-19 disease. We examined the alteration of the gut microbiome biodiversity in COVID-19 patients compared to healthy individuals by using the CLC Microbial Genomics Module 20.1.1 (Qiagen). The results of the metagenomic analysis revealed that the intestinal microbiome in healthy individuals has a higher biodiversity than COVID-19 patients. By demonstrating the relationship between the intestinal microbiome and disease, the use of probiotics to reinforce the intestinal microbiome composition for the prevention or treatment of COVID-19 should be evaluated.

Keyword: Microbiome, gut, COVID-19, healthy individuals, Biodiversity.

The Discovery of Potential Inhibitory Compounds for Human Carbonic Anhydrase IX from Achillea millefolium L.: Molecular Docking and Pharmacokinetic Study

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Abstract

The human carbonic anhydrase IX (hCA IX) enzyme is an important factor in the development of various types of cancer, and currently, targeting this enzyme is considered as a suitable way to find new anticancer drugs and prevent the growth of hypoxic tumors. This study was carried out to evaluate the antitumor properties of phytochemical compounds in *Achillea millefolium* L. By using the FOODB database, the phytochemical library of *A. millefolium* L compounds was prepared. The Discovery Studio 4.5 and AutoDockTools 1.5.6 (ADT) software were used to prepare the files. The AutoDock Vina software was used for structure-based drug screening. The compound Y01, a cocrystal ligand of hCA IX protein, was used as a positive control. Drug similarity and toxicity were investigated using swissadme.ch software and pkCSM server, respectively. Evaluation of pharmacokinetic of the lead compounds was directed by using ADME parameters. The results showed that 7 compounds of this plant had binding energy equal to or less than Y01 and had no cardiac and hepatic toxicity and did not cause skin allergies. According to the results of this study, the compounds of this plant promise to produce drugs for inhibition of the hCA IX enzyme and help prevention of the of solid tumors growth. However, more laboratory studies are needed to prove the identified ligands as lead compounds.

Keyword: Carbonic anhydrase IX (CAIX), Inhibitor compounds, *Achillea millefolium* L., Molecular docking, Pharmacokinetic.

In silico design of an affimer specific for carcinoembryonic antigen

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Abstract

Affirmers are engineered binding proteins that bind to target molecules with high specificity and affinity. These affinity reagents are considered as a suitable alternative to antibodies due to the lack of animals sacrificing and saving time and cost of production. In the present study, the in silico design of an affimer for CEA tumor marker using Dr adhesin protein was investigated. Molecular docking identified part of the Dr adhesin amino acid sequence that binds to CEA. After modeling of shortened sequence, the stability of its interaction with CEA was investigated. Considering the residues involved in the Dr adhesin -CEA interaction, a 110 amino acid sequence was selected. The interaction of the three-dimensional structure of the resulting affimer with CEA was highly stable and the RMSD was 3.95. Utilizing in silico methods and without performing laboratory work, an affirmer with high stability was designed for CEA that can be used in diagnosis and treatment.

Keyword: Affinity reagents, Affimer, Carcinoembryonic antigen, In silico design.

Bioinformatician evaluation of FAD (fatty acid desaturase) gene family structure related to flavor in diploid wild strawberry *Fragaria vesca*

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Abstract

FAD enzymes have been identified as a candidate for the flavor in strawberry fruit. In this study, 25 protein sequences predicted as FAD were identified by searching the database. All sequences had a FAD protein domain. The genes related to the obtained protein sequences were distributed on all chromosomes of wild strawberry except chromosome number 5 and the number of introns in them was between 0 to 8. Phylogenetic studies showed that the sequences were divided into 5 different groups, each group was belonging to one of the FAD family classes. The results of genetic structure analysis and composition of motifs showed high preservation in each group of the FAD family in the arrangement and dispersion of motifs. Most of the studied motifs had characteristics of the FAD family, including the presence of histidine boxes. The study of regulatory regions of Omega 6 FAD genes as candidates involved in strawberry fruit aroma showed the inductance of these genes under the influence of various environmental stimuli, especially light. Investigation of gene families in wild relatives of polyploid domesticated plants can provide a suitable framework for designing breeding programs for these plants, so in this study, we analyzed the FAD gene family in diploid wild strawberry *Fragaria vesca*.

Keyword: Strawberry, Aroma, FAD gene family, Bioinformatics.

Design of a nucleic acid circuit for intelligent detection of biomarkers using DNA strand displacement process

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Abstract

DNA nanotechnology uses DNA molecules to design advanced one- or three-dimensional structures. In this research, through dynamic DNA nanotechnology, an intelligent system of DNA nanostructures has been created; the system has the ability to detect a target nucleic acid biomarker using DNA strand displacement process. This reaction exploited two hairpin structures, a duplex DNA structure, and a target DNA strand, which were designed using the Nupack software package. In the next steps, the desired structures and complexes were assembled in the laboratory and DNA strand displacement reaction started in the presence of the target strand (input 1). The electrophoretic movement of DNA showed that the strand displacement process has been appropriately performed and the desire structures formed. Therefore, due to the high efficiency of this nanostructure in the intelligent detection of nucleic acid biomarkers, this method can be used as an accurate and inexpensive tool to identify the cancer biomarkers like microRNAs.

Keyword: Nucleic acid circuit, Intelligent detection, DNA strand displacement, DNA nanostructure.

Design and production of DNA nanostructure based on dynamic DNA nanotechnology for using in molecular applications

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Abstract

DNA with its own features, such as programming capability, predictable and reversible hybridization, ability to identify different molecules, and production of 2D and 3D structural nanostructures have received more attention. Understanding the thermodynamic properties of DNA hybridization enables us to predict and design different nanostructures and interactions based on this molecule, which is categorized in a new science called DNA nanotechnology. In recent years, many researchers have been interested in using this science for detecting various biomarkers due to the growing development in the DNA nanotechnology field. In this research, a nanostructure is designed from DNA, which can be used in sensing various types of biomarkers, such as nucleic acid and proteins. At first, oligonucleotides were used for the DNA nanostructure designed manually, then encoded, corrected, and completed with the aid of the NUPAK server. The probability of the assembly process of the nanostructure was calculated 99%, by the NUPACK server. The accuracy of the formation of this nanostructure was investigated through electrophoresis gel. To confirm the formation, strands were added step by step to each other to confirm the correct binding of each strand in the formation of the nanostructure. With the addition of each strand, the nanostructure's molecular weight increased and was located at a higher position than the previous stage. Therefore, the pattern obtained from the gel electrophoresis represents the correct binding of each strand in DNA nanostructure. This nanostructure can be used to identify the presence of nucleic acids, such as microRNA and Cell-free DNA in a variety of diseases.

Keyword: DNA nanostructures, DNA nanotechnology, dynamic DNA nanotechnology, molecular detection.

Identification of core defense transcriptional responses against various pathogens using meta-analysis

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Abstract

Plants defend themselves using multiple defense mechanisms to prevent biotic stresses. Despite significant advances in understanding complex plant-pathogen interaction, further elucidation is required to identify intricate molecules, signaling pathways and strategies induced by host plants against infectious agents. New meta-genomic techniques, nevertheless, have proven useful in providing an overall image of plant-pathogen interactions. In this study, techniques of meta-analysis and systems-biology analysis were employed to search for general molecular plant defense responses among different transcriptomic data reported from different pathogen attacks in Arabidopsis thaliana. Data from eight studies were subjected to meta-analysis and revealed a total of 3694 differentially expressed genes (DEGs) in a comparative manner whereby healthy and infected plants were considered. Using network analysis, we highlight the importance of WRKY40, WRKY46 and STZ and in suggesting that they serve as major points in protein-protein interactions. This is especially true regarding networks of composite-metabolic responses by pathogens. In summary, this research provides a new approach that illuminates how different mechanisms of transcriptome responses can be activated in plants under biotic stress conditions.

Keyword: Plant-pathogen interaction, Transcriptomic responses, RNA-seq, Systems-biology.

Design and production of nanostructure containing a peroxidase DNA-zyme for using in molecular application

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Abstract

The unique DNA features, such as the predictable and reversible hybridization, easy manipulation of sequencing and programming capability, have become an ideal option for building nanoscale tools. The enzymatic activity of certain DNA sequences has advantages over protein enzymes that have led to its use as a catalyst for some reactions. The combination of DNA catalysts and DNA nanostructures can be effective in identifying and detecting biological factors. In this study, a DNA nanostructure was designed that has catalytic DNA with peroxidation properties and can oxidation colorless ABTS and produce the green ABTS• product. The design of this structure was first done manually and finally completed and optimized with the aid of the NUPACK server. The probability of the assembly process of the nanostructure was calculated 100%, by NUPACK. Gel electrophoresis was used to confirm the formation of this structure. The constituent strands were first added separately and then step by step to confirm the accuracy of binding them to each other and formation the nanostructure. Increasing the molecular weight of the nanostructure due to the binding of each strand, and the higher position of the band of each step compared to the previous step, indicates correct binding the strands to each other and finally the correct formation of the nanostructure. In the next step, the colorimetric technique was used to finally confirm the correctness of DNAzyme performance and correct structure formation. The dye absorption produced by this nanostructure was measured 2.33 at a wavelength of 416 nm, which indicates its correct activity. This structure can be used as a model for the construction of identification tools and molecular assays to detect the presence of nucleic acids and proteins in a variety of diseases.

Keyword: DNA nanotechnology, DNAzyme, colorimetry, G-quadruple.

Temperature Effect on Structure of Type III Antifreeze Protein (QAE isoform) with Considering Role of Sodium Citrate Cosolute

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Abstract

Antifreeze proteins (AFPs) by inhibiting the ice growth and its recrystallization guarantee the survive of organisms under subzero temperatures. Type III AFPs as the potent ice inhibitors preserve their stable folding in the different conditions. In this study, we provided a molecular insight into the effect of temperature and cosolute on the structure of type III AFP (QAE isoform) by molecular dynamics (MD) simulation to specify the conditions with the greatest structural stability of the protein. The MD simulation was performed for the protein in the absence and presence of the citrate molecules at 225 and 300 K. The RMSD confirmed the structural stability of all simulated systems after 500 ns MD simulation. The cosolute presence altered the flexibility of some residues of the protein at 225 K. The presence of the citrate molecules decreased the number of β-sheet and α-helix structures at 225 K that resulted in the reduction of the hydrophilic surface of the protein. So, the citrate cosolute had the highest effect on the structure of the protein at 225 K. The RDF analyses and density maps also proved the obtained results. As a consequence, the structural stability of the protein was the highest in the presence of the cosolute at 300 K.

Keyword: Antifreeze protein, Cosolute, MD Simulation, Citrate ion, Temperature.

HORIZONTAL TRANSMISSION OF MIRNA GENES BETWEEN TWO MAIN PLANT MONOPHYLETIC LINEAGES SPECIES

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Abstract

Horizontal transposition for any DNA sequence, active genes, or TEs is likely to occur naturally between two unrelated plant species by some well-documented mechanisms. In this study, horizontal microRNA gene (*MIR*) transfer investigates in18 diverse plant species by considering their *MIR* repertoire in the species/direct ancestors throughout Liliopsida and Eudicotyledons (two monophyletic flowering plant lines). Dollo maximum parsimony analysis was employed to carefully compare gains and possible expansions of *MIR* families based on their taxonomic-relevant phylogenetic tree, separately. Then, the hypothetical ancestor *MIR* families' repertoire is estimated. The *MIR* families who were not observed in their universal ancestors but found in the unrelated species/their direct ancestor considered, and the transfer time was equally determined. Next, the sequence similarity of the marked *MIR*s in the species analyzed for enough per-miRNA sequence similarities. Herein, 21 miRNA gene families were detected to be the same between species of Eudicotyledons and Liliopsida but were absent in their universal ancestor(s) after they departed from Mesangiospermae. Due to these two lines are believed to be monophyletic, and due to their enough pre-miRNA sequence similarities, horizontal gene transfer can speculate among the species. They may occur through direct plant-to-plant (*e.g.*, grafting) and, or vector-mediated (*e.g.*, viral infection) horizontal gene transfer, as we see in the case of protein-encoding genes.

Keyword: Dollo maximum parsimony, Eudicotyledons, Horizontal gene transfer, Liliopsida, MicroRNA gene.

Meta-Analysis of Microarray Data to identify Drought Stress Responsive Genes in Maize

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Abstract

Drought stress is one of the major constraints reducing crop yield worldwide. Maize (Zea mays), as one of the staple crops with diverse uses, is sensitive to drought stress throughout growth stages. In order to genetically improve drought tolerance in plants, it is essential to properly understand the molecular basis of mechanisms involved in response to drought stress. In this study, meta-analysis of the microarray data obtained from four drought-stressed experiments in maize was performed to identify differentially expressed genes (DEGs) in response to drought stress. Meta-analysis revealed 222 DEGs including 119 up- and 103 down-regulated ones. Biochemical pathway analysis indicated that the DEGs were enriched in metabolic pathways, biosynthesis of secondary metabolites, starch and sucrose metabolism, and alanine, aspartate, and glutamate metabolism. 14 transcription factor encoding genes as well as three protein kinase encoding genes were detected among the DEGs. Promoter analysis led to identify 13 enriched protein binding motifs within the promoter regions of the DEGs. Functional analysis of the motifs demonstrated that they are involved in response to auxin, DNA dependant transcription regulation, gibberellin, water deprivation, abscisic acid, cold, injury, and carotenoid biosynthesis

Keyword: *Zea mays*, Meta-analysis, Differentially expressed genes, Gene ontology, Transcription factors, Promoter.

Modeling the behavior of magnetic bacteria in the presence of a magnetic field

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Abstract

Magnetotactic bacteria are living organisms that absorb iron from their surroundings and produce ferromagnetic crystals called magnetosomes. These magnetosomes have magnetic properties. The movement of this bacterial could be controlled by applying an external magnetic field. The application of these phenomena is targeted drug delivery into living tissues. Therefore, it is important to study the behavior of these bacteria in the external magnetic field. In a previous study, we investigated the motion of a group of magnetotactic bacteria called Magnetospirillum gryphiswaldense (MSR-1) in presence of a uniform magnetic field. We cultured bacteria, preparing a suspension of them and then applying an external uniform magnetic field and their movement was recorded. The videos were analyzed by the image processing toolbox of MATLAB**.** This research is based on simulating Brownian dynamics and simplifying the structure of bacteria as a self-propelled magnetic particle. We obtained equations of motion. In this model, the bacteria are magnetic self-propelled spherical particles that moving inside a box. We investigated these floating magnetic particle particles in the absence and presence of a magnetic field. Study of the distribution function of magnetic particles in the absence of an external magnetic field shows that the self-propelled particles are uniformly distributed in the box. And in the presence of an external magnetic field, they concentered on the boundaries. Our method can be used to focus and segregate magnetic particles in lab-on-a-chip devices

Keyword: magnetotactic bacteria, Spherical self-propelled particles, Magnetic field, Brownian dynamic simulation.

Evaluation of the ligand independent activation of anti CD19 SynNotch receptor by dual luciferase assay

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Abstract

SynNotch receptors have been developed to sense and respond to a specific extracellular signal by the expression of a protein. However, Despite its superior properties and a broad range of applications, the ligand-independent activity of this receptor is a critical issue that can block its way to the clinic. So the precise evaluation of the performance of this receptor is of high value. In this study, an anti CD19 SynNotch was designed and constructed while in the antigen recognition domain ScFv was replaced with camelid VHH because of its smaller size, lower immunogenicity, and higher stability. For the accurate evaluation of the receptor activity, dual-luciferase assay was utilized for its high precision. Reporter expression was observed in the absence of antigen with increasing concentration of transfected SynNotch expressing vector. However, reporter expression was significantly higher in the presence of antigen (eight and six-fold when compared to control in the CD19+ and CD19- samples respectively-p-value: 0.02). Generally, the outcomes of this study demonstrate the high level of LIA in SynNotch receptors that can hinder their functionality. However various approaches such as decreasing SynNotch expressing vector concentration, adding EGF repeats before VHH or QHGQLWF coding sequence after notch core are suggested to be tested in future studies.

Keyword: SynNotch Receptor،Dual luciferase assay ،VHH ،CD19 ،LIA.

Integration of gene expression and DNA methylation data revealed pathways involved in stomach cancer tumorigenesis

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Abstract

Gastric cancer (GC) is one of the most prevalent cancers and the fourth most lethal cancer in 2020 in the world. The TCGA (The Cancer Genome Atlas) is one of the databases constructed to identify the molecular basis of cancers. In this study, we used gene expression and DNA methylation data of the TCGA database to examine their potential alterations in gastric cancer. The aim of this study was to investigate the changes in gene expression along with DNA methylation alterations in adenoma and adenocarcinoma samples of gastric cancer in order to understand the molecular basis and identify the pathways involved in the development and progression of gastric cancer. Gene expression and DNA methylation data of adenoma and adenocarcinoma samples of gastric cancer were downloaded and analyzed using the TCGAbiolinks package. Enrichment analysis was then performed using genes that had simultaneous alterations in gene expression and DNA methylation with Enrichr tools. We obtained 4514 genes with differences in expression and 1857 probes with differences in methylation levels. After integration, we obtained 629 genes with simultaneous altered gene expression and methylation and used them to identify pathways involved in GC tumorigenesis. In this study, we used a new approach to find important signaling pathways that are effective in gastric cancer formation and progression. This information can be applied in future to introduce new biomarkers for gastric cancer diagnosis and prognosis.

Keyword: Stomach neoplasms, Gene expression analysis, DNA methylation analysis, Systems Biology, Bioinformatics.

A bioinformatics screen uncovers involving pathways of transcribed ultraconserved regions (T-UCRs) in gastric cancer

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Abstract

The transcribed ultraconserved regions (T-UCRs) are non-coding RNAs that are nearly 100% conserved between the genome of three species: human, rat and mouse. Altered expression of several T-UCRs has been reported in cancer and evidence highlights functional roles of the T-UCRs in the pathophysiology of neoplasms, as well as offering potential new strategies for diagnosis and prognosis. In the current study, we aimed to investigate the expression profile alterations and putative functions of all T-UCRs in gastric cancer (GC). RNA-seq data in the BAM file format were downloaded from the cancer genome atlas (TCGA) database, then the feature Counts were utilized to quantitate the number of reads mapping into each T-UCR. Differential expression analysis was then performed using DESeq2. The fold change values of individual T-UCR levels were calculated and differentially expressed T-UCRs with |log2 (fold change) | > 1 and adjusted P-value < 0.05 were considered to be statistically significant. Then, functional enrichment analysis was performed using the PANTHER algorithm. A total of 348 samples were analyzed in this study, including 318 GC and 30 normal tissues. Using the cut-off criteria, 34 differentially expressed T-UCRs were identified between GC and normal tissues. Metabolism was the top biological function and binding, transcription regulation and catalytic activity were the top molecular functional categories associated with T-UCRs**.** These in silico predictions provide useful information in selecting the candidate T-UCRs with a functional impact on the GC and may serve in the future as potential diagnostic/ prognostic biomarkers in GC patients.

Keyword: Gastric cancer, long non-coding RNAs, RNA-seq analysis, The Transcribed UltraConserved Regions (T-UCRs), The Cancer Genome Atlas (TCGA).

Integrative transcriptome data mining for identification of key protein kinases in pancreatic cancer

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Abstract

The absence of symptoms at primary tumor stages, as well as high aggressiveness of the tumor, can lead to high mortality in cancer patients. The treatment of Pancreatic ductal adenocarcinoma (PDAC) is limited due to difficulties associated with surgical removal, and poor sensitivity to radiotherapy and chemotherapy. Therefore, identification of an effective therapeutic target is required. Currently, protein kinases (PKs) are one of the largest and most diverse gene families. PKs have key roles in a considerable number of human diseases such as cancers. The objective of this study was to identify some PKs that have a critical role in PDAC and can be considered as therapeutic targets. In this study, the expression data related to PDAC were retrieved from Gene Expression Omnibus (GEO) database and with a Rank-prod algorithm, we identified some commonly differentially expressed genes (DEGs). Furthermore, enrichment analyses were performed to explore the function and pathway of DEGs. PKs were identified using the GSEA database. The STRING database was applied to evaluate the interactions of PKs. CytoHubba plugin was employed to identify the top hub genes in PKs. The results indicated that 1074 DEGs were between tumor and normal tissues. Sixty-four PKs were identified among the DEGs, which belonged to 12 different PKs families. The network was constructed from PKs which contains 50 nodes and 82 edges. Finally, *EGFR*, *PIK3CA* and *CDK1* genes were identified as the hub genes with top node degrees. The identified PKs will provide potential targets for the diagnosis and treatment of PDAC.

Keyword: Transcriptome data, Pancreatic ductal adenocarcinoma, Protein kinases, PPI Networks.

In silico Methods to Evaluate the Physiochemical Structural and Functional Characterization of two novel mussel-inpired adhesives

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Abstract

Underwater adhesion in mussels (Mytilus californianus) is an extreme adaptation to achieve robust and firm wet adhesion in the ocean, which is biochemically shaped through millions of years. The protein-based adhesion has huge prospective in various fields like industry, medical, etc. recently, no comprehensive records related to the systematic documentation of structural and functional properties of Mussel foot proteins (Mfps). In this study, we evaluated the novel mussel-inspired adhesive originated from Mytilus californianus and Anabaena flos-aquae: Mussel foot protein 5-Gas Vesicle Protein A (GvpA) and GvpA-Mp-3. The in silico characterization revealed the specific physio-chemical structural and functional characters of each Mfps-GvpA. The outcome of the works has huge applications for designing biomimetic materials in the future.

Keyword: Mussel foot protein, Gas Vesicle Protein A, Molecular Modeling, Physio-chemical characterization, Chemical structural characterization.

Bioinformatics study of three-dimensional structure and comparison of TERT sequence of telomerase enzyme in plants

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Abstract

Telomere and telomerase have been the focus of much research on eukaryotic organisms over the past decade. Telomerase is an enzyme that synthesizes telomere without the need for a template. This enzyme consists of three parts and the most important part is related to the protein part (TERT) of Telomerase Reverse Transcriptase with enzymatic action. The region encoding this part in the plant Arabidopsis has 3372 nucleotides, which translates to 1124 amino acids in the polypeptide strand. Different plant species with at least 80% similarity were selected to study this section. The nucleotide sequences of the TERT fragment were aligned with each other by Bioedit and Mega5 software, and then the evolutionary tree of the organisms was plotted. Scanprosite software was also used to search for protein patterns. The three-dimensional structure of this part of the protein, along with all the information of the constituent amino acids and the isoelectric point, was also plotted by ExPASy and Swiss model programs. Due to the similarity of the TERT protein moiety between different plant species, in the N-terminal and C-terminal moieties, specific fragments of protected amino acids were found. The second reverse version at the protein level was seen as a protected role in all studied species. . Study of different polypeptide sequences and point mutations created among plant species in the sequence of this section can be a good goal to express or not express telomerase, which ultimately leads to increasing and decreasing the length of chromosomal telomeres and the survival of chromosomes of different species. Be plant-based.

Keyword: Telomerase, TERT, Bioinformatics, Evolutionary Tree.

Identification of non-ribosomal peptide synthetase gene clusters through genome mining in *Acinetobacter* species

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Abstract

Non-ribosomal peptide synthetases (NRPSs) are multimodular enzymes, which produce a large portion of bacterial secondary metabolites. Genome mining provides a rapid approach for finding new biosynthetic gene clusters. The aim of this study is to analyze NRPS gene clusters among *Acinetobacter* species by genome mining, and the phylogenetic study of adenylation domain within NRPS clusters. The antiSMASH 6.0 was used for the prediction of NRPS gene clusters within the complete genome of 100 *Acinetobacter* species. The phylogenetic tree of adenylation domain predicted in the NRPS clusters was drawn with MEGA X. The substrate of each adenylation domain was predicted by NRPSpredictor2. NRPS gene cluster was identified among 23 *Acinetobacter* species, and their genes were similar to the clusters producing acinetobactin and fimsbactin. Adenylation domain sequences were arranged into two clades in phylogenetic analysis. Hydrophobic aliphatic and aromatic amino acids can be used as the substrate by these two adenylation domain clades. These results open a window into the diversity of NRPS gene clusters and their adenylation domains among *Acinetobacter* species and suggest that *Acinetobacter* species are a good candidate for further analysis to identify new secondary metabolites.

Keyword: *Acinetobacter*, Bioinformatics, Biosynthetic gene cluster.

Modeling and optimization percentage and rate of callus induction in in vivo culture using Multilayer Perceptron-Single point discrete GA

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Abstract

Callus induction is the first step to optimize plant tissue culture. Suitable embryogenesis and stem formation depends on the suitable callus. Artificial intelligence in combination with genetic algorithms helps to optimize callus induction. In this study, the aim of this study was to optimize the percentage and rate of callus induction in carrots using Multilayer Perceptron-Single point discrete GA. In this study, the output is included the percentage and rate of callus induction, while the inputs are included different types and concentrations of plant growth regulators (0. 5, 0.2 mg/l 2,4-D, 0.3, 0.2, 0.5 mg/l BAP, 1, 0.2 mg/l Kin, and 2 mg/l NAA), different explants (roots, stems, leaves and nodes), differences in concentration of compounds from MS medium (1x MS, 4x MS, and 8x MS) and sampling time. MLP was used for sensitivity analysis and optimization. The results showed that R2 in training and testing data was 95% and 95% in order to induce callusing percentage, respectively. However, at the rate of callus induction, 94% and 95% were obtained, respectively. It was found that the highest sensitivity was related to the concentration of MS compounds and the lowest sensitivity was related to the sampling time. The results of MLP-Single point discrete GA showed that the best results were obtained from stem

explant, 1x MS medium, 0.5 mg/l 2,4-D and 0.5 mg/l BAP. Finally, it was found that MLP-Single point discrete GA is a powerful tool to optimize *in vitro* tissue culture such as callus induction.

Keyword: Artificial Neural Network, Perceptron- Single point discrete GA model, micropropagation of carrot.

Selecting the best embryogenesis media in carrots using data mining technology

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Abstract

Tissue Culture of cells, tissue or organ is one of the ways to produce more and faster plants, production of virus-free plants and production of secondary metabolites. However, optimization of tissue culture for each plant and even the varieties of a plant and different explants is time-consuming and costly. For this reason, scientists used artificial neural networks by data mining method to reduce time and cost to obtain the best treatment to optimize tissue culture. The model used for this purpose is the RBF model. Using the RBF model, it was found that the highest sensitivity was related to variety and the lowest sensitivity was related to agar percentage per liter. In the RBF model, the percentage of embryogenesis was 62.5% and in the laboratory, the percentage of embryogenesis in carrots in the laboratory was 75%. The results showed that the RBF model is a powerful model for predicting and determining the best treatment.

Keyword: Artificial Neural Network ،Data Mining ،RBF model, Micropropagation, Carrot.

Bioinformatics study of the OAS-TL gene family in the Arabidopsis genome

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Abstract

The amino acid cysteine is a sulfurous organic compound that plays a key role in the defense of plants against stress. Cysteine is synthesized in plants in two stages and the enzyme O-acetylserin (Thiol) lyase (OASTL; EC 2.5.1.47) is involved in the final stage of cysteine synthesis. OAS-TL is a conserved enzyme synthesized in the cytosol, mitochondria, and plastids. The aim of this study was to investigate the physicochemical properties, phylogenetic tree and expression profile of *OASTL-Like* genes. As a result of Arabidopsis genome search, 20 *OASTL-Like* genes were identified using bioinformatics methods, which were named from *AtOASTL1* to *AtOASTL20* based on their chromosomal position. All of these genes have specific and protected PALP domains. Phylogenetic analysis showed that this gene family includes the well-known BSAS gene subfamily, which forms a separate cluster on the phylogenetic tree due to its conserved evolutionary pathway. Physicochemical studies have shown that these proteins differ in the number of amino acids, molecular weight and pI. The proteins of this family range from 250 amino acids (in *AtOASTL8*) up to 592 amino acids (in *AtOASTL7*) and have a molecular weight of 26.46 to 64.63 kDa. The isoelectric point range of these proteins varies from 5.23 in *AtOASTL6* to 9 in *AtOASTL5*. The complex spatial/temporal expression profile of the *AtOASTL* gene family showed that these genes play an important role in responding to abiotic stresses in the roots and stems of Arabidopsis.

Keywordes: *Arabidopsis Thaliana*, Bioinformatics, Evolution, OASTL, Phylogenetic tree.

Analysis of genes and expression data obtained from rice germination by inoculation with *Sinorhizobium meliloti* 1021

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Abstract

In this study, comparative transcriptome data obtained from rice seedlings in response to inoculation with live and non-living *S. meliloti* 1021 were examined. Exposed buds of LS and DS were collected 1, 2, 5 and 8 days after inoculation for microarray analysis. A total of 2414 different expressed genes were identified. Also, $q \leq 0.05$ and 1.5-fold change was used as the cutoff line and it was found that there is a significant difference between LS and DS groups. Gene ontology and pathway analysis showed that these differentially expressed genes were significantly involved in biotic stress, signal transduction, cellular regulation, plant hormone transduction, cell cycle and cell division. Among transcription factors, some major gene regulatory families such as WRKYs, NAC, bZIP, Y MYB and ZIM were involved in defense responses, while others, such as AUX, E2F / DP, BES1 and GASR, were related to growth and development. Microarrays are used to provide an overview of the expression of genes under the influence of various biological and non-biological factors and Specifically in this study, the effect of *S. meliloti* 1021 on the plant is used to identify genes and regulated pathways of the plant in relation to these factors.

Keyword: Differently Expressed Genes, *S. Meliloti* 1021 live and non-live, Microarray, Gene Ontology.

In silico identification and promoter analysis of SOD gene family in rapeseed (*Brassica napus* L.)

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Abstract

Superoxide dismutase (SOD) is one of the essential enzymes in the plant antioxidant defense system response to reactive oxygen species (ROS) produced by oxidative stress. In this study, identification and *in silico* promoter analysis of the *SOD* gene family in the *Brassica napus* genome have been investigated for the first time. 30 genes encoding SODs were identified which belong to three groups, including Cu/ZnSOD (14 genes), FeSOD (10 genes), and MnSOD (6 genes). Analysis of physicochemical properties of SOD proteins showed that the length of BnSOD proteins varied from 152 to 318. The molecular weight of these proteins ranges from 15.13 to 34.4 kDa and the isoelectric point varies from 4.83 to 8.49. The cellular location of SOD proteins was predicted and results showed that *BnCSD* genes are located in the cytoplasm, chloroplast, and extracellular space. All *BnFSD* genes are located in the chloroplast except *BnFSD2* which is located in the chloroplast or cytoplasm. *BnMSD* genes are active in mitochondria, cytoplasm, and extracellular space as well. *Cis*-regulatory elements (CREs) were also analyzed using 1.5 kb upstream of the start codon for each gene to predict their biological functions. The abundance of stress and hormonal responsive CREs found signifies *BnSODs* possible role in stress response, which may facilitate finding related transcription factors during stress and hormonal response.

Keyword: Bioinformatics, Cis acting elements, Gene Expression, Reactive oxygen species.

Identification of key genes and modules in response to fungal pathogen in barley by weighted gene co-expression network analysis

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Abstract

In molecular plant pathology, fungal pathogens are considered to be the dominant pathogens responsible for a serious decrease in growth, and quality of crop especially in the barley. In generally, for successful incursion into plants, fungi have to contend with the physical and chemical defense barriers of plants. Fungal parasites use natural openings or wounds in order to incursion plants, but true phytopathogenic fungi pass the plant's external structural defense (the cuticle and epidermal cell wall). In this study, a system-biology analysis was performed to understand the underlying transcriptomic mechanisms that are critical for response to these pathogens. Weighted gene co-expression network analysis (WGCNA) is one of the most powerful approaches for interpretation of large transcriptomic datasets. It enables characterization of modules of co-expressed genes that may share biological functional linkages. Based on the results, WGCNA uncovered six distinct co-expression modules. All module groups were significantly associated with genes involved in response to biotic stresses. The network analysis also determined hub genes such as *SCY1*, *SHMT1* and *PDE334*, or genes with unknown functions which may be involved in regulating pathogen responses. The discoveries could help to understand the mechanisms of response to fungal pathogens and to identify candidate genes that may be effective to barley plant breeding programs.

Keyword: Co-expression, Transcriptome data, fungal pathogens, barley.

In silico study of Arabidopsis *FRY1* homologous expression in response to abiotic stresses

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Abstract

The *FRY1* gene encodes a bifunctional enzyme involved in the catabolism of inositol 1, 4, 5-trisphosphate (IP3) and 3′-phosphoadenosine-5′-phosphate (PAP) and act as a negative regulator of stress-responsive gene expression and later shown to be required for suppression of RNA silencing. The aim of the current research was to characterize the *FRY1* homologous genes in Arabidopsis. A total of 9 *AtFRY1. Like* genes were identified in the Arabidopsis genome. Physico-chemical studies of the AtFRY1.Like proteins by the ExPASy ProtParam tool showed a great range of variation in the number of amino acids, molecular weight, and isoelectric point (pI). The length of proteins of this family is from 268 to 407 amino acids and its molecular weight is from 28.8 to 43.5 kDa. Their pI was in the range of 5.21 to 6.2. The localization analyses of the AtFRY1.Like proteins using CELLO showed that they are localized in the cytoplasm and chloroplast. 38 different *miRNA* variants were identified that regulate the expression of these genes through cleavage or inhibition of translation. In order to gain insights into the functional roles of the identified *AtFRY1.Like,* digital gene expression analysis was carried out and the results revealed that the expression levels of *AtFRY1.Like*s in root and shoot varied under abiotic stress conditions. Based on the expression analysis tow genes including *AtFRY1. Like5* and *AtFRY1.Like*8 are early responsive genes to abiotic stress and also have a specific role in genotoxic and wounding stresses.

Keyword: Bioinformatics, Genome Wide, Signaling, Stress, *FRY1* gene.

Identification and characterization of genes coding the superoxide dismutase protein family in wild cherries (*Prunus avium* L)

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Abstract

Environmental stresses such as salinity, cold, dehydration, heavy metals and pathogens affect the yield and quality of wild cherry (*Prunus avium* L), which has antioxidant, anti-inflammatory and anti-cancer properties. Superoxide dismutase (SOD) is the main enzyme in the antioxidant system that plays an important role in protecting plants from various biological and abiotic stresses by inhibiting reactive oxygen radicals (ROS). However, little is known about the SOD gene family. In the present study, using bioinformatics methods, 9 *SOD* genes were identified in the wild cherry genome, which are divided into 3 groups: FeSOD, MnSOD and Cu / ZnSOD based on phylogenetic and conserved domain relationships. Subcellular localization study, predicted the activity of proteins in plastids and cytosols. Analysis of gene structure indicates the existence of similar exon-intron patterns in each group. The presence of several regulatory elements responsive to the developmental process, stresses and hormones in the promoter of these genes, suggests their possible role in responding to various stresses and processes. The SSR marker identified in the gene sequence can be used in breeding programs to identify cultivars with genetic polymorphisms in response to stress and selection using markers. The results of this study provide the baseline data for further research on the role of SOD genes in wild cherries.

Keyword:; Bioinformatics, Phylogenetic study, Reactive oxygen, Stress, Superoxide dismutase .

Brain network graph comprehensive analysis during stages of Alzheimer's disease

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Abstract

Graph theory provides a popular tool for studying complex networks, including the brain network. Alzheimer's disease (AD) is the most common form of dementia, which, as an incurable, progressive and neurological disease, decreases cognition and memory. Brain networks for such a disease can be made up using a variety of imaging techniques. In this paper, we examined the weighted graph created from 3-tesla whole-brain diffusion-weighted images from 202 participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) – 50 healthy controls, 72 with early MCI (eMCI) and 38 with late MCI (lMCI) and 42 AD patients. At first, we briefly mention some important and basic features of graph analysis. Then we will calculate these characteristics such as clustering coefficient, transitivity, average path length, scale-free, small world, etc. in four stages: healthy, eMCI, lMCI and AD. We then plot diagrams of changes in the most important and challenging characteristics as Alzheimer's disease progresses and show its trend. The results show new findings, that interpretation of each, will lead to new knowledge in this field and open new horizons. To the best of our knowledge, such research has not been conducted specifically for the various stages of Alzheimer's disease.

Keyword: Alzheimer's disease, graph theory, topological features.

Identification of genomic regions associated with salt tolernce in bread wheat

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Abstract

Salinity stress is one of the most important environmental factors restricting wheat production worldwide. Several studies have been conducted to identify QTLs controlling traits involved in salinity tolerance in wheat. The application of such QTLs in breeding programs is limited due to instability in various genetic backgrounds and different environments. Meta-analysis of QTLs can result in the identification of consensus QTLs and refine the QTL positions on the genetic map. Therefore, in the current research, out of 8 studies, 123 QTLs associated with traits involved in salt stress tolerance located on chromosome groups 1 and 2 of bread wheat were collected. The extracted QTLs were based on SSR, RFLP and DART genetic markers and included RIL and DH populations. After identifying the QTL positions on the prepared reference map, a meta-analysis of QTLs was done using BioMercator V4.2 software. The results of this study led to the identification of 17 Meta-QTLs on the 6 evaluated chromosomes that had a confidence interval of 2.38 times lower than the mean of the initial QTLs.

Keyword: Bread wheat, salt stress, Meta-QTL.

Bioinformatics evaluation of targeting signaling pathways has-miR92a-2-5p related function of *TYK2*, rs12720334 in COVID-19 patients

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Abstract

Coronavirus disease 2019 (COVID-19) has caused the current pandemic in the worldwide. The major reason of this study is to investigate the bioinformatics relationship of one of the genes involved in COVID-19 by its inhibitor. Tyrosine kinase 2 (TYK2) is the first of the Janus kinase family and is involved in cytokine signaling and mediates signaling of many antivirals characterized by increased interleukin (IL) 10, IL-7, IL-2, IL-6, etc. Micro-RNAs (miRNAs) are non-coding ribonucleic acids that regulate the expression of the target gene after transcription and are usually negatively regulated. Single nucleotide polymorphism (SNP) is single nucleotide diversity in specific genetic regions. Methods: To find of the bioinformatics relationship between these three components (SNP, miRNA, Gene), NCBI sites, miRNASNP, miRBase, and DAVID were used. Results: Our review of this SNP (rs12720334) made it clear that the allele T is converted to C which may affect the function and binding of the microRNAs associated with this region. If according to bioinformatics prediction, the binding site of this microRNA is precisely the rs12720334 polymorphism allele and also the mutant allele. Due to the role of the negative regulatory function of miRNAs the expression of has-miR92a-2-5p is expected to decrease and consequently increase the expression of the target gene. Studies done on the has-miR92a-2-5p determined that this microRNA binds to the 3 UTR of the TYK2 gene transcription with high power and acts on its inhibitory action, leading to TYK2 activation in the “JAK-STAT Signaling pathways”.

Keyword: COVID-19, Bioinformatics, miRNA, SNP, TYK2 gene.

In silico characterization and expression analysis of FtsH family in potato

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Abstract

Abiotic stresses are among the major factors limiting crop yields, and FtsH protein kinases are one of the key regulators of plant response to abiotic stresses. FtsH is an ATP-dependent metalloprotease in prokaryotes and eukaryotes. Due to the economic importance and cultivation area of potato to the abiotic stresses, identification and characterization of FtsH family members in potato is performed in present research Homology-based analysis was applied to determine 10 *FtsH* genes in the potato genome. The distribution of these *FtsH* genes on each chromosome displayed a clear preference for some chromosomes such as chromosome 8 of potato. Some of FtsH proteins were subcellular located to the nucleus and chloroplast. The members of FtsH could be categorized into eight groups. Determination of chromosomal localization, promoter analysis and gene structure was also performed as well as the gene expression pattern of each gene was surveyed. The number of introns in the gene family members varied from 3 to 14. Totally, 33 kinds of transcription factor binding sites (TFBS) including abiotic stress-responsive elements were found in FtsH promoter sequences. Expression of FtsH was expressed by different tissues which

implementing its important role in plant growth and development. Two TFBS had the highest number among TFBS namely MYB and WRKY which were involved in abiotic stresses. It is expected that this gene could be used in plant manipulation and breeding programs aimed for tolerance enhancement to abiotic stresses especially drought.

Keyword: FtsH, TFBS, Phylogenetic, Structure, Protein.

Genome Wide Identification And Characterization Of A Novel Antimicrobial Peptide From Ajwain (*Trachyspermum Ammi*)

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Abstract

Bacterial resistance to antibiotics is a major threat to plant products, animals, and human health, and over the years the situation has become increasingly widespread around the world. On the other hand, plants are promising sources of antimicrobial agents like antimicrobial peptides (AMPs) which have various antimicrobial properties and are capable of degrading microbial membranes leading to control or stop the growth of various microorganisms. Ajwain (*Trachyspermum ammi* L), a plant in the family Apiaceae, is a valuable species from a medicinal point of view and has been widely used in traditional medicine for a long time. In this paper, a novel AMPs of the gamma-thionins family was identified from Ajwain for the first time via NGS data analysis. For this purpose, the assembled whole transcriptome of Ajwain was searched using Hidden Markov Model (HMM) and PHMMER software. The results identified a 228 bp coding sequence encoding a 75 amino acid peptide that belongs to the plant gamma-thionins family. In addition, further analysis showed that the first 28 amino acids are signal peptides which after cleavage may result to a mature peptide containing 47 amino acids with a molecular weight of 5337.15 daltons. Analysis of the Secondary structure of this peptide showed that the final mature peptide has

a three-dimensional structure with three beta-sheets, one alpha-helix and four disulfide bonds. In addition, phylogenetic studies confirmed that this peptide is most closely related to the gamma thionin peptide from the carrot and Chinese ginseng plant, all three of which are in the same plant order.

Keyword: Anti-microbial peptide, Ajwain, Gamma-thionin, Next Generation Sequencing.

Identification and in silico characterization of a novel L-asparaginase from coastal water microbial metagenomics data

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Abstract

L-asparaginase is an enzyme that hydrolyzes the amino acid asparagine to aspartic acid and ammonia. This enzyme is used as a drug to treat leukemia and lymphoma. L-asparaginase activity has been widely reported in plants, animals, and microorganisms, but only asparaginase produced by *Escherichia coli* and *Erwinia chrysanthemum* is used to treat leukemia and lymphoma, and the Searches are ongoing to find new sources for this enzyme. In the present study, we identified a novel gene encoding a new L-asparaginase by analysis of MGnify microbial metagenomics database by HMMER software. A novel gene of 1,059 bp and its corresponded protein of 353 amino acids were identified. The results showed that the identified L-asparaginase protein sequence was 45.87 and 50% % similar to *Erwinia chrysanthemum* and *Escherichia coli* L-asparaginase, respectively. However, despite the low sequence similarity, the structural studies have shown that the three-dimensional structure of this protein is very similar to *Escherichia coli* L-asparaginase. These findings can be used to generate and optimize the enzymatic kinetics of current therapeutic asparaginases.

Keyword: L-asparaginase, leukemia, metagenomics, bioinformatics.

Study on interactions between iteron like sequence of betasatellite with replication associated proteins encoded by helper viruses

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Abstract

Betasatellites, as single-stranded circular DNAs associated with begomoviruses (*Geminiviridae* family), are multifunctional agents that work as symptoms determinant for begomoviral disease complexes. Begomoviruses are replicated by species-specific interactions between the viral replication-associated protein (Rep) and iteron sequence elements. However, betasatellites replication is promiscuous, so that members of different genera can support replication of betasatellites. In the present study, the interaction of Reps encoded by *Cotton leaf curl Multan virus* (CLCuMV, as cognate helper virus) and *Beet curly top virus* (BCTV, as non-cognate helper virus) with the iteron like the sequence of betasatellite, 5́-GAGGACC-3́, was investigated by in silico analysis. Nucleotide sequences of two Rep-encoding genes were obtained from the GenBank database of NCBI. Amino acid sequences of Rep proteins were aligned and their physicochemical characteristics, as well as their secondary and tertiary structures, were predicted by SOMPA tool and I-TASSER servers, respectively. The binding affinity of best-predicted models of both proteins toward betasatellite iteron-like sequence was assessed by docking simulations. The results represented reliable tertiary structures for tested proteins and showed structural similarity for Rep proteins between different geminiviruses. Interaction analysis revealed a higher binding affinity of the CLCuMV-encoded Rep with the iteron-like sequence of betasatellite in comparison to the BCTV-encoded Rep. The results verified more trans-replication activity of cognate viruses for replication of betasatellite genomes and emphasized the role of iteron-like sequences in interactions with Rep from helperviruses.

Keyword: betasatellite, begomovirus, replication associated protein, genome replication .

Synthetic Biology: Advances and Biosafety Management

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Abstract

In recent decades, synthetic biology has made significant advances in the fields of life sciences, industrial development, and environmental bio-remediation. The development and application of synthetic biology have, willingly or unwillingly, raised concerns about biosafety, biosafety and even cyber biosecurity. Also, some countries in Europe, the United States and Asia have enacted laws and regulations to control synthetic biology in basic and applied research. Synthetic biology has led to the success of many researches. There has been much speculation about the novel coronavirus SARS-CoV-2 during the outbreak of the Covid-19 virus, and the true origin of the coronavirus remains unclear. Concerns about synthetic biology are due to the potential and uncertainty in the synthesis and engineering of living organisms. Strict control measures and related laws are necessary to ensure the proper application and development of synthetic biology and to strengthen the oversight of research related to pathogens. These laws relate to biological hazards and the limitations of synthetic biology.

Keyword: Synthetic biology, biosafety, rules and regulations, synthetic life .

Evaluation of point mutation of position of 138 electrostatic loops of superoxide dismutase enzyme by Molecular dynamics simulation

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurological disorder caused by misfolding and aggregation of superoxide dismutase (SOD1). Oxidative stress increases in the spinal nerves of patients with ALS, which damages the structure of the SOD enzyme and alters its function. However, the main cause of this disease is unknown. Considering the relationship between amyotrophic lateral sclerosis and mutations in the gene encoding SOD1, it is important to evaluate the point mutation at position 138 enzyme (G138E) of the electrostatic loop. The aim of this study was to investigate the formation of protein aggregates and the toxicity effect of SOD1 aggregates to identify methods for the prevention or treatment of ALS using molecular dynamics simulation studies. Mutations in this position cause the spatial deformation of the enzyme and cause the misfolding that was associated with pathogenesis. According to the results, mutations in the SOD1 encoded gene at this position are associated with sclerosed amyotrophic lateral disease, which alters protein stability and enhances prone to aggregation.

Keyword: Amyotrophic lateral sclerosis, molecular dynamics , electrostatic loops , G138E. Nucleic acids research, 39(17), e118-e118.

Identification of microRNAs involved in response to cold stress in Chickpea

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Abstract

Chickpea (*Cicer arietinum*) is one of the most important legumes classified as a cold-sensitive species and yearly, the sudden drop of temperature in autumn, freezing temperatures in winter and late spring chill leads to significant yield loss in the crop. Identification of genes, regulatory factors and gene networks involved in response to cold stress can pave the way for the development of cold-tolerant cultivars through genetic engineering and molecular breeding approaches. miRNAs are highly conserved small non-coding RNAs that regulate expression of their target genes by degradation of their mRNAs or suppressing their translation and their roles have been proved in cold stress response in various plants. In the current study, in order to identify miRNAs and their target genes involved in cold stress tolerance in chickpeas, following sequencing transcriptome of a cold stress-tolerant cultivar (Saral) and a cold stress-sensitive line (ILC533) in response to cold stress and identifying cold-responsive genes, sequences of the responsive genes were analyzed by c-mii software to identify possible miRNAs Finally, the target genes of a number of im-

portant miRNAs were identified using psRNA Target software. The results showed that 30 and 23 miRNAs responded to cold stress in tolerant and sensitive genotypes, respectively. By comparing the response of miRNAs in contrast genotypes, miR319, miR339, miR334 and miR159 were identified as candidate miRNAs involved in cold tolerance and their roles was examined in more detail.

Keyword: Chickpea, microRNAs, Cold stress.

Phylogenetic Relationships and Promoter Analysis of Betaine Aldehyde Dehydrogenase Encoding Gene in Some Plant Species

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Abstract

Glycinebetaine is an important osmoprotectant, which is accumulated under various stresses in higher plants. The gene encoding Betaine aldehyde dehydrogenase (BADH) is one of the important genes involved in the biosynthetic pathway of glycinebetaine, and its introduction has led to an increased tolerance to a variety of abiotic stresses in different plant species. In this study, the phylogenetic relationships and promoter analysis of betaine aldehyde dehydrogenase encoding gene in some plant species were done by using bioinformatics approaches. This study suggests that protein motifs are very close to each other and revealed that the BADH gene function conserved during evolution because the distance evolutionary of orthologous under study were very far from each other. Promoter comparative analysis revealed the presence of 14 enriched protein binding motifs within the promoter regions of BADH gene abiotic stress-responsive cis-elements like abre, myb, myc, gata, gt1, etc., salicylic acid, gibberellin, light-responsive motifs and identify. Genome ontology analysis of the enriched motifs demonstrated that they are involved in some biological processes such as protein amino acid phosphorylation, transmembrane receptor protein tyrosine, regulation of transcription, response to auxin stimulus, transmembrane receptor protein tyrosine kinase signal pathway, response to ethylene stimulus and lipid transport.

Keyword: BADH, Glycine betaine, promoter, Cis regulatory elements, phylogentic.

Bioinformatic Analysis of Transcription Factors in Tobacco

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Abstract

Transcription factor (AP2 / ERF) is one of the largest families of transcription factors in plants which plays an important role in many biological processes as well as response to biological and non-biological stresses. In this study, using bioinformatics techniques, the members of this family in tobacco and its orthologous genes in *Arabidopsis* and tomatoes have been studied and identified. Protein sequences of this family were extracted using different databases and after confirming the presence of the AP2 domain, this family was divided into 3 main subdivisions based on DNA binding domain including ERF and DREB, RAV, and AP2 in tobacco. Three sequences in tobacco were also classified as a soloist based on their high similarity to the AT4G13040 sequence in *Arabidopsis*. In this study, 93 genes in *Arabidopsis* and 136 genes in tomatoes were identified as orthologous genes during the speciation process. The results of gene ontology showed that in biological processes: DNA-connected transcription regulation, DNA-connected transcription, and signaling pathway of ethylene activity, in cell components group: nucleus, and in molecular function group: DNA binding and transcription factor activity and DNA binding to specific sequence are dominant.

Keyword: Transcription factor, AP2/ERF family, Bioinformatics, Tobacum.

Bioinformatics Analysis of AP2 / ERF Family in *Arabidopsis thaliana*

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Abstract

Environmental stresses such as temperature, drought, and salinity greatly have a great impact on plant growth, survival, and reproduction. Transcription factors are effective in regulating gene expression in environmental stresses and may be considered as the main regulators in response to these stresses. Among these transcription factor families, APETALA2 / ETHYLENE RESPONSIVE FACTOR (AP2/ ERF) has emerged as the main regulators in response to various environmental stresses. In this study, using bioinformatic analysis, the members of this family in *Arabidopsis* have been studied and identified. One hundred seventy-two protein sequences of this family were extracted in *Arabidopsis* using the PlantTFDB database and after confirming the presence of the AP2 domain, members of this family were classified into four main subfamilies including DREB, RAV, AP2, and ERF, as well as a number of unknown sequences called soloist. The results of gene ontology represented that in biological processes: regulation of DNA-bound transcription, DNA-linked transcription, the signaling pathway of ethylene activity; in the group of cellular components: nucleus; and in the molecular function group: DNA binding and transcription factor activity and DNA binding to a particular sequence are dominant. Promoter analysis of genes in the AP2 / ERF family identified 15 repetitive and significant motifs in the promoter region of the studied genes. Functional analysis of the motifs revealed that they responded to auxin, abscisic acid, water deprivation, cold, wounding, DNA-dependent transcription regulation, jasmonic acid, polygalacturonase activity, and pectinesterase activity. The results revealed that the members of this family are involved in a regulatory network, which plays an important role in response to different stresses.

Keyword: Transcription factors, Bioinformatic analysis, AP2 / ERF family, *Arabidopsis.*

In Silico Epitope Mapping of Human Lysosomal Acid α-Glucosidase

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Abstract

Human Lysosomal Acid α-Glucosidase (GAA) is an enzyme necessary for degrading glycogen to glucose. The deficiency of the enzyme due to the various mutations in the coding gene, results in Pompe disease which is a metabolic autosomal recessive disorder. The Pompe disease includes a spectrum of symptoms depending on the type of mutations. The symptoms can vary from dysfunction of skeletal muscles to breathing problems which can result in death. The only approved treatment for Pompe disease is enzyme replacement therapy (ERT) using recombinant human Lysosomal Acid α-Glucosidase (rhGAA). The immune system's reactions and antibody production is caused by epitopes or antigenic determinants. Epitope is the part of an antigen recognizable by the immune system. Identifying the epitopes recognized by B-cells plays an important role in the developing of diagnostic kits. The early diagnosis of the Pompe disease is vital for starting the therapy, saving lives and improving the quality of it. *In silico* analysis and categorization of experimentally recognized epitopes has helped to develop algorithms for predicting epitopes. Using such algorithms in databases like IEDB and analyzing the data, results in high accuracy predicted epitopes which helps with saving time and decreasing the costs. In this research, we have postulated six regions containing high-score epitopes in Human Lysosomal Acid α-Glucosidase structure. Upon in vivo confirmation, these regions could be used to raise antiserum for developing diagnostic kits in order to diagnose the Pompe disease.

Keyword: Epitope Mapping, Bioinformatics, *In silico*, Human Lysosomal Acid α-glucosidase, Pompe Disease.

Use of RNA-Seq data to study the expression of queen bee brain genes in the mating process

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Abstract

Mating in honey bees is a complex process that is often associated with behavioral and physiological changes. However, understanding the genetic basis of these changes is limited. In addition to their own direct production, honey bees are involved in the pollination of most agricultural products. As a result, understanding the mating process of the queen honey bee has practical value. For the present study, from the total RNA sequence, 10 samples of European honey bees (*Apis mellifera carnica*) including 5 samples of virgin queen honey bees and 5 samples of queen injected with semen into hemocoel by aligning and locating RNA-Seq readings on the genome. Honey bee reference version Amel_4.5 sorted was used. After analyzing the differential expression of genes, 15853 genes were identified on the transcript of these samples and 971 genes with a p-value <0.05 had a significant difference between the treatments of the virgin queen and the queen injected with semen. Gene expression patterns differed between virgin queens and queens injected with semen into the hemocoel. Analysis of the gene ontology and biological pathways showed that some of these genes are involved in purine metabolism, biosynthesis of secondary metabolites, N-methylglycine glycine activity, ECM receptor interaction, oxidoreductase activity, acyl-coenzyme reductase activity, neurotransmitter activity Fat carriers are associated. The results showed that the injection of sperm into the queen honey bee's hemocoel, like natural mating, altered gene expression in the brain and thus provided a unique insight into the biology of the mating process in bees.

Keyword: honey bee, Mating, Gene expression, Transcriptomics, RNAseq.

Use of RNA-Seq data to study the expression of queen bee brain genes in the mating process

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Abstract

Mating in honey bees is a complex process that is often associated with behavioral and physiological changes. However, understanding the genetic basis of these changes is limited. In addition to their own direct production, honey bees are involved in the pollination of most agricultural products. As a result, understanding the mating process of the queen honey bee has practical value. For the present study, from the total RNA sequence, 10 samples of European honey bees (*Apis mellifera carnica*) including 5 samples of virgin queen honey bees and 5 samples of queen injected with semen into hemocoel by aligning and locating RNA-Seq readings on the genome. Honey bee reference version Amel_4.5 sorted was used. After analyzing the differential expression of genes, 15853 genes were identified on the transcript of these samples and 971 genes with a p-value <0.05 had a significant difference between the treatments of the virgin queen and the queen injected with semen. Gene expression patterns differed between virgin queens and queens injected with semen into the hemocoel. Analysis of the gene ontology and biological pathways showed that some of these genes are involved in purine metabolism, biosynthesis of secondary metabolites, N-methylglycine glycine activity, ECM receptor interaction, oxidoreductase activity, acyl-coenzyme reductase activity, neurotransmitter activity Fat carriers are associated. The results showed that the injection of sperm into the queen honey bee's hemocoel, like natural mating, altered gene expression in the brain and thus provided a unique insight into the biology of the mating process in bees.

Keyword: honey bee, Mating, Gene expression, Transcriptomics, RNAseq.

Integrated analysis of a competitive endogenous RNA network reveals potential regulatory axes in gastric cancer

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Abstract

Gastric cancer (GC) is one of the most common malignant tumors worldwide. GC is a complex disease and its genetic alterations are not fully understood. Different types of RNAs can compete with each other for binding to the same microRNAs (miRNAs). These interactions make competing endogenous (ceRNA) networks that are highly informative and can introduce new diagnostic and prognostic biomarkers. In the current study, we constructed a ceRNA network and analyzed the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) of the mRNAs from the network. RNAseq and miRNAseq data from The Cancer Genome Atlas (TCGA) database of GC were downloaded using the TCGAbiolinks package in the RStudio software. Batch effects correction were performed by sva package. DESeq2 package was utilized for getting differentially expressed RNAs between cancerous and normal adjacent GC tissues and the data were annotated by biomaRt package. Interaction prediction of differentially expressed miRNAs (DEmiRNAs) with differentially expressed mRNAs (DEmRNAs) and differentially expressed lncRNAs (DElncRNAs) were done by using a multimer package and RNAInter database, respectively. Cytoscape (version 3.8.1) was utilized for constructing the network. KEGG and GO analysis were done by KOBAS (3.0) database. Based on cut-offs ($|logFC| > 2$, adjusted p-value < 0.001), 1597 DEmRNAs, 722 DElncRNAs and 67 DEmiRNAs were identified. Then a ceRNA network including 15 miRNAs interacting with both lncRNAs and mRNAs, 229 mRNAs and 15 lncRNAs was constructed. Enrichment analysis revealed important KEGG pathways and GO terms relating to the network. Our findings provide novel insights on ceRNA regulation in GC and a new perspective for GC pathogenesis research as well as introduction of new GC diagnostic/prognostic biomarkers in future studies.

Keyword: *Gastric cancer, ceRNA network, lncRNA, miRNA, mRNA.*

Phenylpropanoid gene expression analysis in flax using transcriptome data

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Abstract

Linum usitatissimum has a wide range of applications in the food, textile, and pharmaceutical industries. Medicinal properties, especially its anti-cancer properties, are due to lignan compounds, which play an important role in defending the plant against stress. The major lignan compounds produced in this genus include pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, 6-methoxy podophyllotoxin, and podophyllotoxin. In the biosynthetic pathway of these lignan compounds, the enzyme secoisolariciresinol dehydrogenase is a key enzyme that oxidizes secoisolariciresinol to matairesinol and is sequentially converted to other lignans. To analyze the transcript data of the SDH gene, RNA-Seq sequencing data were used in flaxseed under biotic and abiotic stresses. In this study, data quality was measured with FastQC software. After splicing the readings, the data were mapped, and then the expression level of each gene was examined. The results showed that in addition to genes that showed an increase or decrease in expression in response to both biotic and abiotic stresses, genes in the biosynthesis pathway of secondary metabolites, including lignan compounds, also up-regulated or down-regulated. SDH gene transcript showed significant changes in both biotic and abiotic stresses based on P-value value .050.05. Expression change in isoform LUS10035258 was also obtained under all three conditions of potassium deficiency, drought, and aluminum.

An increase in metabolites occurs in the early stages of stress. With increasing stress intensity, free radical scavenging enzymes are activated. Therefore, by investigating the expression profile of genes related to different stresses, we can first determine the genes involved in stress response and then obtain the stress threshold to produce the highest amount of secondary metabolites.

Keyword: abiotic stress, biotic stess, RNA-Seq, lignan.

Identification of genomic regions associated with drought tolerance in foxtail millet

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Abstract

Abiotic stresses, especially drought, are one of the most important obstacles to sustainable agricultural development, especially with the spread of droughts and climate change in the world. Foxtail millet has a short growing season and it will be necessary to develop new crop varieties which can produce more grain while using less water in favorable environmental conditions. Identification of key genes involved in drought tolerance can be useful in promoting this trait in millet and other cereals by molecular modification or gene transfer. In this study, a total of 448 QTL from 8 published studies related to performance control traits in tropical millet were collected. These traits include plant height, water consumption, biomass, root length, panicle length. The extracted QTLs were based on SSR and SNP genetic markers and included RIL populations. Under normal conditions of moisture and drought stress, 287 and 96QTL were collected, respectively, and were mapped to the consistency map. Meta-analysis was performed using Biomercator v4.2 (https://urgi.versailles.inra.fr/Tools/BioMercator-V4 /). In total, 32 Meta-QTLs (MQTL) were detected on 9 foxtail millet chromosomes. Their confidence interval were 3.49 folds lower than the average of the initial QTLs. Relevant genomic regions will be used to identify involved candidate genes.

Keyword: foxtail millet, Drought Stress, Meta-QTL.

Genetic and bioinformatics study of phytoplasma isolates of tomato big buds in West Azarbaijan

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Abstract

Tomato bud (Big bud), a phytoplasma disease, is one of the most important and economical tomatoes diseases in the world. It causes by phytoplasma. To study on the genetic and bioinformatics aspects of group 6 phytoplasma isolates associated with tomato big buds in Iran, sampling was performed from tomato fields in West Azerbaijan province. Universal primers P1/P7 and R16F2n/R16R2 were used for amplification of the Phytoplasma 16S rDNA gene by nested PCR. PCR products were digested using different enzymes. *In silico* method was performed using Neb Cutter software and compared with RFLP profiles obtained from similar sequences isolated from Zanjan, Yazd and Khorasan Razavi provinces. Comparison of Single Nucleotide Polymorphism (SNP) of 16S rDNA sequences of isolates was performed with MEGA software. Profile of digestion analysis with *Tsp*451, *Hpa*I, *Dra*I, *Hae*III and *Eco*RI enzymes are distinguishable in Iranian isolates. In addition, SNP analysis of these sequences showed that eight isolates were similar to *Candidatus* phytoplasma trifolii Ct1 and among which four groups were separable. Results of these studies indicated that Iranian big bud isolates have a high genetic diversity.

Keyword: Big bud, SNP, genetic diversity, Tomato, phytoplasma.

Bioinformatics analysis of miRNAas affecting some genes involved in the Calvin cycle and the photorespiratory pathway of rapeseed (*Brassica napus* L.)

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Abstract

Rapeseed (*Brassica napus L.*) is the third largest source of vegetable oil in the world due to its 40-45% oil per grain. The Calvin cycle and the photorespiratory pathway are two very important processes in the plant, which Better knowledge of the factors that affect them can help improve the production system of plants with abundant economic value, like canola. MicroRNAs are key factors that regulate posttranscriptional gene expression in eukaryotic organisms. In this study, miRNAs affecting GlyK, ME, PRK, FBPas and FTR, which are five genes involved in the Calvin cycle and photorespiration, were bioinformatically identified using the NCBI database and psRNATrget web-based software. Finally, the relations between the target genes and their associated miRNAs were mapped using Cytoscape software. Based on the results obtained in this study, in rapeseed, for the GlyK gene, four microRNAs of the miR172 family were identified, including bna-miR172a, bna-miR172b, bna-miR172c and bna-miR172d. It was further found that bna-miR167a, bna-miR167b, bna-miR167c and bna-miR167d are four members of the miR167 family that affect the ME gene. For the other genes in this study, no effective miRNA was detected in Rapeseed.

Keyword: Bioinformatics, Calvin cycle, photorespiration, psRNATrget, Brassicaceae

Potential of a novel metagenome-derived laccase with stable performance in biorefinery of lignocellulosic biomass

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Abstract

Laccases have been broadly applied as a biocatalyst in various industries, but their applications tend to be limited by easy deactivation, lack of adequate stability, and susceptibility under the harsh condition. Thermostable laccases that can tolerate extreme conditions are excellent catalysts for solving many industrial problems and providing pollution-free by-products. Therefore, this study concerns identifying the novel stable laccase with stable performance in complex downstream reactions. The PersiLaccase1 was mined from the metagenomic data through a hybrid multi-stage in-silico screening approach. The enzyme was cloned, expressed, purified and its activity and stability under various conditions were studied. The purified laccase demonstrated high resistance under abnormal conditions including extreme temperature, pH, long-term storage, presence of inhibitors, surfactants, organic solvents, and metal ions. The efficiency of a stable PersiLaccase1 in bio-ethanol production from the fermented quinoa hull and rice straw was performed. The enzyme effectively degraded biomass substrates and improved ethanol production, followed by a reduction of reducing sugar during fermentation. According to the results, the long-term stability of PersiLaccase1 in high temperature leads to the efficient production of bioethanol from fermented agricultural waste and reflects the versatile abilities of the enzyme, including delignification, detoxification, and biodegradation of the lignocellulosic substrates.

Keyword: Stability, Laccase, Metagenome, Agricultural waste, Bio-ethanol, Bioremediation.

Metagenome derived laccase enzymes with application in plastic degradation

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Abstract

Many microorganisms and bacteria present in the metagenome environment cannot be cultured in the laboratory. Therefore, metagenomics can be described as the study of the whole microbial community and its related enzymes directly isolated from an environment. Due to the growing usage of plastic products and the consequent increase in plastic waste, global concern about the harmful effects on the environment has also increased. In recent years, a number of microbial enzymes capable of modifying or degrading recalcitrant synthetic polymers have been identified. Laccases are the most important enzymes that were introduced to degrade plastics. This study summarizes the main findings on plastics degrading laccase enzymes. Metagenomics analysis is performed in several steps and for each step, some software are designed. The quality of raw data are checked by FastQC and verified. MetaPhlAn3 are used for taxonomic profiling of the sample. The MEGAHIT assembler are used to build contigs from raw sequencing reads. To reconstruct population genome bins, contigs are clustered based on their coverage and tetranucleotide frequency, using MetaBAT2 software. Generated bins are analyzed using ECpred software. In this study, the main finding is the identification of plastic-degrading laccase enzymes in the metagenome environment. In a recent study of a plastic-contaminated metagenomic environment, the identification of a group of new enzymes involved in plastic degradation is considered. Based on the results of MetaPhlAn3, the classification distribution of microorganisms at different levels, including Phylum, is obtained from metagenome samples. According to the reported results, laccase enzymes under plastic stress-contaminated environments play an effective role in plastic degra-

dation. Using metagenome data obtained from this study, the population of dominant microorganisms in the candidate environment is identified and by examining their genes, the candidate genes in plastic digestion can be identified. Also, by identifying plastic digestive enzymes, candidates are identified that, if expressed recombinantly, can be effective for plastic digestion and reduction of environmental pollutants.

Keyword: Laccase , Metagenome , plastic , polymer , Biodegradation

Discovery of Statins Targeting c-Met Kinase Domain using Molecular Dynamics Simulation Studies

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Abstract

Tyrosine kinase proteins with enzymatic ability are a novel therapeutic target for many cancers. Among these, deregulated activation of c-Met signaling pathway via multiple mechanisms such as activation, mutation, gene amplification and heterodimerization has been reported to correlate with high tumor grade and lower survival in most cancers. Thus, inhibition of HGF/c-Met axis has been proposed as a novel treatment to overcome cancer progress. To achieve novel small-molecule c-Met inhibitor, a docking approach was performed using Autodock 4.2 to screen statin molecules against the c-Met kinase domain. The best binding pose with the lowest binding energy was subjected to molecular dynamic (MD) simulations to elucidate intermolecular contacts in protein-ligand complexes. Analysis of MD simulations and MM/PBSA binding free energy uncovered that pravastatin provided the least binding free energy and reasonable hydrogen bonds while pitavastatin forms the least stable complex with c-Met receptor among whole statin family members. MD and MMPBSA results can contrive further studies over the anti-cancerous effects of statins to bring a bright future in cancer therapy

Keyword: c-Met inhibitor, Statins, Autodock 4.2, Molecular Dynamics, Binding free energy.

Genome-wide of 14-3-3 gene family in tomato

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Abstract

GF14 proteins are a family of conserved dimeric proteins that regulate several cellular processes, ranging from metabolism to transport, growth, development, and stress response. However, the little report is known about 14-3-3 genes in potatoes (*Solanum tuberosum*). In this study, twelve *14-3-3* genes were detected in the potato genome. Based on their phylogenetic relationships, the *StGF14* family members were categorized into two classes. The number of exons in *SlGF14* genes was from one to eleven and most of these genes in the same subfamily had the same exon-intron pattern. The intron-exon patterns validated the 14-3-3 gene family phylogenetic classification. Also, the intron-exon patterns validated the SlGF14 phylogenetic classification. Based on the results of genome distribution, SlGF14 genes were located unevenly on the 12 *S.lycopersicum* chromosomes. The results showed that gene duplication may play an important role in the genome expansion of *S.lycopersicum.* Furthermore, genome evolution of *S.lycopersicum* using orthologous and paralogous identification was surveyed. Our finding showed that were orthologous gene pairs between *S.lycopersicum* and *A.thaliana.* The results of this study will be useful in the investigation of the functional role and molecular mechanisms of GF14 genes in response to different stresses.

Keyword: Tomato, Phylogenetic, Paralogous, Orthologous, Duplication.

Genomine; Cloud-based platform for NGS data analysis

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Abstract

Next-generation sequencing has made major progress in the area of precision medicine and accordingly the number of studies based on large sequencing data sets is growing. Deeply investigation of these data requires researchers to use large-scale computational resources. In this regard, cloud computing can be a suitable method for genomics research whereby users can rent computers and storage from their data analysis. By developing an automated genomic data analysis pipeline on cloud computing platforms, studying genomics data from a huge amount of samples will lead to a more accurate understanding of normal and disease diversity. By applying this method combining different data types, global accessibility and availability, the security of the data, and high-performance data processing will no longer be a major issue. However, this approach requires technical knowledge and ever-growing compute and storage resources. Accordingly, our well- established product, "Genomine" is developed to achieve this objective. In Genome, raw NGS data particularly genomic data can be processed through automated well-defined workflows consisting series of steps as part of a pipeline that can be modified by users to transform into a form that is ready for analysis. In order to fulfill this objective, previously validated bioinformatics tools and pipelines such as GATK have been implemented on the cloud-based services which facilitate the process of NGS data analysis by users. As we know, the NGS market is highly competitive and growing with high speed, so having highly efficient and accurate platforms like Genomine that allow for fast adoption of new methods and technologies is critical in this area.

Keyword: NGS, Genome, genomic data, Genomine, Data analysis.

Genome Wide Identification And Characterization Of A Novel Antimicrobial Peroxidase From Ginger (*Zingiber officinale*)

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Abstract

Peroxidases are a large group of enzymes that catalyze various oxidative reactions and usually oxidize their substrate by transferring electrons to H2O2. Heme peroxidases are found in animals, plants and microorganisms and are generally divided into three groups (Animal peroxidases, Catalases and non-animal peroxidases) based on catalytic structure and properties. Plant peroxidases class III (EC 1.11.1.7, POX) is one of the most widely used enzymes known so far and is widely used in various fields such as industry, pharmacy, medicine, biotechnology, etc. Ginger (*Zingiber officinale*) is one of the most common spices in the world. Ginger has a long history of medicinal use which back to 2500 years ago. Some of the compounds found in ginger have strong antioxidant and anti-inflammatory activities, and some of them show cancer-preventing properties. In this study, a novel peroxidase of the Class III peroxidase family was identified from Ginger for the first time via NGS data analysis. For this purpose, the assembled whole transcriptome of Ginger was searched using Peroxidase Hidden Markov Model (HMM) and PHMMER software. Identified peroxidase candidate had a 966 bp coding sequence encoding a 321 amino acid protein. In addition, further analysis showed that the first 24 amino acids are a signal

peptide which after cleavage may result in a mature protein comprising 297 amino acids with a molecular weight of ~32 kDa and a theoretical isoelectric point of 7.66. Further analysis confirmed that identified peroxidase is a plant peroxidase class III with 44.34% identity to Horseradish peroxidase CA1. To the best of our knowledge, this is the first report of the Peroxidase gene from Ginger.

Keyword: Peroxidase, ginger, Bioinformatics, Next Generation Sequencing.

Genome-wide of U-box gene family and analysis of their expression growth of different stages in tomato

Abstract

The Plant U-box (PUB), ubiquitin ligase gene, has a highly conserved domain in tomatoes. However, little information is known about U-box genes in potatoes (*Solanum tuberosum*). In this study, the phylogenetic relationship, gene structure, and gene expression were investigated. In this study, 60 U-box genes were detected in the tomato genome. Based on *in silico* analysis, most of SlU-boxs included a U-box domain and some of these genes lacked harbored domain the ARM, Pkinase_Tyr, and other domains. Based on their phylogenetic relationships, the *SlU-box* family members were categorized into four classes. The gene expression profiles of U-box E3 family members show involvement in biotic stress as well as different developmental stages. We found remarkable participation of the *U-box* gene family in vegetative and reproductive tissue development. Our study provides a comprehensive picture of distribution, structural features, promoter elements, evolutionary relationship, and gene expression of the U-box gene family in the tomato. According to the results of genome distribution, *SlU-box* genes were located unevenly on the 12 *S.lycopersicum* chromosomes. The results of this study will be useful for further investigation of the functional role and molecular mechanisms of U-box genes in response to different stresses.

Keyword: U-box, ARM, tomato, gene structure, phylogenetic.

Investigation of PON1 Protein Function and Pathogenicity of its L55M-polymorph: An *in silico* Analysis Study

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Abstract

PON1, PON2 and PON3 are members of paraoxonase gene family which PON1 is the newest member of this family. PON3 shows high lactonase, low arylesterase, and nearly no paraoxonase activity. PON2 displays lactonase and very low arylesterase activity. PON1 is an antioxidant calcium-dpendent enzyme that appears to play an important role in the development of a large variety of diseases. This enzyme possesses three enzymatic activities: lactonase, arylesterase, and paraoxonase activity. PON1 is mainly synthesized in the liver, then transported from the liver to several tissues and released into the blood circulation in which it binds to cell membranes and protects lipids against peroxidation. L55M and Q192R are important functional genetic polymorphisms which are identified in PON1 gene which in coding region. In current study, we used different bioanformatics tools such as., I-Mutant 2.0, PolyPhen, SNP & GO, SIFT, MutPred, Hit Predict, STRING and PhD-SNP databases (tools) in order to study L55M polymorphism. Our studies, like other researchers, showed that the L55M motif effects on the paraoxonase activity of the PON1 protein and its stability. On the other hand, this motif has three phenotypes LL, LM and MM in different individuals, which in MM phenotype, addition to reducing the number of PON1 transcripts, also reduces the activity of this enzyme. Our study showed that the well-known polymorphism of L55M can lead to various diseases such as Parkinson's, Breast cancer, Inflammatory bowel disease (IBD) and infertility. This study could provide the way for further clinical studies.

Keywords: PON1, Polymorphism, Missense, Bioanformatics

Identification of pathogenic SNPs of *MLH1* gene using bioinformatics tools

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Abstract

Hereditary nonpolyposis colorectal cancer or HNPCC accounts for about 1-7% of colorectal cancer and has high mortality rate. Mutations in *MLH1*, responsible for repairing of DNA, have been reported in almost all of patients. Identification of cancer-related nsSNPs is crucial in facilitating future genetic studies and drug development, and using of bioinformatics methods is expanding. The aim of the present study was to determine the pathogenic SNPs of the *MLH1* and to investigate the effect of these mutations on the structure and function of the protein. For this purpose, the possibility of pathogenicity of 119 SNPs were predicted by four databases SIFT, PANTHER, PhD SNP and POLYPHEN and protein stability predicted by I-MUTANT. Then, 57 SNPs, predicted as pathogenic by all four databases, were evaluated by JPRED and PyMOL for their effect on the secondary and tertiary structures of mutant proteins, respectively. The results showed that in most cases, mutations increase the number of hydrogen bonds between the mutant residue and surrounding residues, increase the number of helixes, and change the number of beta sheets in the overall structure of the mutant protein. Also, conformational changes in one region of the protein can alter the overall stability of the protein and reduce its stability. In general, in this study, using bioinformatics tools, pathogenic mutations that alter the structure and consequently function of the MLH1 protein were identified. Identification of these SNPs will greatly contribute to the genetic and therapeutic studies of patients with inherited non-polyposis colorectal cancer in the future.

Keywords: Colorectal cancer, SNP, *MLH1*, Bioinformatics

Single-cell transcriptomics in cancer: computational challenges and opportunities

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Abstract

Intratumor heterogeneity is a common characteristic across diverse cancer types and presents challenges to current standards of treatment. Advancements in high-throughput sequencing and imaging technologies provide opportunities to identify and characterize these aspects of heterogeneity. Notably, transcriptomic profiling at a single-cell resolution enables quantitative measurements of the molecular activity that underlies the phenotypic diversity of cells within a tumor. Such high-dimensional data require computational analysis to extract relevant biological insights about the cell types and states that drive cancer development, pathogenesis, and clinical outcomes. In this talk, we will highlight emerging themes in the computational analysis of single-cell transcriptomics data and their applications to cancer research.

Keywords: cancer, opportunities, computational challenges

Plant Biotechnology

Biotechnology and Religion
Bioethics
Biotechnology Law and Intellectual Property Right
Biotechnology policy, Regulation and Biosecurit
Biotech Commercialization

The effect of 2,4-D on callus growth rate in different explants of Silene conoidea

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Abstract

Plant tissue culture technology is widely used for plant propagation for commercial applications. The choice of explants along with the selection of appropriate plant growth regulators has a significant effect on callus induction as well as regeneration of shoots. In this study, for callus induction, leaf, root and hypocotyl explants of Silene conoidea were cultured on MS medium containing different concentrations of 2,4-D and it was observed that all concentrations of 2,4-D resulted in 100% callus induction in root explants. Culture of leaf explants at all concentrations of 2,4-D except 0.5 mg / l resulted in 100% callus induction. Based on the results, the highest and lowest fresh weight of callus were also associated with root explants cultured at 1 mg / l and hypocotyl explants cultured at 4 mg / l (mean 0.27 and 0.0133 g, respectively).

Keyword: Callus, in vitro culture, plant growth regulators, *Silene conoidea.*

The effect of plant growth regulators on callus induction in leaf explants of *Silene conoidea*

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Abstract

Silene conoidea belongs to the family *Caryophyllaceae*. This plant has high medicinal value as various parts of it are used as herbal medicine to treat various diseases. Nowadays, medicinal plants have more therapeutic effects and are of particular importance due to lack of side effects and greater biocompatibility with human tissues. Plant tissue culture technology is widely used for plant propagation for commercial applications. To induce callus, *Silene* leaf explants were cultured on MS medium containing different concentrations of 2,4-D and BA and it was observed that most of the hormonal compounds used resulted in 100% callus induction in leaf explants. Moreover, the callus growth rate was highest in MS culture medium containing 1 mg / l 2,4-D and 0.1 mg / l BA (mean 0.420 g).

Keyword: Callus, in vitro culture, plant growth regulators, *Silene conoidea.*

Effect of *Faecalibacterium prausnitzii* on RUNX gene expression involved in EMT in colorectal cancer cell-line

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Abstract

Epithelial-mesenchymal transition (EMT) is a morphogenetic program, undertaken by embryonic and adult cells. This change in cell behavior involves the loss of epithelial characteristics and the acquisition of migratory properties. The EMT process is an initial determining step in the metastatic cascade. The RUNX family of transcription factors are important players in cell fate determination. Their expression often overlaps with the occurrence of EMT. Dysregulation of RUNX expression and functions are increasingly linked to the aberrant induction of EMT in cancer. *Faecalibacterium prausnitzii* is a commensal bacterium in human intestinal tract with potential therapeutic and anti-inflammatory effects. The aim of this study was to evaluate the effect of *F. prausnitzii* on RUNX expression in colorectal cancer cell line (HCT-116). HCT-116 was treated with *F. prausnitzii* strain A2-165. RNA extraction was done using TRIzol®. cDNA was synthesized and Real-time PCR was done. Analysis of data showed that in *F. prausnitzii* treatment sample, RUNX2 and RUNX3 expressions were down-regulated compared to control. However, RUNX1 expression was up-regulated notably. Therefore *F. prausnitzii* had suppressive effects on RUNX2 and RUNX3 expression, but strongly induced RUNX1 expression. The results demonstrated that *F. prausnitzii* could be an EMT interferer, given that RUNX2 and RUNX3 are positive regulators of Snai2 (EMT's primary inducer). On the other hand, the precise transcriptional program maintained by RUNX1 remains unknown; So there are no certainties for RUNX1's effect on EMT. However, further investigations are

necessary for this potential bacterial treatment in clinical practices, especially as metastasis suppressor in cancer therapy.

Keyword: Gut microbiota, Transcription factors, Epithelial-Mesenchymal Transition (EMT), Colorectal Cancer (CRC), Real-time PCR.

Direct and indirect regeneration in black cumin medicinal plant (*Nigella sativa*)

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Abstract

Nigella sativa L. belonging to the family of Ranunculaceae, annual plant and diploid (2n = 2x = 12) is one of the important medicinal plants that produces many types of secondary plant metabolites including terpenes. In the present work of direct and indirect regeneration of black cumin was examined. After seedling growth, seed, root, stem and leaf explants of 0.5 cm diameter were used to induce callogenesis. The explants were cultured on MS or half MS medium containing different concentrations of hormone at 6 different treatment levels. Cultures were placed at 26 ℃ and 16 h light and 8 h dark condition. *In vitro* cultured samples were counted regularly and the number of explants that produced callus was counted and the percentage of callus formation was calculated for each treatment and explant type separately. The experiment was performed in three replicates with four explants. After 3 to 4 subcultures, the grown calluses were transferred to the culture medium for regeneration. The culture medium was used for regeneration, MS basic medium with different levels of BA and Kinetin (1BA + 0 KT), (0BA + 1KT), (0.5BA, 0.5KT), (1 BA + 1KT). After transferring calli to the regeneration medium, the samples were exposed to 26 ℃, 16 h light and 8 h dark condition. Results showed successful direct and indirect regeneration of black cumin.

Keyword: Callus, Regeneration, Black cumin, Hormone

Effect of explant type and plant growth regulators on in vitro callus induction in Pelargonium quercetorum Agnew

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Abstract

Pelargonium quercetorum Agnew is a medicinal plant belonging to the family *Geraniaceae*, which is found in northwestern of Iran, especially in Kurdistan province. Extracts and essential oils of this plant are economically valuable and used in the pharmaceutical, cosmetic and food industries. Today, *in vitro* culture techniques, mainly callus production, are highly appreciated in terms of production of secondary metabolites, plant propagation, plant breeding and genetic manipulation, and are considered as one of the new methods in plant biotechnology. In the present study, the effect of explant type and different concentrations of 2,4-D and NAA in combination with BAP was investigated on *in vitro* callus production in this species. Based on the our results, combinations of 0.5 mg/L 2,4-D and 0.5 mg/L BAP, 1 mg/L 2,4-D and 0.5 mg/L BAP were introduced as the most efficient hormonal treatments in callus induction from all three studied explants of cotyledon, hypocotyl and leaf. In addition, the cotyledon explants had a higher ability to induce callus compared to other explants. In general, according to the growth index of callus tissues, the highest fresh weight of calli in cotyledon, hypocotyl and leaf explants was obtained in combinations of 0.5 mg/L BAP and 0.1 mg/L NAA, 2 mg/L BAP and 0.1 mg/L NAA, as well as 2 mg/L 2,4-D and 0.5 mg/L BAP, respectively. To sum up, our findings can be used to study the secondary compounds present in callus, to investigate the possibility of indirect regeneration, to induce polyploidy and to study other aspects related to *in vitro* callus formation.

Keyword: Explant, Hormonal combinations, Callus, *Pelargonium quercetorum* Agnew

Identification of informative markers using regression analysis association between tree characteristics and molecular markers in ber

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Abstract

Ber (*Zizphus spina-christi*) is a fruit tree species of Rhamnaceae family. This tree is considered as a hardy plant species to various environmental stresses and hence is a good choice for land validation and agriculture extension in the vicinity of non-arable lands.

The main purpose of this work was to investigate the association of phenotypic traits and molecular markers in *Z. spina-christi* for probable identification of informative markers and subsequent utilization in ber improvement programs.

In the present study four *Z. spina-christi* populations including 41 wild accessions were evaluated using seventeen fruit and tree morphological traits and 161 SCoT markers. The SPSS software was used to investigate trait-marker connection using multiple regression analysis (MRA) by using molecular data as independent variables and phenotypical attribute as dependent variables.

High level of association was detected between different morphological traits and SCoT amplicons. SCoT31.4 was one of the fragments that showed significant association with some of the fruit related traits such as fresh and dry weight as well as fruit and stone dimensions. Fruit moisture and dry weight matter were somewhat associated to the SCoT23-2 marker. Moreover, leaf length and leaf width showed significant association with SCoT20-4 and SCoT28-3, respectively.

Some of the SCoT fragments showed to be linked with a number of traits indicating there are pleiotropic effects about those traits. Results of present study offer an unprecedented clue for marker assisted selection (MAS) and subsequent ber improvement. Some of the identified fragments show to be highly promising for development of more precise and co-dominant sequence-characterized amplified regions (SCARs) markers, which could be highly beneficial in ber breeding programs where no other genetic information is available.

Keyword: Ziziphus spina-christi; association analysis; morphological traits; molecular markers

Investigation of ISSR Markers for predict Genetic Diversity in Juniper Populations

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Abstract

Juniperus is a durable and evergreen genus of conifer that exist in ecological landscape in Southern Iran. In this study efficiency of inter simple sequence repeat (ISSR) markers were investigated for detect genetic diversity of juniper populations that grown in Southern Iran (Fasa, Sepidan, Khabr, Rabor, and Genow). Twelve ISSR markers were assessed for genetic diversity detection. leaf sample of 10 diverse accessions were collected of population of each habitat (totally 50 accessions) for DNA extraction. Results of ISSRs investigation were shown all markers amplifications predicted 75 total loci (TL) in 100–1000 bp for all investigated accessions of Juniperus. Overall, 89.33% of all predicted loci were shown polymorphic. Results were shown difference in total loci and polymorphic loci among all amplified ISSR primers. The mean fraction of polymorphism (FP) for evaluated primers is 0.88, results shown FP in a range of the lowest 0.60 to the highest 1. Two primers (UBC812 and UBC835) had the highest Polymorphic information content (PIC) (0.50) and UBC825 has the lowest PIC (0.38), mean PIC for all primers was 0.45. Results of ISSR primer index (ISPI) are shown the UBC807 primer had the highest and UBC810 had the lowest ISPI. Mean suggested ISPI for evaluated markers in this study is 2.38. The highest resolving power (Rp) was exhibited by UBC807 (5.72) and the lowest was belonged to UBC810 (2.16), and total mean Rp was 3.78. Therefore, multiplex ratio (MR), effective multiplex ratio (EMR), and marker index (MI) were observed 6.25, 5.50, and 2.47 respectively.

Keyword: Assessment, Marker, *Juniperus*, Population

Identification of polyphenol oxidase (PPO) gene in grass pea (Lathyrus Sativus)

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Abstract

Polyphenol oxidases (PPOs) are copper-containing enzymes that use oxygen to oxidize common orthodiphenolic compounds. These enzymes are involved in the plant's defence against biological and abiotic stresses.

In this study, the polyphenol oxidase (PPO) gene was isolated and identified in Grass pea (*Lathyrus sativus* L.), an annual and diploid (2n = 2x = 14) pulse crop, for the first time. Therefore, sampling from grass pea plant (*Lathyrus sativus* L.) (Golestan population) was carried out in 50% podding stage and drought treatment (25% of field capacity – FC) with three replications. Following total RNA isolation from leaves using Ribospin Plant kit, cDNA of interest gene was synthesized. Then, the PPO gene was amplified by polymerase chain reaction (PCR) with degenerate primers. The PCR product was analyzed by agarose gel electrophoresis for insert size, amplification quality, and quantity.

Consequently, the gene was sequenced. The result indicated that PPO was expressed in leaves cultured in drought treatment. This gene was confirmed via two methods, PCR and sequencing. Sequencing revealed that the partial coding region of the PPO gene from Iranian native *Lathyrus sativus* has 100% similarity with the coding region of *Medicago sativa* in NCBI. This gene is recorded in NCBI with an MT210156.1 accession number.

Keyword: Grass pea, *Lathyrus sativus*, polyphenol oxidase (PPO), Degenerate primers

Effect of plant growth regulators on proliferation of anise (Pimpinella anisum L.)

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Abstract

Anise is a valuable medicinal plant with the scientific name of Pimpinella anisum L from the genus Apiaceae (Umbelliferea and genus Pimpinella. About 80 to 90% of its essential oil compounds are anethole. Many studies have been done on its pharmacological properties, essential oils and oils. The aim of this study was optimization of direct regeneration of this plant in an in vitro condition. In this research, different methods for germination of plant seeds were first investigated. Then different disinfection treatments were tested to decontaminate the explants. The effect of different compounds of BA, Kin and NAA on the regeneration of hypocotyl bud explants was tested with three replications. These experiments were performed in a completely randomized design. The best germination response of 70% was obtained in 24 hours of seed hydro priming and disinfection with 70% ethanol and 1.5% sodium hypochlorite in 1.2 MS medium. Also, the best direct regenerative response in terms of petiole length is related to MS supplemented with 0.5 mg/l BA + 0.5 mg/l Kin + 0.5 mg/l NAA with 4.33 cm and in terms of leaves number was related to MS supplemented with 0.5 mg /l Kin + 1 g/l and 0.25 mg/l BA + 0.25 mg/l Kin + 1 mg/l NAA with 47 leaves were obtained. Regeneration of this plant can be a prerequisite for further studies

Keyword: Anise*, Pimpinella anisum*, direct regeneration, BA, Kin, NAA.

Effects of precursor feeding with ornithine on phytochemical characteristics of Hyoscyamus reticulatus L. hairy roots

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Abstract

Different species of Hyoscyamus contain metabolites such as tropane alkaloids (hyoscyamine and scopolamine), flavonoids, chromogenic acid and tannins. Various biotechnological methods such as hairy root have been used to increase the production of important medicinal compounds. In the present study, the effect of different ornithine precursor concentrations (0, 3, 5 and 10 mM) at different exposure times (24, 48 and 72 h) on phenolic and flavonoid content, antioxidant capacity and tropane alkaloids production in *H. reticulatus* hairy roots were investigated. The highest total phenol (12.67 mg GAE per g FW) and flavonoid (1.54 mg QUE per g FW) content were obtained in hairy roots were fed with 10 mM ornithine during 72 hours of exposure time and control, respectively. The highest antioxidant capacity by IC50 and FRAP methods (1.36 µg/ml and 13.92 mmol Fe^{+2} g^{-1} FW, respectively) was observed at 5 and 10 mM ornithine and 24 hour of exposure time. HPLC results showed that the highest levels of hyoscyamine (35.15µg. g^{-1} DW) and scopolamine (94.3 µg. g^{-1} DW) were obtained in hairy roots fed with 5 mM ornithine concentration after 24 hours, which was 1.39 and 1.23 times than control. According to results, the ornithine amino acid can be used as effective precursor to improve the plant secondary metabolites, such as tropane alkaloids production.

Keyword: Hyoscyamine, Ornithine, Precursor, scopolamine

Cytotoxicity and Apoptosis Investigations of Purslane (Portulaca oleracea) Alcoholic Extract in AGS Gastric Cancer Cell Line

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Abstract

Cancer is one of the most important problems in medical science and patients with this disease are increasingly spreading. Due to the need to find the effective methods and drugs with the least complication in the treatment of cancer, herbal medicines are characterized as one of the most approved suggestions. Several studies represented the beneficial effects of Portulaca oleracea and its compounds in inflammatory conditions including toxicity and apoptosis. For this reason, the effect of this plant on the cytotoxicity of the AGS cell line of the gastric cancer model, as one of the most common and deadly cancers in the country and the world, was considered. furthermore, due to the major role of apoptosis in cancer treatment, as one of the mechanisms of action for drugs, stimulation of the BCL-2 and BAX genes expression in inhibiting and regulating the apoptosis in AGS gastric cancer cell line by Portulaca oleracea extract was designed and proposed.

Methanolic extract of aerial parts of the Portulaca oleracea plant (leaves and stems) was collected and the AGS cells were then treated with different concentrations of this extract. First of all, MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) method was used to evaluate the cell viability rate and to determine the optimal dose concentration after 24 and 48 hours treatment. The treated cells were then collected at the optimum dose concentration obtained from the MTT assay for RNA extraction followed by cDNA synthesis. Finally, Real-time PCR was per-

formed using the BAX and Bcl-2 apoptotic genes. Finally, an annexin V-PE kit for flow cytometry assay was also used to evaluate the apoptosis level. Each experiment was performed with three replications and the results were statistically analyzed by Prism software.

The IC50 inhibitory concentration of cytotoxicity assay for AGS cells was approximately 4 mg/ml and 2 mg/ml in 24-hour and 48-hour incubation time respectively. Real time analysis showed that BAX gene expression was significantly increased and BCL-2 gene expression was significantly decreased in AGS cell line compared to the control group. The IC50 extract dose concentrations also could induce significant apoptosis levels in the AGS cell line.

The cytotoxicity of Portulaca oleracea extracts on AGS cell line represented valuable information in the cancer treatment process. Utilization of this plant can introduce a significant method to induce the apoptosis pathway in cancer cells (which is the most effective mechanism in cancer cell suppression). This study proposes a potentially attractive plant for further experimental research to evaluate the comprehensive estimations to introduce an effective drug in cancer treatment.

Keyword: AGS Gastric Cancer, Purslane (*Portulaca oleracea*), BCL-2 and BAX Genes, Apoptosis .

The central role of salicylic acid in control of Fusarium head blight in wheat and its importance in agricultural biotechnology

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Abstract

Fusarium head blight is a disease of cereal crops caused by a group of trichothecene producing Fusarium species such as *Fusarium graminearum*. It is the main disease of wheat in different areas of Iran, such as Mazandaran, Gorgan and Moghan regions. Wheat has evolved complex mechanisms to defend against the pathogen. Salicylic acid has been shown to have a central role in defense against Fusarium head blight in wheat. In this review, we aim at outlining the studies conducted on Fusarium head blight in wheat with the emphasis on the SA role in resistance against the pathogen. Exogenous application of SA can activate the plant defense mechanisms before pathogen attack without environmental side effects of protective chemical agents. In addition, a summary of the physiological and molecular responses of wheat to exogenous application of SA were provided.

Keyword: Fusarium head blight, Trichothecene, Salicylic acid, Wheat, Defense mechanisms.

Quinoa, a review on genetic diversity and some applications in biotechnology

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Abstract

Quinoa (Chenopodium quinoa Willd.) is a pseudo-cereal grain originating from the Andean region in and one of the main nutrition of the ancient inhabitants of Andes. In the late 1970s, quinoa production in South America began to revive for both domestic consumptions and exportations. Due to its high-quality protein content and balanced essential amino acids, quinoa has received an engrossed attention in the rest of the world; so that 2013 was named the International Year of Quinoa. Quinoa is well adapted to different latitudes and production under adverse environmental conditions such as drought and salinity, heights and plains, range of 40% to 88% humidity, and -4 to 38°C temperature. Hence, quinoa crop plays an important role in food security due to its wide genetic diversity and adaptation to a vast range of agricultural conditions. In addition to nutritional value, two important compounds within the quinoa seeds, starch and saponin, have recently been used in biotechnology applications. Starch, as the main component of quinoa's seed, comprises 30 and 70% of dry matter and is used as a bioplastic polymer or the nanoparticle's shield for therapeutic compounds encapsulation. Saponin is a component of episperm with a bitter taste and is removed for the seed consumption and the quinoa is divided into sweet (<0.11% w/w saponin) and bitter (>0.11% w/w saponin) crops based on the saponin content. The saponins are also been used as biocompatible natural surfactants in biotechnological industry.

Keyword: Quinoa, Genetic diversity, Genetic modification, Saponin, Starch.

Hairy root induction in *Securigera securidaca by Rhizobacterium rhizogenes*

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Abstract

Securigera securidaca (L.) that belongs to Fabaceae family has been used as a medicinal plant due to its therapeutic effects. This important plant possesses many vital metabolites such as flavonoids, alkaloids, saponins, steroids, tannins, coumarins, and cardiac glycosides Secondary metabolites is very low in the plants biomass and traditional methods are not cost-effective for obtaining this limited amount of pharmaceutical metabolites. Due to this, various plant tissue cultures such as cell suspension, callus culture, and hairy root culture have been developed.

In this study, *Rhizobacterium rhizogenes* strain A13 was used for induction of hairy roots in different plantlets of *S. securidaca* (meaning hypocotyles, cotyledons, and seedlings). There was a significant difference in the number of induced hairy roots of different plantlets. The number of induced hairy roots in cotyledons and seedlings was doubled compared to the hypocotyledons.

Transgenic status of the hairy roots was confirmed by PCR using *rol* A-B-specific primers. Among the obtained hairy root lines, the line L12 was selected as the fastest growing clone for making higher yield of biomass and the production of pharmaceutically important metabolites of *S. securidaca.*

Keyword: Securigera securidaca, hairy root, explant, Agrobacterium.

Application of fingerprinting method in diversity and distinction of flavonoid markers in *Stachys* species

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Abstract

The genus *Stachys* L. belonging to Lamiaceae is a rich source of secondary metabolites. Due to the fact that there is no specific taxonomic boundary in some species of this genus, they are considered as complex groups. Consequently, this research aimed to identify the diversity and distinction of flavonoid markers in three *Stachys* species belonging to section *Fragilicaulis*. Accordingly, nine accessions were collected from different natural habitats from center, west and south-west of Iran. The extracted flavonoids of dried leaf from each species were investigated through thin layer chromatography with BuOH-C_2H4O_2-H_2O solvent system, column chromatography and high performance liquid chromatography based on mass spectrometry (HPLC-MS/MS). In order to study the differences between species, cluster analysis with UPGMA method and PAST 3.14 was used. According to the results of this research, a total of 22 flavonoid compounds were identified from which 14 compounds were considered as species markers. Some of these markers were assigned to bi-apigenin and dihydroxy-isoflavone hexoside in *St. kermanshahensis*, quercetin-hexoside and trihydroxy-dimethoxy flavone in *St. megalodonta*, and trihydroxy-prenyl isoflavone and pentahydroxy-methoxy flavone in *St. graveolens*. Moreover, the highest flavonoid diversity was observed in *St. graveolens* with 11 compounds. The results of cluster analysis indicate the separation of three studied species. Consequently, the complexities of three taxa have been exactly resolved using flavonoid markers and HPLC-MS/MS technique. Moreover, the proposed species markers can be used for purposes of pharmaceutical technologies.

Keyword: Mass spectrometry, diversity, *Stachys*, Lamiaceae, flavonoid marker

The Effect of nano Tio2 elicitor on phenolic and flavonoid compounds of the *Crataegus Oxyacantha* on *in Vitro* Conditions

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Abstract

The genus *Crataegus* L. are deciduous trees or shrubs belonging to the Rosaceae family. This experiment was conducted with the aim of effecting titanium dioxide nanoparticles on increasing the production of phenolic and flavonoid compounds in Crataegus hawthorn cell suspension culture. Plant growth regulator 2, 4-D (0, 1, 2 and 4 mg/l) were used for callus production on MS medium. TiO_2 elicitor (0, 100 and 150 µmol) for 24 and 48 hours were used in cell suspension culture. Three replicates were considered for each experiment. The results of this study showed that wet and dry callus weight was influenced by different concentrations of 2.4-D growth regulator and showed a significant difference at 1% probability level. Concentration of 1 mg/l of this regulator had a significant difference from 2 and 4 mg/l. The results of ANOVA for phenolic and flavonoid compounds in response to Tio_2 at different times of cell harvesting also showed that the interaction of Tio2 × time had a significant difference at 5% level on rutin flavonoids and quercetin. Tio2 nanoparticle had a greater effect on the increase of phenolic and flavonoid compounds at lower concentration and 100 µM and time 24 h.

Keyword: Elicitor, Callus, Cell suspension, secondary metabolites

Effect of plant growth regulators on in vitro callus production of coffee plant (Coffea Arabica)

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Abstract

The coffee plant is an evergreen tree called coffea. The plant is a flowering plant of the *Rubiaceae* family that grows in the tropics regions. In order to investigate the effect of plant growth regulators on callus induction and in vitro production of coffee plant in MS culture medium, different growth regulators including kin and 2, 4-D at a concentration of 2 mg /l were used to induce calluses. Callus size, fresh and dry weight of calluses were recorded. In the results, the best growth regulators that callus production were combination of Kin + 2,4-D (2 + 2) mg / l.

Keyword: Tissue culture , callus , coffea, plant growth regulators .

Evaluation of the effect of different treatments on germination of Stachys infalata Benth

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Abstract

The medicinal plant, *Stachys inflata* Benth belongs to the Labiatae family. This herb has valuable secondary compounds that are widely used in traditional medicine. Germination is one of the most important stages of plant growth that in natural environments may be affected by various stresses. This plant has a long dormancy period and its mass reproduction in *in vitro* conditions requires the adoption of a strategy to overcome its dormancy period. In this regard, in order to break the dormancy of the seeds and evaluate its germination rate, an experiment with 14 different treatments was performed. Treatments were seed treatment between wet filter paper and culture in 1/2 MS in two temperature conditions (4 and 22 ° C), 500 ppm gibberellin in 24, 48, 72 and 96 hours, seed preservation in wet sand at 4 ° C and combined treatment of 500 ppm gibberellin in 24, 48, 72 and 96 hours in combination with cold treatment for 2 months and embryo culture. The results of this study showed that the highest germination percentage was acheived from embryo culture in 1/2 MS for one week.

Keyword: Germination, Culture medium, Gibberellic acid, Embryo culture, *Stachys inflata* Benth.

The effect of phosphorus on the content of storage proteins in rice grain of Neda cultivar

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Abstract

Phosphorus participates as an essential element in the structure of nucleic acids, phospholipids, proteins, as well as providing the energy needed for processes such as photosynthesis, respiration and nutrient uptake. The present study was performed to evaluate the effect of different levels of phosphorus on germination and the amount of rice grain proteins in the modified cultivar Neda. For this purpose, after disinfection, rice seeds were placed in petri dishes to germinate at 30 °C in the dark. Then, germination percentage and rate, phosphorus content (spectrophotometric method using Barton reagent) and protein content (Bradford method) in different concentrations of phosphorus were examined in 4 replications and 40 seeds per replication. The results showed that the germination percentage was significantly decreased with increasing phosphorus concentration (at concentrations of 160 and 240 kg/ ha). While germination rate did not show any significant differences. Phosphorus content in rice grains also responded positively to the increasing of the content of phosphorus (160 kg/ ha and above that), which was followed by a decrease in total protein content of 24% and 42% at concentrations of 160 and 240 kg/ ha, respectively. Gluten protein was comprised 62% of total grain protein, which was reduced by 15% and 47% at 160 and 240 concentrations, respectively. Globulin protein at concentrations of 160 and 240 was significantly reduced by 35% and 23%, respectively. The content of albumin and prolamin did not show a statistically significant difference between different concentrations.

Keyword: Rice, phosphorus, germination, protein, seeds

Cytotoxicity and Apoptosis Investigations of Purslane (*Portulaca oleracea*) Alcoholic Extract in AGS Gastric Cancer Cell Line

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Abstract

Cancer is one of the most important problems in medical science and patients with this disease are increasingly spreading. Due to the need to find the effective methods and drugs with the least complication in the treatment of cancer, herbal medicines are characterized as one of the most approved suggestions. Several studies represented the beneficial effects of Portulaca oleracea and its compounds in inflammatory conditions including toxicity and apoptosis. For this reason, the effect of this plant on the cytotoxicity of the AGS cell line of the gastric cancer model, as one of the most common and deadly cancers in the country and the world, was considered. furthermore, due to the major role of apoptosis in cancer treatment, as one of the mechanisms of action for drugs, stimulation of the BCL-2 and BAX genes expression in inhibiting and regulating the apoptosis in AGS gastric cancer cell line by Portulaca oleracea extract was designed and proposed. Methanolic extract of aerial parts of the Portulaca oleracea plant (leaves and stems) was collected and the AGS cells were then treated with different concentrations of this extract. First of all, MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) method was used to evaluate the cell viability rate and to determine the optimal dose concentration after 24 and 48 hours treatment. The treated cells were then collected at the optimum dose concentration obtained from the MTT assay for RNA extraction followed by cDNA synthesis. Finally, Real-time PCR was performed using the BAX and Bcl-2

apoptotic genes. Finally, an annexin V-PE kit for flow cytometry assay was also used to evaluate the apoptosis level. Each experiment was performed with three replications and the results were statistically analyzed by Prism software.

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The cytotoxicity of Portulaca oleracea extracts on AGS cell line represented valuable information in the cancer treatment process. Utilization of this plant can introduce a significant method to induce the apoptosis pathway in cancer cells (which is the most effective mechanism in cancer cell suppression). This study proposes a potentially attractive plant for further experimental research to evaluate the comprehensive estimations to introduce an effective drug in cancer treatment.

Keyword: AGS Gastric Cancer, Purslane (*Portulaca oleracea*), BCL-2 and BAX Genes, Apoptosis

Phylogenetic analysis of some Salvia species based on 18S ribosomal RNA gene sequences

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Abstract

Nucleic acid sequences from small-subunit ribosomal RNAs (18S rRNA) have proved useful for phylogenetic analysis in eukaryotes. *Salvia mirzayanii* and *Salvia macrosiphon* are medicinal and aromatic plants belonging to the Lamiaceae family, which have many pharmaceutical properties. The aim of this study is the genetic diversity and phylogenetic relationships of two mentioned species with related species in NCBI database. In the present study, the nucleic acid sequences of small-subunit ribosomal RNAs (18s rRNA) were isolated from *S. mirzayanii* and *S. macrosiphon* and then the phylogenetic relationships of these two species with 29 related Salvia species were investigated. Results show that all Salvia species used formed three major clades based on world distribution and morphological characters. Clade I consisted of 21 species in two subclades (subclade A and B) separately. The two studied species and 9 other species were identified in clade I, subclade B. Interestingly, several of the species in this subclade including *S. mirzayanii*, *S. macrosiphon*, *S. scalerea*, *S. aethiopis* and *S. verbascifolia* are all endemic plants in Iran. In conclusion, the small subunit rRNA (18S) has played a dominant role in the informative estimation of relationships among Salvia species from molecular data.

Keyword: Molecular systematic, *Salvia mirzayanii, Salvia macrosiphon,* medicinal plant

Alignment and phylogenetic tree analysis of some Iranian and Chinese wheat cultivars based on DREB partial gene sequences

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Abstract

Dehydration responsive element binding (DREB) protein is a subfamily of AP2/ERF transcription factors which control expression of many osmotic stress-inducible genes. Eight bread wheat (*Triticum aestivum* L.) Iranian and ten Chinese wheat cultivars DREB partial gene sequences were taken from NCBI database. Alignment was done by multalin software. Phylogenetic tree was drawn by neighbor-joining method. The Iranian TaDREB DNA sequences were about 500 bp in length. The alignment result displayed that the Iranian sequences and the Chinese sequences are very similar near to 90 percentage. Phylogenetic tree shows that the Chinese sequences were completely separated from Iranian sequences but the Iranian sequences are subdivided to seven groups and the Chinese sequences to only three groups. It means that the Iranian sequences have more biodiversity.

Keyword: Alignment, Phylogenetic tree, Wheat, Biodiversity, NCBI database

Interaction effects of root endophyte fungus and zinc oxide nanoparticle on growth and total phenol of garden cress (*Lepidium sativum*)

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Abstract

The root endophytic fungus *Piriformospora indica* stimulates plant growth and increases its resistance to environmental stresses. In addition, the role of nanoscale fertilizers in sustaining agricultural goals has been reported. In this study, the effects of *P. indica* and zinc oxide nanoparticles on some growth indices of *Lepidium Sativum* were investigated, in 1.5 kg pots during growth period 60 days. Two levels fungus (presence of the fungus and absence of fungus) and five levels zinc oxide nanoparticles (0, 5, 10, 15 and 20 mg/L as spray at 15-day intervals) were applied as the treatments. In plants without fungal treatment, the application of zinc nanoparticles at some levels, especially at the level 5 mg/L, had a positive effect on shoot and root dry weights. Presence of endophyte fungus significantly increased shoot and root dry weights under most levels of nanoparticle. Maximum level of total phenol in non-inoculated plants, was observed at 20 mg/L nanoparticle. Presence of the fungus increased total phenol content under all levels of nanoparticle, particularly in 10 and 20 mg/L. According to the results of this experiment, it can be said that *P. indica* as a biofertilizer increases the growth indices and total phenol of the *L. sativum*. Also, zinc oxide nanoparticle increases the plant dry weight at the level of 5 mg/L and total phenol under 20 mg/L.

Keyword: *Piriformospora indica*, *Lepidium Sativum,* Zinc oxide nanoparticle, Dry weight

The changes in antioxidant enzymes activity of garden cress under Piriformospora indica and zinc nanoparticle treatments

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Abstract

Root endophytic fungus *Piriformospora indica*, with similarities to arbuscular mycorrhizal fungi interacts with roots of many different plants and promotes growth and nutrient uptake, and allows plant to survive under biotic and abiotic stresses. Nano-fertilizers are superior to conventional fertilizers due to the slow and controlled release of nutrients. This greenhouse experiment was conducted to examine the changes in antioxidant enzyme activities of garden cress plants inoculated with *P. indica* fungus under varying levels of zinc oxide nanoparticles (0, 5, 10, 15 and 20 mg/L as spray at 15-day intervals). The sampled shoots and roots at 60 days after sowing were measured for activity of the antioxidant enzymes. In plants without fungal treatment, the highest activity of root ascorbate peroxidase and shoot catalase was at 5 mg/L, root catalase at 5 and 15 mg/L and shoot ascorbate peroxidase at 0 mg/L nanoparticles. Presence of endophytic fungus increased the activity of catalase and ascorbate peroxidase of shoot and root at all nanoparticle levels. Also, in fungus treated plants, the highest activity of shoot ascorbate peroxidase was at 0, root ascorbate peroxidase at 5, shoot catalase at 5 and root catalase at 15 mg/L nanoparticles. The results showed that the coexistence of garden cress plants and *P. indica* along with zinc oxide nanoparticles application (especially at 5 mg/L), has an important role in increasing the activity of plant antioxidant enzymes.

Keyword: Nano-fertilizer, Garden cress, endophyte fungus, antioxidant enzyme

Assessment of growth regulator on In vitro micropropagation of *Streptocarpus* x *hybridus* as ornamental plant

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Abstract

The use of tissue culture technology can be one of the most desirable methods of rapid simplification of ornamental plants in a short time and limited space. The effect of different growth regulators on shoot production and rooting rate was measured. Streptocarpus x hybridus Ladyslippers cultivar young leaves were collected, and after disinfection, were divided into pieces with dimensions of 1cm*1cm. Therefore, effect of different combination of BAP and IBA on shoot regeneration from leaf segments was investigated. Subsequently, different root induction mediums including MS with different levels of NAA and MS without growth regulators were investigated. The obtained data was analyzed by factorial experiment in a completely randomized design with 4 replications. The results showed that 1 mg/l BAP in combination with 0.5 mg/l IBA is most suitable for shoot production. A better performance for root induction happened in MS medium with 0.5 mg/l NAA with an average of 26.23 roots per plantlet. Acclimation of rooted plants were done successfully. The system developed in this study, due to the high efficiency of this protocol for plant regeneration offers new possibilities for micropropagation of Streptocarpus plants.

Keyword: *Streptocarpus* x *hybridus, Leaf explant,* shoot proliferation, Auxin, Cytokinin

In vitro plant regeneration from ornamental plant of Gloxinia (*Sinningia specios*)

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Abstract

Gloxina is a genius of the family Gesneriaceae and has been grown as an ornamental plant due to its attractive flowers. Because it is difficult to obtain large numbers of this plants by conventional methods, the aim of this study is to establishment of a Convenient and efficient combination of growth regulator in order to in vitro micropropagation of this plant from leaf explants. For this purpose, young leaves of *Sinningia speciosa* were isolated and after surface sterilization, cutted into pieces measuring 1 cm^2 and placed in the MS medium containing different levels of BAP and IBA growth regulators with the adaxial side. The results of this study showed that 2 mg/l BAP and 0.5 mg/l IBA were the most efficient treatments in shoot proliferation from the leaf explants of this plant. Plantlets rooted on the MS medium with 0.1 mg/l NAA and acclimation were done successfully. The system developed in this study, due to the high efficiency of this protocol for plant regeneration offers new possibilities for micropropagation of Gloxinia plants.

Keyword: *Sinningia speciosa*, leaf explant, shoot proliferation, growth regulator

Graphene Nanoparticles: A Biodegradable and Efficient nano-additive for Increasing of Asexual Embryogenesis of Date Palm

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Abstract

The use of nanotechnology in the culture of date tissues can help increase the efficiency of callus propagation. The aim of this study was to prepare graphene nanoparticles (GNP), use nanocomposites in culture medium and evaluate their efficiency in increasing callus proliferation. Three experiments were performed with different concentrations of growth regulators and GNP with 5 replications (each repetition containing 3 petrile) on callus propagation. The experiments were performed in a completely randomized and factorial design in a completely randomized design. Based on the results of the first experiment, treatments of 10 mg/l NAA + 30 mg/l 2ip and 10 mg/l NAA + 0.05 mg/l 2ip were selected as the best callus propagation treatments. In the second experiment, it was found that among the examined treatments, there is no statistically significant difference between 10 mg/l NAA + 30 mg/l BAP and10 mg/l NAA + 10 mg/l BAP with the other BAP treatments. The results of the third experiment showed that the use of treatments of 30 mg/l GNP + 10 mg/l NAA + 30 mg/l BAP could produce the most calluses. Due to the positive effect on weight gain of calli, it seems that this treatment is the most suitable option for propagating date callus of Medjool cultivar.

Keyword: Date palm, Tissue culture, Nanotechnology, Graphene nanoparticles.

Study of single-walled and multi-walled carbon nanotubes effect on callus induction of medicinal plant *Catharanthus roseus* L. under in vitro conditions

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Abstract

In recent years, carbon nanomaterials have attracted considerable attention among other nanostructured materials due to their unique biological and physicochemical properties. The present study was carried out to explore the potential effects of single-walled and multi-walled carbon nanotubes on callus induction, weight and total antioxidant activity of callus in *Catharanthus roseus* L. under in vitro conditions. To induce in vitro callus, explants from leaf of four-week-old growing seedlings were obtained and cultured on B5 basal medium supplemented with single-walled and multi-walled carbon nanotubes (0, 0.50, 100 and 200 mg L^{-1}) as well as in combination with l mg L^{-1} 2, 4-D and 0.1 mg L^{-1} kinetin. The results showed that the carbon nanotube treatments alone had no effect on callus induction and calli growth. As compared with the control, the highest fresh weight of callus was observed in 200 mg L^{-1} nanotubes along with the hormones, while the highest dry weight and dry matter content were obtained in 100 mg L^{-1} nanotubes and the hormones. Also, total antioxidant activity enhanced with increasing the nanotube concentrations. However, neither was any difference observed between single-walled and multi-walled carbon nanotube treatments with respect to these parameters. Our findings suggested that B5 medium supplemented with 1 mg L^{-1} 2-4D + 0.1 mg L^{-1} Kin + 200 mg L^{-1} carbon nanotube was the optimum medium for callus induction and growth.

Keyword: Callogenesis; Carbon nanotubes; Periwinkle

Marker assisted selection for developing melon cultivars resistant to virus and aphids

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Abstract

Melon (*Cucumis melo*; Cucurbitaceae; 2n=2x=24) is one of the most important vegetables that its cultivation has a long history in most parts of Iran. 'Samsoori' and 'Saveh' cantaloupes are landraces that are very popular among farmers and consumers due to the quality of the fruit such as green flesh color, soft and juicy flesh texture, unique flavor and early ripening. However, one of the major problems of these cultivars is their high susceptibility to many viral diseases, which causes a lot of damage and economically reduces fruit yield and quality. *Aphis gossypii* plays an important role in virus transmission in melon. Viruses such as CMV, ZYMV, PRSV and WMV are transmitted by aphids from one plant to another. The *Vat* (Virus Aphid Transmission) gene in melon is a single dominant gene that causes dual resistance to aphids and viruses transmitted them. Coding DNA sequence of this gene is publically available and molecular markers based on this gene have been developed. 'Ginsen Makuwa' cultivar is resistant to viral diseases and to aphids. This cultivar is shown to harbor *Vat* gene. In this experiment "Ginsen Makuwa" as the resistant parent was crossed to susceptible Iranian cultivars ("Samsoori" and "Saveh"). F1 plants were back crossed to "Samsoori" and "Saveh". The functional marker developed previously by Dogimont et. al. (2014) was used for marker assisted selection of resistant heterozygote plants in BC1. BC5. Finally, the resistant plants were self-pollinated to produce *VatVat* homozygote genotypes. The resistant homozygote plants were cultivated for seed production.

Keyword: Back Cross, *Vat*, HRM

Genotyping of *SlERF1* in F6 lines of melon

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Abstract

Melon (*Cucumis melo*; Cucurbitaceae; 2n=2x=24) is one of the most important vegetables that its cultivation has a long history in most parts of Iran. 'Samsoori' cantaloupe is a landrace that is very popular among farmers and consumers due to the quality of the fruit such as green flesh color, soft and juicy flesh texture, unique flavor and early ripening. However, the sugar content of this cultivar is low. This research was carried out with the aim of improving fruit sugar content in Samsoori. The 'Samsoori' was crossed with 'Galia' and the F2 population was generated. The plant in F2 generation were self-pollinated until F6 generation to produce inbred lines. 50 F6 lines were evaluated in farm and sugar content in fruits was measured using a refractometer. HRM technique was used to determine the genotype of a candidate SNP marker (SlERF1) which previously had been reported strongly associated with sugar content in fruit. The HRM analysis was carried out using Real Time PCR. 'Galia' and 'Samsoori" genotypes had A_1A_1 and A_2A_2 genotypes, respectively. T-test analysis showed that these two groups had a significant difference in sugar content. The average of sugar content in brix unit (%) for A_1A_1 and A_2A_2 genotypes was 12.8 and 10.8, respectively. These results indicate that the marker can well select the plants that have higher sugar content than the parent of Samsoori.

Keyword: HRM, fruit sugar content, SNP

Genetic Stability Against Somaclonal Variation Using ISSR Markers in Callus and Seeds of *Arnebia Pulchra* (family: Boraginaceae)

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Abstract

This is the first report on analysis ISSR markers for assessing the genetic variation in *Arnebia pulchra.* The present study was performed to investigate genetic variation in two *in vitro* culture samples of the medicinal plant *A. pulchra* including new (one month old) and old (four years old) calli versus seeds by Inter Simple Sequence Repeat (ISSR) marker. 79 bands were produced by 10 ISSR primers. Cluster analysis was performed to indicate in the form of dendrogram the genetic stability of the samples. UPGMA tree discriminated samples in two major groups and principal component analysis (PCoA) confirmed clustering. The first major cluster included seeds and the second involved new and old calli. Although it was thought that the morphological changes of *A. pulchra* could be due to the somaclonal variation caused by successive subcultures however, the results indicated that the new and old calli are clustered in the same group.

Keyword: Cluster analysis; ISSR markers; somaclonal variation; successive subculture; tissue culture.

The effect of different concentrations of polyethylene glycol and sorbitol on the production of potato (*Solanum tuberosum*) microtubules *in vitro*

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Abstract

Potato (*Solanum tuberosum* L.) is one of the most important crops worldwide. It is vegetatively propagated using pieces or whole potato tubers, but a virus infection causes crop reduction to almost a half or even one third, which varies from place to place and from season to another. Micropropagation is the practice of rapidly multiplying stock plant material to produce many progeny plants, using modern plant tissue culture methods. Microtuberization in potato needs the right interaction between several factors such as cyto, sucrose and osmotic stress. The purpose of present study was to investigate microtuberization efficiency of three potato cultivars under osmotic stress and *in vitro* conditions. This experiment was carried out based on completely randomized design (CRD) in three replications. Potato seeds of three cultivars (Agria, Savalan and HPS-II/67) were cultured on Murashige and Skoog (MS) medium. After proliferation, plantlets were transferred to the media containing nine treatments with polyethylene glycol hydrogel (PEG) at four levels (0.003, 0.006, 0.009, 0.012 M), sorbitol at four levels (0.1, 0.2, 0.3, and 0.4 M). For this purpose, potato quality, microtuberization percentage were measured. The results showed that plantlets grown under non-stressed conditions (control) possessed higher rates of new shoot length and proliferation index compared to the under stressed conditions. Agria cultivar presented better growth characteristics under stressed conditions compared to other experimental cultivars. The highest potato destruction was observed at 0.012 M PEG in Savalan cultivar followed by 0.4 M sorbitol in all cultivars. Microtuberization percentage increased in all three cultivars by stress conditions, in which it increased with progressing in stress levels. The greatest percentage of microtuber-

ization was obtained from Agria cultivar at 0.003 M PEG. Generally, based on the results, maximum rates of microtuberization and viability were observed in Agria followed by HPS-II/67, and the highest microtuber weight was obtained in HPS-II/67 cultivar. When plants are faced to stress conditions like osmotic stress, free radicals such as reactive oxygen species (ROS) increases. On the other hand, plants use different strategies to scavenge the generated ROS. The reduction of chlorophyll content and increases of antioxidant enzyme activities are due to increase of ROS under osmotic stress conditions. At slight stress conditions, potato plants utilize the strategy to generate more tuber. Therefore, the lower concentrations of PEG can be introduced as a stimulator of microtuber.

Keyword: osmotic stress, PEG, sorbitol, potato.

Effect of different cytokinines on Shoot proliferation of *Begonia* × *hiemalis Fotsch*

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Abstract

Cytokinines are effective at all stages of plant growth. These compounds are involved in plant metabolism including biosynthesis and enzyme activity at different stages of growth, organ emergence and nutrient transport in plants. Begonia Himalayas is an ornamental and perennial plant that is popular due to its beauty, high variety of colors and easy maintenance. In the present study, we investigated the shoot generation and proliferation of begonia Himalayas in a completely randomized design under the influence of plant growth regulators cytokinine (benzyl adenine (BA) and kintine (KIN) at levels of zero, 0.5 and 1/5 mg/L) with three replications *in vitro*. The studied traits were fresh and dry weight of shoot and root, number of leaves, shoot length, necrosis percentage and proliferation rate. All the mentioned traits were affected by different concentrations of cytokinines and showed a significant difference at the level of one percent probability. In control conditions (no use of growth regulator) the lowest rate of regeneration and proliferation efficiency was observed, however, all cytokinine treatments had a significant positive and incremental effect on fertility coefficient, shoot length, number of leaves, fresh and dry weight of roots and shoots. In general, the type of cytokinine origin for proliferation and shooting of begonia plant was not much different and both types of kintine and benzyl adenine increased the proliferation rate in the plant that could be used and considered commercially.

Keyword: benzyl adenine, Begonia Himalayas, proliferation, shooting.

Diversity of AvrStb6 affecting Gene for Gene relation between Zymoseptoria tritici and wheat

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Abstract

In this study, in order to evaluate the evolution of *AvrStb6* effector in some Iranian isolates of *Z. tritici*, 78 isolates were isolated and purified from 30 specimens collected from East Azarbaijan and Ardabil provinces. Isolates were identified based on morphological characteristics (colonies features and yeast-like growing macropicinidiospores). The pathogenicity of 78 isolates of pathogen was evaluated on susceptible wheat cultivar was called "Tajan". Between them, four isolates were asymptomatic and non-pathogenic. According to the pathogenicity test results, 40 isolates were selected for pathogenicity test on resistant cultivar called "Shafir" containing *Stb6* gene. In pathogenicity test on Shafir cultivar, among the selected isolates, four isolates showed no symptom, three isolates with disease severity 1 with a small number of spots caused by hypersensitivity and without the formation of pycnidia, nine isolates with disease severity 2 by connecting some spots at the tip of the leaf to each other and small pycnidia, seven isolates with disease severity 3 with the connection of spots at the tips and margins of the leaves to each other and the number of moderate pycnidia and one isolate with disease severity 4 infected more than 70% of leaves with intermittent necrotic spots with pycnidia, 16 isolates with disease severity 5 covered more than 80% of the leaves with interconnected necrotic spots and abundant pycnidia. Then 14 isolates were selected based on the disease severity for sequencing and studying the nucleotide sequence variation of *AvrStb6* gene. The results of sequences analysis showed that the isolates are classified based on geographical area and disease severity in separate clades. The results of this study also showed the occurrence of mutations in the sequence of nucleotides in the exon regions, which led to changes in the amino acid sequence, followed by the emergence of symptoms with different severity on "Shafir" cultivar containing *Stb6* gene.

Keyword: Septoria Tritici Blotch, Wheat, *AvrStb6*, *Zymoseptoria tritici*

Investigation of regeneration of *(satureja spicigera* L.) medicinal plant in vitro culture conditions

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Abstract

Satureja spicigera L., is one of the most important medicinal plants belongs to Nepetaceae, which is of special importance due to its valuable medicinal compounds such as thymol, paracetamol, gamatripenene and carvacrol. Optimization of in vitro culture through callogenesis is proposed to be an alternative for vegetative propagation. In present study, an efficient protocol has been developed for callus induction of *S. spicigera* using different explants and concentrations of plant growth regulators on MS basal medium.

The results showed that the highest rate of callogenesis was in MS medium supplemented with 1 mg / l BAP and 0.5 mg / l NAA in dark conditions. On the other hand, node and root explants were the most efficient explants for callus induction. The node explants in callogenesis medium went directly to regeneration and was completely regenerated in medium with 1 mg / l KIN and 1 mg / l BAP and 0.5 mg / l NAA, and also regeneration was performed in MS medium supplemented with 1 mg / l BAP with 0.1 mg / l NAA and also rooting was performed in MS medium at half concentration.

Keyword: Growth regulators, Culture medium, Explants, Callus, *Satureja spicigera* L.

Genome-wide association study (GWAS) of seed germination-related traits in rice

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Abstract

Genome-wide association study (GWAS) has become an accepted and powerful method for understanding the associations between phenotypes and genotypes. In agricultural production, uniform and rapid germination is an important prerequisite in crop production. Here, a rice (*Oryza sativa* L.) GWAS with 33,934 SNPs (MAF > 0.05) for eight germination traits including germination percentage (GP), shoot (SL) and root length (RL), root (RFW) and shoot fresh weight (SFW), root (RDW) and shoot (SDW) dry weight, and number of days to germinate (NDG) was performed to define genomic regions influencing seed germination. GWAS revealed Loci (47) with 75 significant germination-associated markers were detected across all rice chromosomes. Some of candidate associated genes were: LOC_Os01g26210 (OsWAK6) co-located with #2183 that is seed vigor QTL, LOC_Os07g23944 (GH31) with an α-glucosidases /starch lyase activity; id7000519 marker that corresponds to a gene cluster containing glutathione *S*-transferase and glucan endo-1,3-β-glucosidase, LOC_Os06g47640 (calmodulin-related calcium sensor protein 29) involved in the inhibition of ABA during seed germination. Here, the genetic diversity of rice genotypes was put under scrutiny for germination. Our GWAS results identified several likely candidate genes for germination traits that will greatly contribute to our understanding of the genetic complexity underlying the corresponding traits. The associated genes with the germination traits can be generally classified as hydrolytic enzymes and regulatory proteins that can directly or indirectly influence germination.

Keyword: germination, *Oryza sativa,* quantitative trait loci (QTL), seed, single nucleotide polymorphism (SNPs).

Investigation of micro propagation of greenhouse peas (Pisum sativum)

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Abstract

The tissue culture techniques and they have important role in producing of kinds of plant species. The benefits as genetic stability, reducing of cost and time a reason for special important of this field of biotechnology. In this study Greenhouse pesa (Pisum sativum) was selected because high cost of this producing by asexual propagation and its have high axillary difficult in iran country. In this study some media cultures: MS, 1/2 MS. Investigated in factorial statistical design with two hormones called NAA in four Levels (0.5, 1, 1.5, 2) mg/l and BAP in tri levels (0, 0.1, 0.2) mg/l and by Tri repetition. Results showed most suitable treatment for explant (shoot tip) sterilization of samples is using %96 etanol and %15 vaitex and washing with water in proliferation,and then was sub culture rooting stage. also Result showed1/2MS media is more suitable relative to others media and high doze of NAA (2 mg/l) and low doze of BAP (0 mg/l) in rooting stage is suitable.

Keyword: Greenhouse pesa , Tissue culture, Micro propagation, Rooting stage.

Genetic and molecular analysis of trichome development in *Arabis alpina*

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Abstract

The genetic and molecular analysis of trichome development in *Arabidopsis thaliana* has generated a detailed knowledge about the underlying regulatory genes and networks. However, how rapidly these mechanisms diverge during evolution is unknown. To address this problem, we used an unbiased forward genetic approach to identify most genes involved in trichome development in the related crucifer species *Arabis alpina.* In general, we found most trichome mutant classes known in *A. thaliana*. We identified orthologous genes of the relevant *A. thaliana* genes by sequence similarity and synteny and sequenced candidate genes in the *A. alpina* mutants. While in most cases we found a highly similar gene-phenotype relationship as known from Arabidopsis, there were also striking differences in the regulation of trichome patterning, differentiation and morphogenesis. Our analysis of trichome patterning suggests that the formation of two classes of trichomes is regulated differentially by the homeodomain transcription factor *AaGL2*. Moreover, we show that overexpression of the *GL3* bHLH transcription factor in *A. alpina* leads to the opposite phenotype as described in *A. thaliana*. Mathematical modeling indicates that this non-intuitive behavior can be explained by different ratios of GL3 and GL1

in the two species.

Keyword: Arabis alpina, trichome genetics, trichome patterning

Investigation of antibacterial properties of plant extracts Centella asiatica and Green tea On *Staphylococcus aureus* and *Escherichia coli* bacteria

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Abstract

Today, the increasing spread of drug resistance among bacteria has led to the use of other methods, including the use of herbal medicines to control human infections around the world. This study investigated the antibacterial properties of plant extracts of centella asiatica and green tea on *Staphylococcus aureus* and *Escherichia coli* microorganisms.

This experimental study was performed on standard strains of *Staphylococcus aureus* and *Escherichia coli*. Centella asiatica ana Green tea extracts was used. Then, the antibacterial activity of the extracts with concentrations of 100, 200 and 500 mg / ml was investigated by disk diffusion in agar and bacterial counting on the standard strain of the mentioned bacteria.

The highest antimicrobial susceptibility of Centella asiatica extract against *Staphylococcus aureus* and green tea extract was obtained from *Escherichia coli*. The highest percentage of lethality was shown by Centella asiatica extract against *Staphylococcus aureus* and green tea extract against *Escherichia coli*. Extracts of Centella asiatica and green tea in low concentrations also showed good antibacterial properties.

Plant extracts have shown significant antimicrobial and lethal susceptibility against the tested bacteria. Therefore, these plant extracts can be a good option for future studies in vitro for the preparation of antibacterial drugs.

Keyword: Antibacterial properties, centella asiatica plant extract, Green tea plant extract, Agar agar disk diffusion method, Bacterial counting method

The use of hairy root for the expression of recombinant proteins

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Abstract

The use of plants as bio-factories for producing recombinant proteins for expression of interest is relatively high in protein and low cost. Agrobacterium rhizogenes increases the expression of auxin in transgenic cells by transferring auxin-producing genes to plant cells, thereby creating hairy roots in the plant. Features such as rapid growth, genetic stability, ease of maintenance and for a long time on the medium and the ability to secrete extra cellular proteins expressed the possibility of using the body to express the full-scale range of secondary metabolites and recombinant proteins provided has brought. In the present study the hairy root brief introduction to the latest findings on the use of these organs will refer to expression of recombinant proteins.

Keyword: Recombinant protein, Agrobacterium rhizogenesis, hairy root

Optimization of Protoplast Isolation, Regeneration and PEG-Mediated Gene Transfer in Potato (*Solanum tuberosum*) cv. Agria

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Abstract

Protoplast systems have been proven to be a powerful tool in modern plant biology and genetic engineering. However, isolation of abundant viable and intact protoplasts is a challenge in this method. Although conventional breeding approaches has improved potato yield, heterozygous nature and tetrasomic inheritance makes potato researches and breeding programs through traditional crossbreeding challenging.

The aim of this study is optimizing of protoplast isolation, regeneration and transformation from mesophyll cells of potato cv Agria and analysis of cell division and callus production, for further use in genetic transformation.

About 0.5 g leaf material from 4-6 weeks-old plants were enzymatically hydrolyzed for 16-18 h in the dark and in enzyme solution containing 1% Cellulase, 0.2% Macerozyme and 0.2 M mannitol and glucose. $2x10^6$ protoplasts/ml were obtained from about 0.5 g leaf mesophyll cells. The effect of various concentrations and combinations of plant growth regulators on callus induction were investigated.

Maximum rate of callus was recorded when 5 mg/L NA and 0.1 mg/L BAP was used. Protoplasts cultured in MS medium containing optimized hormone concentration. First protoplast division was started after 4 days. After one-month micro-calli were visible with disarmed eyes. In addition, PEG- mediated transformation efficiency, was estimated approximately upto 50% by using pBin-61 vector. Based on this result, our described protoplast extraction system, can be used for functional analysis of genetic transformation and expression in potato.

Keyword: Potato, Protoplast isolation, Genetic transformation, Polyethylene glycol

RFLP Markers for Study Phytoplasmas Associated with Mungbean Seed Pod Abortion in Iran

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Abstract

Mungbean (*Vigna radiata*) plants showed aborted seed pods symptoms were analysis. Thirty samples were collected from symptomatic and non-symptomatic plants, followed by PCR amplification, DNA sequencing, RFLP and phylogenetic analysis of 16S ribosomal RNA gene. RFLP results indicated that two different phytoplasmas, were associated with different symptomatic plants samples from the same field. *In silico* RFLP and phylogenetic analysis of mungbean seed pod abortion (MubSpa) phytoplasma indicated that the associated phytoplasmas belonged to phytoplasmas of subgroups 16SrVI-A and 16SrII-D. Result of this study indicated that MubSpa phytoplasma strains in Iran are divergence. These results showed similarity in ecological niches of various phytoplasma populations.

Keyword: mungbean, seed pod abortion, In silico RFLP

Genetic diversity of *Tuf* gene in phytoplasmas associated with grape decline in Iran.

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Abstract

Grape is one of the most valuable and the most important horticultural crops in Iran. Recently, phytoplasmas disease has been reported from these Qazvin and Lorestan provinces. Preliminary Analysis shows that different strain associated with this disease. In order to study genetic diversity of phytoplasmas associate with grape decline using *Tuf* gene, different samples collected from these provinces. DNA extraction has been performed by CTAB method. PCR was employed to amplify *Tuf* gene in phytoplasmas using primer pair Tuf C/D. The expected 850 bp fragment of the phytoplasmal 16S rDNA was amplified. In order to study genetic diversity of grape phytoplasmas isolates, RFLP analysis has been performed on *Tuf* gene using *Ecor*I, *Mse*I and *Taq*I enzymes. Results of this study show that *Mse*I enzyme could distinguish different phytoplasmas isolates in grape.

Keyword: Grape, Phytoplasma, RFLP and *Tuf* gene.

Transformation of DRO1 and CKX4 genes in order to modify rice root architecture and improved drought tolerance in rice

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Abstract

The engineering of plant root architecture system can be led to plant tolerance and maintained plant's yield during environmental stresses such as drought, Lodging, and nutrition. Furthermore, a greater root system through improving nutrient and water uptake could result to increase grain yield and optimal seed quality. Considering the water crisis in the country, the production of plants tolerant to drought will be valuable. In this study, to create a drought-tolerant rice plant, two genes involved in root length, mass, and root angle respectively, were cloned and constructed in a multi-gene vector and transferred to Hashemi cultivar. The OCKX4 and DRO1 genes that originated from the wild rice variety were isolated and cloned into the T-DNA region of binary vector under control of root-specific and constitutive cloning promoters respectively. The resulted recombinant vector called pUhrCkDro, introduced into the local cotton variety Hashemi by using an Agrobacterium-mediated gene transformation. Transformed calli were selected on MS medium containing 50 mg / l hygromycin. Putative calli were subsequently regenerated into full plants in the selective medium. Fully developed plants were to Yoshida solution and then to pots. Polymerase chain reaction was used to confirm the integration of OCKX4 and DRO1 transgenes in the T1 plant's genome. Independent events were analyzed by inverse PCR reaction to distinguish the events. The location of the transgene was identified in three of the eight events and is ongoing for other events. Comparison of root phenotype with untransformed control plant showed an apparent difference in root

structure but did not show any other differences. Transgenic plants were sown in the transgenic greenhouse of the Agricultural Biotechnology Research Institute and underwent more molecular analysis in T1 and T2 generations. The resulting multigene contract can also be used to transfer genes to other plants to change root structure and drought tolerance. It is hoped that the production of transgenic rice by increasing the root mass and changing the root architecture system can increase drought tolerance in this important crop and reduce water consumption in rice cultivation.

Keyword: Transgenic rice, Drought tolerance, Multi-gene vector, Root architecture

Evaluation of antioxidant and anti-diabetic effect of Eryngium billardieri plant in Hamedan province

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Abstract

Alpha-glucosidase enzyme evaluates antioxidant activity by ABTS radical scavenging test and determines the anti-diabetic activity of borage extract using alpha-glucosidase activity. The sample of bougainvillea plant was changed from powder to a soluble state by the desired solvent of bougainvillea plant (water, ethanol), then by concentration, the extract was concentrated in concentrations (1.25, 1, 0.75, / 5). 0.25, 0.125 µg / µl from high to low concentration of each concentration in three replications (dilution). The anti-diabetic properties of borage plant were evaluated using alpha-glucosidase enzyme and its substrate PNPG (para-nitrophenyl phosphate guanine) and by ELISA device. And ABTS was used. The measurement of inhibitory activity of antioxidant properties is as follows: Plant extract was diluted in 6 concentrations in three concentrations in three replications and then converted to nanoparticles by ultrasonics and stored in a falcon in a dark environment with the addition of ABTS. Ethyl benzothiazoline sulfonic acid was poured onto a 96-well plate and read by a UV device at a wavelength of 740 nm.

Keyword: Bougainvillea, Diabetes, Alpha-glucosidase enzyme, Antioxidant properties, Anti-diabetic properties.

Expression of the key gene Hypericin synthase under the effect of salicylic acid and methyl jasmonate in St. John's wort (*Hypericum perforatum*

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Abstract

Perforate St John's-wort (*Hypericum perforatum*) is a valuable medicinal plant that has unique secondary metabolites, including naphthodianthrone such as hypericin and hyperforin. Hypericin is the most important secondary metabolites of St. John's wort, which has antidepressant property. In order to find a way to increase hypericin production through the genes involved in the hypericin biosynthetic pathway, the hypericin synthase (*Hyp-1*) expression was studied. Salicylic acid and methyl Jasmonate elicitors at concentrations of 0, 10 and 100 micromolar (in the pre-flowering stage) were used to assay induction of gene expression. Leaves were sampled at 0, 12, 24 and 48 hours after treatments. The expression of hypericin synthase gene was investigated by semi-quantitative RT-PCR. The results of salicylic acid and methyl jasmonate treatments confirmed that hypericin synthase shows higher level of expression in the presence of these elicitors and both 10 and 100 µM concentrations are significantly different from the control (zero concentration). Study of the expression of *Hyp-1* in the hypericin biosynthesis pathway indicates that hypericin synthase is affected by salicylic acid and methyl jasmonate and hypericin is likely to increase in response to these treatments.

Keyword: Gene expression, Elicitor, Biosynthetic pathway, Hypericin, *Hypericum perforatum*

Gene expression analysis of some key flavonoids synthesis genes on *Vitis vinifera* cv. Syrah

under regulated deficit irrigation condition

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Abstract

Grapes constitute a major source of nutrients and other compounds with various protective and antioxidant roles. Among those, flavonoids are produced by the secondary metabolism of higher plants and their biosynthesis is reported to depend on various environmental factors such as light, temperature, and water availability conditions. Several studies have shown that water deficit conditions induce the biosynthesis of flavonoids in grapes. The current study aims to investigate the effects of drought in the regulation of the expression of seven biosynthetic genes (*CHS2, CHI1, FLS1, DFR, ANR, LAR1*and *UFGT*) and a transcription factor (*MybA1*) implicated in the flavonoid biosynthetic pathway in grape, in seven developmental stages (from the start of veraison to full maturity). The trial was conducted during two consecutive seasons (2017-2018) in a commercial vineyard in Epanomi Thessalonikis (Greece) planted with *Vitis vinifera* L. cv. Syrah grafted onto 1103P rootstock. Vines were subjected to three irrigation regimes starting at berry set [full irrigation (FI): 100% of crop evapotranspiration (ETc), deficit irrigation (DI): 50%] triplicated in randomized blocks. To study gene expression regulation during flavonoid biosynthesis, molecular analysis was performed following a real-time RT-PCR approach with *UBQ* as a housekeeping gene. Results showed that drought stress affected the expression levels of all tested genes. General induction of the genes implicated in flavonoid biosynthesis was observed at the full maturity stage under drought stress. Moreover, no differences were observed in the overall

regulation of gene expression between the two growth seasons.

Keyword: grape berry, anthocyanin, antioxidant, drought stress, gene expression

Investigating the Effect of Darkness on Germination of Lettuce Seeds in *invitro* Condition

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Abstract

Lettuce with the scientific name of *Lactuca sativa* is an annual plant of the chicory family. This plant is one of the most important commercial plants in the world, which is mostly grown as a vegetable, but sometimes it is also found in the form of stems or seeds. Lettuce is one of the plants that has leaves with high nutritional value and is also harvested faster due to its short growing period on farms and is always available. Therefore, this plant with these properties is a suitable plant for use in diets as well as research work such as the production of antibodies, vaccines and other medicinal proteins. One of the most important stages in the life cycle of plants is seed germination. Germination is the process by which a plant begins to grow from seed or a seed-like structure. Seed germination depends on both internal and external seed conditions. The most important external factors influencing germination are temperature, water, oxygen, and light or darkness. In this study, the effect of darkness and lack of light on the germination of lettuce seeds under in vitro conditions was evaluated. The results showed that dark treatment has a very important role in accelerating the germination of lettuce seeds in vitro.

Keyword: Darkness, Germination, *In vitro* culture, Lettuce

Comparison of the effect of two different concentrations of sodium hypochlorite on the removal of surface contaminants and the rate of damage to leaf tissue in gerbera *in vitro* culture

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Abstract

Gerbera with the scientific name of *Gerbera jamesonii* is a valuable ornamental plant species. This plant is cut as a branch flower and has gained a lot of popularity in many countries of the world over the past few years due to its characteristics such as beauty, various colors and long shelf life. In order to commercialize gerbera and meet its growth needs, tissue and organ culture techniques are used as an alternative to traditional plant propagation in many countries. One of the problems in plant tissue culture is the damage to plant tissue and its growth areas due to the use of sterilizing chemicals. In this study, we investigated the effect of two different concentrations of sodium hypochlorite on the rate of contamination of different parts of the leaf tissues of gerbera. The results showed that the rate of contamination in the use of 15% sodium hypochlorite solution for 5 minutes was less than 0.1% sodium hypochlorite for 10 minutes in different parts of leaf tissue. Also, the use of these concentrations of sodium hypochlorite during the tested period did not have an adverse effect on the viability of cells in different parts of the leaf. It is added that in both methods, prior to application of sodium hypochlorite, the tissues were treated with 70% ethanol for 1 minute. Therefore, a concentration of 15% sodium hypochlorite for 5 minutes is recommended for efficient sterilization of gerbera leaf tissue.

Keyword: Sterilization method, Gerbera, *In vitro* culture, Sterilizing chemicals

The effect of alcohol and sodium hypochlorite on the control of contamination in grape micropropagation

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Abstract

Grape is one of the most important plants in the world. Tissue culture technology is a new method for healthy, massive and off-season propagation of specific mother plants in this plant. The first essential step in the successful micropropagation of this plant is the removal of endogenous and exogenous contamination of its explants. The aim of this study was to evaluate the effect of different levels of alcohol and sodium hypochlorite on the control of infection and growth of tissue culture seedlings of Cardinal cultivar. Accordingly, the simultaneous effect of 3 levels of alcohol (96% alcohol for 4 minutes, 96% alcohol for 2 minutes and 70% alcohol for 4 minutes) and 2 levels of sodium hypochlorite (2.5% sodium hypochlorite for 10 minutes and sodium hypochlorite 5% for 5 minutes) was evaluated as a factorial experiment in a completely randomized design with 3 replications. After disinfection with the desired treatments, the explants were placed in solid MS medium containing 0.5 mg / l BAP. The presence or absence of contamination (bacteria or fungi) in the cultured explants was assessed daily. After 4 weeks, seedling growth traits were recorded. The results showed that the interaction of alcohol and sodium hypochlorite was significant for all traits at the level of 1%. Simultaneous use of 96% alcohol treatment for 2 minutes and 2.5% sodium hypochlorite for 10 minutes was considered the most desirable pollution control treatment (without pollution) and the use of this treatment, led to the highest rate of intracellular growth of seedlings.

Key word: Micropropagation of grapes, Cardinal cultivar, disinfection in tissue culture, growth traits.

Application of magnetic water in improving the micropropagation of Labroska grape

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Abstract

Grapes are one of the most important horticultural products in the world in terms of area under cultivation as well as nutritional and economic value. Plant tissue culture technology is one of the important methods in plant propagation. Magnetic water improves the quality and quantity of growth characteristics in the plant by affecting the chemical reactions and photosynthetic activities of the plant. In this study, in order to optimize the micropropagation of nodal segments of Labrosca cultivar, the effect of four different treatments (ordinary distilled water with 0 mg/l BAP hormone, ordinary distilled water with 0.5 mg/l BAP hormone, 48-hour magnetic water With 0.5 mg/l BAP and 72 hours of magnetized water with 0.5 mg/l BAP in 0.5MS culture medium was evaluated in a completely randomized design with 3 replications. After 4 weeks, seedling growth traits including leaf number, stem length, root length and total plant length were measured. The results showed that the most desirable seedlings with the best and highest growth traits were created in a treatment that used 48 hours of magnetized water with 0.5 mg/l of BAP hormone. According to the results, the use of magnetic water to improve micropropagation in grapes is recommended.

Keyword: Grape seedling, Tissue culture, Growth traits, explants, Nodal segments

Optimization of tissue culture, callus induction, and regeneration of Melissa officinalis L

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Abstract

Lemongrass is one of the most important medicinal plants in the *Laminaceae*, which is highly regarded in the medical, cosmetic and food industries. This study was performed to investigate the possibility of micropropagation, to determine the best type and composition of plant growth regulators for Lemongrass (Melissa officinalis L.) in vitro. In this experiment, the effect of three types of growth regulators, Banzyl amino purin (BAP), Naphthalene acetic acid (NAA) and Dichlorophenoxy acetic acid (2.4-D) in different concentrations on callus induction and regeneration of four types of hypocotyl, node, Stem, and leaf were examined. The results of Anova analysis showed that the highest rate of callus induction in the medium containing BAP 2 mg / l + NAA 0.5 mg / l and also BAP 5 mg / l + NAA 0.5 mg / l and the highest regeneration in the environment Contained with BAP hormone at a concentration of 5 mg / l with NAA at a concentration of 0.5 mg / l. Also, explants of nodal and hypocotyl fragments had the best response for callus induction and regeneration, respectively.

Keyword: Lemongrass, *Melissa officinalis* L., Callus induction, Regeneration, Growth regulators.

Effect of NAA and IAA Hormones on Lateral Buds in Saffron Greenhouse Cultivation (*Crocus sativus* L.)

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Abstract

Saffron (*Crocus sativus* L.) as a medicinal plant is known one of the most important herbs around the world. It is valuable in terms of exports to the whole world. Quality and mother corm size are the most important factors in the yield of saffron which affect the flowering potential, vegetative growth, and daughter corm production. Mother corm with higher weight generally has a higher nutritional reserve. Given that the growth of daughter corm is dependent on a mother's bush. The mother corm size can directly affect the daughter corm formation. A large number of active lateral buds in the mother corm may produce small corms. In the present study, two auxin hormones, including IAA and NAA, in the concentrations of 0.5, 1 and 2 mg l^{-1}were used to control the number of active lateral saffron buds. The experiment was performed in a completely randomized design (CRD) with three replicates at greenhouse condition. Hormonal treatments were applied by direct injection and the number of active lateral buds was evaluated 20 - 30 days after treatments. The results showed a significant difference effects between NAA and IAA treatments. Compared to the IAA treatment, the highest effect was observed in NAA at 2 mg l^{-1}. The number of active lateral buds showed a significant decrease with increasing the concentration of NAA and IAA hormones. The results showed that the concentration of 1 mg l^{-1} of NAA hormone reduced the number of active lateral buds to 48/65%. While the number of active lateral buds under the influence of IAA treatment with a concentration of 0.5 mg l^{-1} decreased to 9/66% compared to the control.

Keyword: Apical dominance, Plant hormones, Lateral bud control, Saffron yield

Enhancement of silymarin accumulation in tissue culture of milk thistle using elicitor feeding and red light

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Abstract

In vitro culture of *S. marianum* has been considered as an economic alternative for the production of silymarin instead of conventional extraction from dried fruits of field grown plants. The elicitation is one of the approaches employed to increase accumulation of plant valuable secondary metabolites. Present study assessed the combinational effect of chemical (Methyl jasmonate) and physical (red light) elicitors on the production of silymarin in *S. marianum* leaf and root explant tissue cultures. Undifferentiated cells (callus) from *S. marianum* leaf and root explants appeared after ~60 days on MS medium along with 1 mg/L each of 2,4-D and 6-Benzyladenine hormones. The root and leaf callus were treated by 20 mg/L MJ for 2 days. Thin layer chromatography (TLC) using; Ethyl acetate/chloroform/methanol/formic acid (1:1:1:1:1drop) as liquid phase confirm the elevation of silymarin only in callus cells derived from root while the amount of silymarin remain invariant on treated leaf originated callus. Moreover, we found that total silymarin content was significantly increased under red light exposure which was 1.7 times compare to the control. The result indicated that combination of chemical elicitor and red light can be used to improve accumulation of secondary metabolites in *in vitro* cultures of milk thistle.

Keyword: Elicitors, Red light, tissue culture, milk thistle, silymarin

Epigenetic regulation in response to salinity

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Abstract

Abiotic stresses, especially salinity, are major factors limiting plant growth and yield. Cultivation of saline soils to feeding world's growing population requires the production of cultivars which can tolerate the harmful effects of environmental changes. The first step to achieve this goal is to fully understand the mechanisms of plants respose to abiotic stresses. Inheritable diversity within populations is the raw material of evolution. But along with genetic factors, non-genetic factors are involved in phenotypic diversity and are known as Epigenetic factors. In this article we have studied various aspects of plants epigenetic regulation in response to stress. Epigenetics is heritable phenotype changes that do not involve alterations in the DNA sequence. Epigenetic changes can vary between genomic regions over time, in response to biological and non-biological stresses, and even between individuals and populations. Epigenetic alterations regulate gene expression in response to changing emvironment and creating a significant part of different species populations phenotypic diversity. Epigenetic alterations leads to alterations in gene expression that enable organisms to rapidly respond to changing environments, thus adapt to new environmental conditions. Methylation, phosphorylation, acetylation and ubiquination are thr most important epigenetic changes. Among the epigenetic changes, DNA and histone methylation can alter the structure of chromatin and make a vase range of specific epigenetic effects that have a strong effect on phenotypes.

Keyword: Abiotic stress, Salinity, Epigenetic, Gene expression, Adaptation

Aquaporins and Salt stress

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Abstract

Many abiotic stresses directly affect the hydraulic conductivity of plants, and plants under such conditions protect their ionic and osmotic homeostasis by absorbing, transporting, and segregating water and solutes. Plants maintain optimal water balance by continuously adjusting the water conduction of their tissues. Vascular tissues and stomatal guard cells play a major role in directing water and controlling transpiration, but water must flow between living cells to enter and exit vascular tissues. The most important pathway for water conduction in living cells is aquatic channel proteins called aquapurins. In ths article, aquaporins the most important proteins in response to salinity were studied from different aspects. Aquapurins are a large, protected family of proteins (MIPs) that have evolved to facilitate the passive flow of small polar molecules such as water or glycerol between membranes. A large number of MIP genes have been identified in plants. Plant MIPs based on their sequence similarity in at least 4 subfamilies including plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs), and class-specific intrinsic proteins (SIPs). They are tied up. Abiotic stresses reduce root hydraulic conductivity, and this response is often associated with reduced MIP activity. Many studies have shown significant expression of several AQP aquapurins in the same tissue, and many of them show significant increases or decreases in expression under different stress conditions. Multiple controls on the function of aquapurins in cells have also been developed to continuously regulate the permeability of membrane water in a rapidly changing environment.

Keyword: Abiotic strss, Salinity, Aquaporins

Evaluating Genetic Diversity of some Iranian Aegilops tauschii accessions using Start Codon Targeted (SCoT) marker

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Abstract

CWR are unique resources for food security and are increasingly used for crop breeding program. In this study, 44 *Aegilops tauschii* accessions collected from different geographical regions of Iran were studied using 14 SCoT primers. In the present study, genetic diversity among 44 Iranian *Aegilops tauschii* accessions collected from different geographical region was determined using inter Start Codon Targeted (SCoT) markers. The mean percentage of polymorphism determined in all the genotypes was 96.71. The number of polymorphic bands for each primer varied from 9 to 17 and a total of 156 replicate bands were scored, of which 151 bands showed polymorphism. The average content of primer information polymorphism (PIC) was estimated to be 0.47 and the SC14 primer showed the highest PIC (0.50). The highest value of marker index (MI), resolution power (RP) and effective multiplier ratio (EMR) belonged to SC9 suggesting the high efficiency of this marker in band diversity distinction. The results of cluster analysis for 14 SCoT primers were performed using Jaccard dissimilarity coefficients and the neighbor -joining algorithm. The accessions were divided into 4 groups based on this analysis. The results of this study showed that SCoT markers can be effectively used to study the genetic diversity of Iranian *Aegilops tauschii* and will provide the possibility of breeding.

Keyword: *Aegilops tauschii*, polymorphic parameter, cluster analysis

Green Synthesis of Fe Nanoparticles Using Aqueous Extract of Gundelia tournefortii Collected from Maragheh

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Abstract

Synthesis of iron nanoparticles (FeNPs) by the recently developed green method is extremely promising because of its non-toxicity and eco-friendly behavior. In this study, iron nanoparticles were synthesized from hexahydrate ferric chloride ($FeCl_3.6H_2O$) with the addition of *Gundelia tournefortii* shoot extract under room-temperature conditions. The shoot extract contains a rich source of phenolic compounds that are used as reducing agents. Polyphenols contained in extract act as reducing agents for iron ions in aqueous solutions, forming thus iron nanoparticles, and stabilize the nanoparticles produced from further oxidation and agglomeration. The synthesis of iron nanoparticles was confirmed by characterization using UV-Vis Spectrophotometry and Fourier Transform Infrared spectrometer (FTIR) studies. The formation of iron nanoparticles was confirmed by changing the color of the solution from yellow to brown. The formation of FeNps was monitored by UV–vis spectrophotometer wavelength, which ranges from 200–600 nm. In the UV region a sharp adsorption band occurs between 260–300 nm. The analysis of FeNPs by FTIR represented the presence of different functional groups. The peak located at 3311.97 cm^{-1} is attributed to O-H and N-H stretching vibrations. The peak at 1637.74 cm^{-1} revealed the presence of C=O and C=C stretching in the extract. The strong peak at 614.99 cm^{-1} corresponds to the inorganic stretching indicates the FeNPs. The results of present research showed that the *Gundelia tournefortii* shoot extract can be used to synthesize iron nanoparticles.

Keyword: Aqueous Extract, Green Synthesis, *Gundelia tournefortii*, Iron Nanoparticles.

The Study of silver nanoparticles application in tissue culture of tall fescue (*Festuca arundinacea*)

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Abstract

Tall fescue (*Festuca arundinancea*) is a cold-season, perennial forage grass that is resistant to a variety of biological and non-biological stresses. The study of tissue culture of this plant provides a way to propagate resistant genotypes of this plant. The aim of this experiment was to investigate the effect of silver Nano particles (Ag) on callus induction and regeneration of this plant. Four concentrations of silver nanoparticles (0, 20, 40, 60 mg / l) were studied in a completely randomized design with three replications. The results of analysis of variance of the studied traits showed that the effect of silver nanoparticles on the number of days to callus induction and regeneration percentage was significant and on the other studied traits including callus formation percentage, callus perimeter and area, callus length and width, fresh weight and callus dry weight was not significant. The results of mean comparisons showed that increasing the concentration of silver nanoparticles had a positive effect on callus formation rate and at a concentration of 20 mg / l callus formation was induced more rapidly. The results also showed that increasing the concentration of silver nanoparticles had a negative effect on regeneration and 60 mg/l Ag nano particles had the lowest means, but it has been very effective in eliminating bacterial and fungal infections.

Keyword: Callus induction, explants, culture medium, regeneration, nanoparticles.

Inter-and intraspecific genetic relationship of *Capsicum frutsicens* and *Capsicum annuum* using ISSR Marker

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Abstract

This study was performed to genetic relationship of *Capsicum frutsicens* and *Capsicum annuum* using ISSR Marker. In this experiment, 15 populations of two species of pepper (*Capsicum annuum*, *Capsicum frutsicens*) collected from different regions of the Iran country and two populations from Italy and the United States. ISSR marker was applied for evaluation of genetic diversity in peppers. According to ISSR results, primers created 68 bands, of which 51 were polymorphic bands and 17 were monomorphism bands, in 15 pepper genotypes. Among them, primer 15 with 13 polymorphic bands and primer 19 with 2 polymorphic bands had the highest and lowest number of polymorphic bands, respectively. Cluster diagrams with a similarity coefficient of 21% revealed the two main groups. According to the results, the ISSR marker was able to differentiate pepper species in terms of geographical origin and climatic similarity of regions.

Keyword: Genotypes, Variation, Medicinal plants, Molecular marker.

Investigation of genetic relationships between Inter and Intra species of 15 pepper genotypes

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Abstract

Few studies have been performed on the genetic diversity of medicinal plants, and the increasing consumption of this group of plants in the wild from natural habitats has reduced their genetic storage (germplasm). This study was performed to genetic relationship of *Capsicum frutsicens* and *Capsicum annuum* using physiological properties. In this experiment, 15 populations of two species of pepper (*Capsicum annuum*, *Capsicum frutsicens*) collected from different regions of the Iran country and two populations from Italy and the United States. Some physiological traits were studied including Chlorophyll, Anthocyanin, Flavonoids, phenol and soluble sugars. Cluster diagrams with a similarity coefficient of 21% revealed the two main groups. There was the least genetic similarity or the greatest genetic difference between Semnan and Kashan genotypes. In the dendrogram, pepper genotypes from the United States and Italy were next to the Iranian samples. In general, the placement of foreign samples with Iranian samples in a dendrogram group may be due to their genetic similarity due to their common primary origin or due to the climatic similarity of these areas.

Keyword: Genotypes, Variation, Medicinal plants.

Optimization of hairy root induction in Iranian basil (*Ocimum basilicum L.*)

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Abstract

Ocimum basilicum L. (Lamiaceae) is cultivated in various ecological conditions and warm regions of India, Africa, and South Asia. It has antidiabetic and antibacterial properties and also it has been recommended for the treatment of asthma, malaria and skin diseases. Due to the importance of secondary metabolites, hairy root culture is a suitable strategy for further production of these substances. In this study, the optimization of hairy root induction in Iranian basil was evaluated using hypocotyl and cotyledon explants under the influence of ATCC-15834 and A4 strains of *Agrobacterium rhizogenes* in a factorial experiment based on a completely randomized design. The results indicated that the interaction effect of explant and strain on hairy root induction rate and hairy root induction percentage was not significant, while their main effects were significant. The results suggested that the rate of hairy root induction in the cotyledon explant (8.33 days) was faster than the hypocotyl (10.16) and the percentage of hairy root induction in the cotyledon explant (80) was higher than the hypocotyl (37 %) and also the ATCC-15834 strain was superior to A4 strain in terms of root induction rate and root induction percentage. The transgenic nature of the produced hairy roots was confirmed by tracing part of the *rolB* gene using a PCR reaction. The results of the present study suggested that hairy root induction in different explants of Iranian basil by *Agrobacterium* is possible and can be used in gene transfer studies and hairy root culture for producing valuable secondary metabolites.

Keyword: Hairy roots, Secondary metabolites, Iranian basil.

The optimization of hairy root induction in broccoli plant

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Abstract

Brassica oleraceae var. Italica (Brassicaceae) provided a good source of nutritional benefits. The broccoli includes many important secondary metabolites specially those containing glucosinolates and their breakdown product. Here we present induction of hairy root of broccoli. As for the importance and application of secondary metabolites, the hairy root is a suitable strategy for the production of these substances. Based on this reason in this study, the optimization of hairy root induction in the broccoli plant was evaluated using hypocotyl and cotyledon explants under influence of ATCC-15834, A4 strains of Agrobacterium rhizogenes in a factorial experiment based on a completely randomized design.in this study, after wounding explants, hairy roots appeared. The result of this study illustrated that the interaction effect of explants and strain of bacteria on the hairy roots induction rate and hairy root induction percentage was not significant. Also, the results illustrated that the hypocotyl and cotyledon explants were significantly different in terms of hairy root induction rate. Nevertheless, the rate of induction in the hypocotyl explant (with a mean of 8.66 days) was faster than the cotyledon (with a mean of 11.33). The transgenic nature of the hairy roots produced was confirmed by tracing part of the rolB gene utilizing a PCR reaction. According to the result of this study, the transgenic and hairy root induction in different explants by different strains of bacteria is possible can be used in gene transfer surveys and hairy root culture to produce valuable secondary metabolites.

Keyword: Hairy root, Secondary Metabolite, Broccoli.

Using hormonal compounds in Order to Optimize Media for Production Suspension Callus in *Catharanthus roseus*

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Abstract

Periwinkle (Catharanthus roseus) has always been considered because of an important alkaloid that are have high medicinal value in drug industry. It has been estimated that active alkaloid content including vincristine and vinblastine in leaves is very low, as well as the low extraction performance from plant tissue and the high-cost extraction have been a challenge for researchers. Therefore, in this study, the optimization of production of suspension callus with high alkaloid content in Periwinkle plant using different hormonal compounds in culture medium was investigated. Results show that the highest volume and fresh weight of callus was observed in treatments containing 0.5 mg /l 2,4-D and 0.2 mg /l Kinetin. Also, the texture obtained from this treatment is brittle and fragile, which is very suitable for callus suspension. Treatments without 2,4-D and NAA showed the lowest callus yield.

Keyword: Callus**,** Periwinkle*,* Suspension, Auxin, Cytokinin

Identification of main and epistatic effects and environmental interactions of QTLs in safflower lines

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Abstract

Important agronomic traits have quantitative heritability and are controlled by many genes, and the environment plays a importent role in controlling them. The main purpose of this study was to identify the main-effect QTLs, epistatic QTLs and QTL-by-environment interactions in genetic control of grain yield and yield components and safflower oil (Carthamus thinctorius L.). The experimental data were collected from a recombinant inbred lines (RILs) population derived from a cross between two Goldasht /Mex.22.191 varieties under normal and drought stress conditions during flowering in the field of Research and Technology Institute of Plant Production of Shahid Bahonar University of Kerman. A linkage map with 69 polymorphic AFLP markers distributed over 12 linkage groups was constructed and QTL analysis performed using QTL IciMapping software (version 4.1). The analysis detected a total of 18 main-effect QTLs for the 8 traits of QTLs *qDW_N-4-2*، *qOY_S-4-1*, *qNC_S-4-1* at linkage group 4 co-located with *qNC_N-5-1, qDW_N-5-1* at linkage group 5. Also, 63 epistatic QTLs (AA) were identified that qSW-N-LG4 / LG5 and qGY-N-LG4 / LG5 explained 8.81% and 8.31% of the phenotypic variations of Plant Seed Weight and grain yield, respectively, were identified As major epistatic QTLs and also 19 epistatic QTLs (AAE) were identified. Majority of the

main-effect QTLs with no QTL × environment interaction were stable, which can be used in breeding programs to improve performance and related traits in new safflower lines.

Keyword: Epistatic QTL, Main-effect QTL, Seed Yield, Drought Stress, Linkage Groups

Effect of combination of three bacteria Pseudomonas fluorescens, Bacillus coagulance and Bacillus subtilis on growth indices of tomato plants infected with tomato yellow leaf curl virus

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Abstract

Tomato yellow leaf complication is one of the most damaging diseases in tomatoes caused by the yellow tomato leaf complication virus (TYLCV). Control of the virus has become important due to the severe damage that the virus does to tomatoes. Given that the control of plant viruses is mainly based on chemical control of vector insects and this method has many environmental hazards, so the development of environmentally friendly methods is of great importance. In this study, in order to achieve the effect of the combination of three bacteria B.subtilis, P.fluorecens and P.agglomerans on the growth indices of tomatoes infected with the tomato yellow leaf complex virus, this study was conducted in a completely randomized design with three treatments and 14 Repeat performed. Treatments included a combination of three bacteria B.subtilis, P.fluorecens and P.agglomerans of healthy plants (negative control) and plants infected with TYLCV (positive control). Tomato plants after inoculation with the mentioned treatments three times were inoculated by Bemisia tabaci containing TYLCV to evaluate the physical growth indices (fresh weight of shoot, dry weight of shoot, height and surface index). Leaves) were treated in greenhouse conditions. The overall results of this experiment showed that plants treated with a combination of three bacteria B.subtilis, P.fluorecens and P.agglomerans increased growth indices. This study provides evidence of increasing growth indices using Provides PGPR bacteria.

Keyword: Growth Stimulating Rhizobacteria, Biofertilizers, Tomato Yellow Leaf Complexity Virus, Physical Indicators of Growth and Tomato

In vitro propagation of Echinacea purpurea from leaf and cotyledon explants

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Abstract

Micropropagation can be utilized for large scale multiplication of pharmaceutical plants hence avoiding an over misuse of natural resources. This study points to create a dependable convention for the in vitro proliferation of *echinacea purpurea*, belongs to *Asteraceae* family. Murashige and Skoog (MS) medium supplemented with 6-benzyl aminopurine (BAP) were be used to induce shoot. The highest multiplication rate (35%) shoot with a high number of shoots per explant (6.7 shoots/ explant) was obtained in MS medium supplemented with BAP (0.2 mg/L). Plantlets were rooted on MS medium free.

Keyword: Micropropagation, *echinacea purpurea,* 6-benzyl aminopurine (BAP), tissue culture, *in vitro*

Effects of NAA and Kinetin on Flower Traits in *Crocus sativus* L

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Abstract

Saffron, the dried stigma of *Crocus sativus* L., is the most expensive spice in the world which is mainly used as a food coloring and flavoring, as well as a medicine. The economic justification for saffron cultivation depends on the stigma yield obtained per unit area Therefore, , attention to different methods in order to increase the amount of saffron flowers and accordingly increase the amount of stigma has been of particular importance.. In the present study,the effects of growth regulators of NAA and Kinetin (0.05, 0.1, 0.2, 0.5 and 1 mg/1^{-1}) were investigated. For this purpose, an experiment was conducted in a completely randomized design under greenhouse conditions and the functional traits of flowers including flower length, flower weight and flowering tube length of saffron plant were studied. Analysis of variance (ANOVA) showed a significant difference between different hormonal treatments on flower traits. Based on the results, the highest increase in flower weight, flower length and closed flowering tube was observed by using 1mg l^{-1} kinetin1 + mg l^{-} NAA.

Keyword: Stigma, closed flowering tube, Flower weight, flower length

Identification and isolation of stress related transcripts in olive and probable mechanisms of stress in this plant

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Abstract

Olive (*Olea europaea* L.) is one of the most important oil producing plants with a long history of cultivation in Middle East and Iran In spite of its commercial importance and its health beneficial properties, molecular mechanism of different aspects of plant including oil production and stress tolerance are unknown. cDNA-amplified fragment length polymorphism (cDNA-AFLP) is an open architecture transcriptome analysis techniques with high potential for detecting differentially expressed transcripts as well as identification of new genes in different plant species. In the present study a cDNA-AFLP technique was employed to investigate the transcript profile of "Mari" (a high quality oil producing cultivar) and "Shenge" (a low quality oil but stress tolerant cultivar) as two important Iranian olive cultivars, during different fruit developmental stages. Altogether, transcript expresion analysis revealed there are substantial variation in gene expression of these two cultivars. According to their present/absence in each cultivars, twenty transcripts were isolated, cloned and sequenced. BLAST analysis showed high level of homology in sequence of 18 transcripts with deposited gene sequences in GeneBank database. Generally, most of those isolated from "Shenge" had high similarity with biotic and abiotic stresses tolerance. Identification of these transcripts at critical stages of fruit ripening provide a valuable clue for defense mechanisms employed by this cultivar to cope with the biotic stresses. Results of this work can provide valuable information about defensive mechanisms in olive tree at molecular level, which can be utilized in the olive breeding programs and subsequent incorporation of high quality oil with stress tolerance characters and subsequent production of superior commercial cultivars with high level of stress tolerant.

Keyword: Olive, biotic stress, abiotic stress, gene expression, breeding, fatty acid

The central role of salicylic acid in control of Fusarium head blight in wheat and its importance in agricultural biotechnology

Abstract

Fusarium head blight is a disease of cereal crops caused by a group of trichothecene producing Fusarium species such as *Fusarium graminearum*. It is the main disease of wheat in different areas of Iran, such as Mazandaran, Gorgan and Moghan regions. Wheat has evolved complex mechanisms to defend against the pathogen. Salicylic acid has been shown to have a central role in defense against Fusarium head blight in wheat. In this review, we aim at outlining the studies conducted on Fusarium head blight in wheat with the emphasis on the SA role in resistance against the pathogen. Exogenous application of SA can activate the plant defense mechanisms before pathogen attack without environmental side effects of protective chemical agents. In addition, a summary of the physiological and molecular responses of wheat to exogenous application of SA were provided.

Keyword: Fusarium head blight, Trichothecene, Salicylic acid, Wheat, Defense mechanisms

Studying the function of some genes in response to salinity stress

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Abstract

Being sessile organisms, plants are constantly exposed to various kinds of environmental, especially abiotic stresses which affect biomass production and yield of major crops up to 70% negetively. At molecular level, often plants response to stress is the result of gene expression alternation, and therefore the identification of related genes and their regulation pattern in response to stress are essential for the production of new cultivars with high salinity tolerance. In this regard, the function and expression changes of a number of genes involved in response to salinity stress were investigated. AOC and AOS genes involved in the biosynthesis pathway of jasmonic acid one of the most important plant hormones, L1S1 a cell death inhibitor gene and participant in response to stress, sHSP protein encoding gene as molecular chaperones involved in proteins and membranes stability, MtN3 gene involved in multiple physiological pathways related with reproductivety, aging and adaptation, RD22 a dehydration-responsive gene, SelBP gene contributed in cell defense and hormonal regulation, PEAMT gene involved in biosynthesis of choline, ond of the membrane main lipids phosphatidyl coline synthesis components, BFRUCT3 gene one of the enzymes involved in biosynthesis of plants invertases which hydrolyzing sucrose to glucose and fructose, OMT gene contributed in enzymatic methylation of oxygen atoms of secondary metabolites and NHX1 gene encoding Na^+/H^+ antiporter of tonoplast membrane and involved in the transport of sodium into the vacuoles are involved in response to abiotic stresses.

Keywords: Abiotic stress, Salinity, Gene expression modification, Jasmonic acid, Molecular chaperons

karyological study of wild saffron *Crocus cancellatus* subspecies *damascenus*

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Abstract

Saffron *Crocus cancellatus* is a wild species of saffron, a perennial, herbaceous, and ornamental plant of the Iridaceae family. This species has several subspecies, among which Iran is one of the main habitats for *damascenus* subspecies. In the present study, in order to obtain more information about the karyotype and to clarify the taxonomy of this group, karyological analyzes of four Iranian ecotypes were performed using growing roots. Comparative cytogenetics showed that the studied ecotypes had a high diversity in the number of chromosomes, so that the ecotypes of Aligudarz and Marivan had chromosome numbers of 2n = 8, Seghez 2n = 10 and Eghlid 2n = 12. In addition, this sub-specious had different chromosomal structures, so that the ecotype of Aligudarz had 8 sub-telocentric chromosomes and Eghlid had 3 sub-metacentric, 8 sub-teleocentric and 2 metacentric chromosomes. Also, in Saghez ecotype, 8 subtelocentric chromosomes and 2 metacentric chromosomes were seen, and finally, we saw 6 subtelocentric chromosomes and 2 submetacentric chromosomes in Marivan ecotype. Therefore, the results of this study suggest the hypothesis of the existence of a new species in the populations of *damascenus* in Iran that additional studies of genetic and morphological diversity should be performed to more accurately assess the taxonomy of this subspecies.

Keywords: chromosome number, *Crocus cancellatus* subsp. *Damascenus,* karyotype

Optimization of regeneration in leaf explants of African violet (*Saintpaulia ionantha*) using response surface methodology (RSM)

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Abstract

The aim of the present study was to optimize regeneration in two cultivars (Grinia and Littel Maya) of African violet (*Saintpaulia ionantha*) using response surface methodology (RSM). Leaf explants were washed thoroughly by distilled water and then sterilized using hypochlorite 50%. The explants were transferred on the MS medium supplemented with different concentrations of BA (0.1 and 0.2 mg/l) and NAA (1 and 2 mg/l). Optimization of regeneration was performed using Design Expert software. Independent variables were benzyl adenine (BA), naphthalene acetic acid (NAA) and cultivars and the response was regeneration. The statistical analysis indicated that all independent variables and interaction between BA and NAA were found to be significant ($p \leq 0.05$). Based on the proposed models by software, the optimum condition for regeneration was as follows; 0.42 mg/l of BA, 1 mg/l of NAA and "Grinia" cultivar.

Keywords: Regeneration, Response surface methodology, Optimization

Comparison of three different nutrient solutions (Hoagland, QL and MS) on growth and some biochemical parameters of *Hyssopus officinalis* L.

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Abstract

Hyssopus officinalis L. is a shrub in the Lamiaceae family has been used in traditional medicine. The purpose of this research was to explore the possibility of hyssop propagation under in vitro conditions. We studied different growth parameters, pigments production, and content of phenolic compounds of the *H. officinalis* germinated and grown on three different media culture (MS, QL and Hoagland) in vitro for 90 days. According to the results, Hoagland supported better growth than QL and MS such that the fresh biomass of plant grown in the Hoagland was 60 and 100% more than that of QL and MS. Interestingly, the development of new organs including new shoots and leaves as well as photosynthesis pigments content in hyssop plants grown in the QL and Hoagland was more than that of the MS. Conversely, the MS grown plants had significantly more phenolic compounds as compared to QL and Hoagland. The probable cause of these findings based on nutrient contents of the media culture was discussed in detail.

Keywords: Hoagland, Hyssopus officinalis, MS, Phenolic Compounds, Photosynthesis Pigments, QL

Evaluation of the activity of defense enzymes of cucumber plant against the F. oxysporum f. sp

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Abstract

Invasion of plants and diseases caused by plant pathogens such as viruses, fungi, nematodes and bacteria reduces the yield and quality of products. Fusarium oxysporum is one of the fungal pathogens that attacks the roots of cucurbitacea plants. In this study, the activity of PAL, Glucanase and Chitinase enzymes under the Fusarium oxysporum in cucumber plant in greenhouse conditions was evaluated. In general, the results showed that the activity of PAL enzyme from the first to the fifth day had an increasing trend and then had a decreasing trend, while this trend in Glucanase and Chitinase enzymes was first decreasing and then increasing. The antifungal activity of chitinase and 3,1-beta-glucanase was demonstrated by the researchers, who, after purifying and placing these proteins in culture medium containing plant fungal pathogens, observed that the growth of these fungi was restricted in the areas where these proteins were present. This can be a good reason for the direct effect of these PR proteins on pathogens. The results of this study also indicate an increase in the expression of these enzymes in fungal treatment compared to the control over time.

Keywords: Cucumber - Fusarium - PAL - Glucanase - Chitinase

Diversity of flavonoid markers in *Teucrium gnaphalodes* using fingerprinting method

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Abstract

The genus *Teucrium* L. belonging to Lamiaceae with different secondary metabolites is one of the well-known medicinal plants in the world. Therefore, different species of this genus have been extensively used in pharmaceutical sciences and treatment of diseases. This research aimed to identify phytochemical diversity with emphasis on flavonoid markers in the eight accessions of *T. gnaphalodes* belonging to the south and south west regions of Iran. Accordingly, the leaf extract of each accession was yielded using 95% methanol. Then, flavonoid isolation and purification were performed using thin layer chromatography and column chromatography with chloroform-methanol solvent system. The identification of flavonoid derivatives was accomplished by high performance liquid chromatography mass spectrometry (HPLC-MS/MS). Phytochemical diversity was performed by principle component analysis (PCA) and PAST 3.14. The results of this research revealed 16 flavonoid compounds, some of which include kaemferol glycoside, quercetin hexoside, dihydronaringenin hexoside, hexahydroxy flavone methyl-ether, and orotinin. In addition, principle component analysis showed two distinct groups for this species. Consequently, the compounds identified in the southern regions were mostly attributed to hydroxy-methoxy flavone and flavone methyl-ether and in southwestern regions were flavonol and flavone hexoside. Based on the results of this research, phytochemical diversity has been accurately detected using HPLC-MS/MS method at the infra-specific level. The data obtained from this research can be useful in the purposes of technology, propagation and conservation of medicinal plants in the country.

Keywords: diversity, Lamiaceae, spectrometry, flavonoid

International Abstract

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دوازدهمین همایش ملی و چهارمین همایش بین المللی
بیوتکنولوژی جمهوری اسلامی ایران

Clinical findings of patients with human bronchial asthma in Basrah, Iraq

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Abstract

This study aimed to describe the clinical features of patients asthma in Basrah south in Iraq. The study showed that (3, 5) age group population were more affected with asthma (27.9%) and the Females were more affected than males in group 2, 3and 5 (6.4%, 15.7% and 14.7%) respectively compared to (4.9%, 12.3% and 13.2). in same group of male. While There were (68.6%) of patients came from urban areas in comparison to (31.4%) of cases who came from rural areas. The Smoking patients with positive (43.1%). and well patients with animal contact positive their proportion was while (49%). Seasonal asthma attack in male (23.5%) more than female (20.6%) the perennial asthma attach was recorded in male (29%) more than female (26.9%) in this study show Asthmatic patients with other allergy about (15.7%) and with chronic diseases (31.9%). The percentage of patients with positive family history were 39.2% of the cases, The pulmonary function test result was recorded below (70%) in all age groups. Skin test where the study found highly percentage to HDm to female (76.8%) and (66.2%) to male and HD to female (69.5%) and (68.7%) to male.

Keyword: clinical parameters, human bronchial asthma

Spirometric criteria for airway obstruction: use percentage of FEV1 ratio as a Pulmonary function test for asthmatic patients in Basrah, Iraq

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Abstract

This study aimed to describe the clinical features of patients asthma in Basrah south in Iraq. The pulmonary function test result was recorded below (70%) in all age groups. The study of pulmonary function test includes study of parameter FEV1.0 showed that age group2 record (63%) for male only, other age groups recorded following rate for male and female respectively (61.17 and 65.85%), (61.88 and 49.50%) and (68.75 and 63.50 %) for age group 3, 4 and 5 respectively according to statistical analysis.

Keyword: Pulmonary function test, human bronchial asthma

Study the Increasing of Brain Toxicity in Newborn Rats and Related to Exposure Their Mothers to Uranyl Acetate

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Abstract

Background: congenital and foetuses malformations was notified increase with abnormalities of brain in infants, and other cases within population of Iraq after first and second Wars of Gulf [2]. Aims: This study aimed to investigate uranyl acetate effects in many areas of rats brain. Materials and methods: Twenty-four rats (albino female Sprague- Dawley) was Adult of with body weight between 200-250 grams, have age ranged 12-15 weeks old were administered orally with 75mg/kg of uranyl acetate dihydrate (UAD) as single dose before mating, with untreated males for two weeks, in addition to pregnancy period and lactation. The newborn at age of third and fifth weeks were scarificed; the brain has been raised. Results: The offspring brain have histopathological change at third week which treated by 75 mg/kg, it's appear blood vessel congestion and lumen have neutrophil aggregation with estrocyte hypertrophy in the parenchyma of brain. The newborn brain sections at fifth weeks administered with 75 mg/kg was show neuron cells vaculaotion and nuclei shrink with blood vessel congestion and nerve cell a rounded with oedema.

Keyword: Uranyl Acetate, Brain toxicity, Uranium, newborn.

Prevalence of Sarcocystis Infection in Slaughtered Sheep and Goats in Duhok Province/ Iraq

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Abstract

A total of 2358 sheep and 532 goats were examined for the presence of macrocystis of *Sarcocystis*. For microcysts, different muscle tissues were randomly taken from 118 sheep and 110 goats. Macrocystis were examined through naked eye inspection, while microcysts were examined microscopically by using histopathology, pepsin digestion, mincing & squeezing and muscle squash method. The overall prevalence of macrocystis was 1.2% in sheep and 2.6% in goats. The intensity rate of the cysts was 4 cysts/ gram in sheep & 3 cysts/ gram in goats, respectively. while, the overall prevalence of microcysts in sheep and goats was 96.5%. The infection rate in sheep was 96.6% and in goats was 96.4%. The total intensity rate of microcysts was 32.4 cysts/ field in sheep and 16.8 cysts/ field in goats, respectively. Histopathological examination found different shapes, size, wall thickness and intensity rate of microcysts in muscle tissues of sheep & goats. The pathological reaction showed mild to moderate granulocytosis and mononuclear cells infiltrated surrounding the microcysts with necrotizing and degeneration of myofibrils. The largest average size of spindle and round shaped cysts (290 ± 89.7 x 76.1 ± 10 µm and 88.8 ± 10.3 µm) in goats and (127.2 ± 18.9 x 53.3 ± 5.4 µm and 74.4 ± 7.5 µm) in sheep, was detected in the esophageal muscle. Statistically, there was significant difference ($P<0.05$) in the prevalence of macrocystis in sheep and goats, while no significant difference ($P>0.05$) was observed in the prevalence of microcysts between the both animal species.

Keyword: Macrocystis, Microcysts, Intensity rate, Measurement size

Molecular Identification of some isolates of Fungi Isolated from Al-Barakia wastewater treatment plant

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Abstract

This study was conducted in the Microbiology Laboratory of the Department of Environmental Sciences - College of Science - University of Kufa with the aim of isolating and diagnosing fungi isolated from the wastewater treatment plant in Al-Barakia in Najaf Governorate. Fungi isolates were diagnosed using the polymerase chain reaction (PCR) technique and the nucleotide sequences of the DNA products multiplied using ITS1 and ITS4 primers. The results of the analysis of the nucleotide sequences of the double DNA products of the isolated fungal isolates in this study and using the BLAST program showed that (19) were diagnosed and it was applied that some fungalates in this study indicated (1, 2, 3, 4, 5, 6) belonging to the fungus Aspergillus caespitosus. Aspergillus flavus (7, 8) showed a difference in the sequence of some nitrogenous bases for the DNA products. Aspergillus.niger (12, 13), Trichoderma asperellum (9, 10, 11), Aspergillus oryzae (14) Alternaria alternata (15), Acremonium sp (16), Aspergillus terrus (17), Cladosporium sphaerospermum (18), Aspergillus tubingensis (19).The results showed that all the fungi isolates isolated in this study that all isolates were recorded globally, and some of them were not recorded in Arabic such as (Acremonium sp, Aspergillus flavus, Alternaria alternata, Cladosporium sphaerospermum, Aspergillus tubingensis) but not previously registered in the Iraq National Center for Biotechnology Information (NCB).

Keyword: wastewater, Molecular Identification, fungi, Polymerase chain reaction (PCR), DNA sequence analysis

Optimum Condition for glutaminase Activity in Crude Extract of Capiscum annum

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Abstract

In this study glutaminase was extracted from fruit part of Capiscum annum then Glutaminase activity was detected and optimised. Optimum conditions for the activity of crude glutaminase were studied. Results showed maximum activity of glutaminase was achieved 1.8 u/ml when the enzyme was incubated with 150 mM of glutamine at 35 °C for 30 minutes in the presence of 0.05 M of potassium phosphate buffer solution at pH 8.

Keyword: Capiscum annum, glutaminase, enzyme activity

Antibacterial Activities of Lemon Oil Against Escherichia coli Bacteria isolated from urine

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Abstract

According to presence of biological active compounds in lemon oil and use of this oil in traditional medicine, it seems that this plant possesses considerable antibacterial activity. The purpose of this study was investigating the antibacterial activity of lemon oil 25, 12.5 and 6.25, 3, 125, 1.56, 0.195 µl/ml concentrations of oil on the ten isolates of *Escherichia coli*. Results showed that lemon oil prevented bacterial growth of *Escherichia coli* 12.5, 6.25 µl/ml concentration of this oil indicated inhibitory effect on *Escherichia coli isolates*.

It can be concluded that despite the inhibitory effects of concentrations of Lemon oil on the growth of pathogenic bacteria especially *E. coli* to introduce it as an alternative to chemical antimicrobial drugs, wider investigation is required.

Antibacterial effects of aqueous and al-coholic extract of Colutea cilicica L plants

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Abstract

In this study, samples were collected from the plant *Colutea cilicica* L, which is a member of the family Papilionoideae (legume family), and it is the only species of the genus *Colutea* L spread in Iraq. Aqueous and alcoholic extract of the plant were prepared for antibacterial efficacy study. All of extracts were tested for their antibacterial activity against (8) pathogenic bacterial three of them Gram positive bacteria (*Staphylococcus albus, Staphylococcus aureus and Streptococcus pyogenes)* and five were negative for Gram stain (*Escherichia coli, Klebsilla pneumoniae, Pseudomonas aeruginosa, Serratia marcescens and Sallmonella typhi)* by perpendicular streak method on Muller - Hinton agar. The extracts have antibacterial activity against pathogenic bacteria. The study proved the effectiveness of the extract against bacteria. The diameter of inhibition zone of the alcoholic extract reached (30) mm against Gram positive bacteria and reached (7.27) mm against Gram negative bacteria. While the inhibition diameter of the aqueous extract reached (29) mm against Gram-positive bacteria (5.30 mm) against Gram-negative bacteria. The results showed that the minimum inhibitors concentration (MICs) of alcoholic extract against bacteria was (0.625) µg / ml for all gram negative and gram positive bacteria except against *E. coli* was (1.25) µg / mL and the minimum bactericidal concentration (MBCs) ranged from (0.312-0.625) µg / ml against Gram-positive and negative bacteria. While minimum concentration inhibitors (MICs) of aqueous extract were (0.625) µg / ml against Gram negative and Gram positive bacteria, (MBCs) were (0.312) µg / mL against Gram positive and negative bacteria.

Keyword: Antibacterial effects; (MICs) ; *Colutea cilicica L*; (MBCs) ; Aqueous extract; alcoholic extract

Optimization of laccase production from Marasimus palmivorus

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Abstract

Marasimus palmivorus was evaluated for their ability of producing extracellular enzymes (Laccase enzyme) using media containing substrate, this media called Guaiacol agar medium. *M. palmivorus* showed high laccase activity at 3rd day of incubation and give red zone around colony. *M. palmivorus* showed maximum activity for laccase production in liquid media (0.166U/ml). The production of laccase enzyme from *M. palmivorus* was investigated using some environmental condition such as; Temperature, PH, and incubation period. The results showed that the optimum incubation period for laccase in *M. palmivorus* was occurred within four days (96 hour). Laccase exhibited maximal activity at pH 5.5, and 25˚C.

Keyword: laccase, *Marasimus palmivorus*

Combined Interferon-Antiviral therapy effectiveness against Hepatitis B viral infection in Babylon Province

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Abstract

Background: Hepatitis can be defined as one of the liver's inflammations, that might be caused via viral infections, toxins, drugs and alcohol. The hepatitis via viral infection can be classified according to the type of viral strain and symptom into (A, B, C, D, E). Also, hepatitis classified according the situation of disease into acute hepatitis, which is specified as a hepatitis of short term, and within six months, the virus is cleared from the body via the immune system, however, the chronic hepatitis is defined as a long-term hepatitis, in which the infection prolong over six months since the immune system of the body has no ability for clearing the virus. In addition, the Hepatitis B is specified as one of the dangerous diseases resulting from a virous that is infecting the liver and has the ability of causing life-long infection, liver failure, liver cancer, cirrhosis (liver scarring) and death. Methodology: The current work is carried out for evaluating the interferon as well as antiviral treatment effect on the viral load and viral activity among certain group of Hepatitis B virus infected patients enrolled to GIT and liver center in Merjan Medical City using Real time PCR for viral load and viral copy number determination. Results: the results show that the all recorded cases in GIT center about (3612 hepatitis virus at both B and C), 2226 for HBV and 1386 for HCV, only 566 HBV patients was analysis by PCR

around the year 2016. The males were high significant than females in hepatitis B infections. The north area of Babylon province had higher percentage than south and middle areas. Low activity of treatment protocol was mentioned on HBV infected patients, where (38.62 %) of patients had final outcome as undetectable viral load after treatment with combined interferon and oral anti–viral drugs. Conclusion: was that the combination treatment of immunological derivative and oral treatment more effective than single treatment used in HBV infection.

Keyword: Hepatitis, HCV, HBV, interferon.

Molecular Study on Efflux Pumps of Uropathogenic Escherichia coli (UPEC) Isolated from Patients with Cystitis

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Abstract

Background: Uropathogenic Escherichia coli regards most important and prevalent Gram negative bacteria among urinary tract infections. UTIs may be the first common infection in both hospital acquired and community acquired infections. Recurrent and chronic infections in UTIs is more frequent than other and may be attributed to antibiotic resistance. One of the highly distributed chromosomally encoded trait of resistance is efflux pumps. The current study was conducted to investigate most common members of 5 classes of efflux pumps among Uropathogenic E. coil isolates. Methodology: E. coli isolates was diagnosed on EMB by green metallic sheen appearance and confirmed by uidA gene. Antibiotic susceptibility test was performed according to CLSI-2019. Efflux pumps genes were investigated by PCR. Results: The antibiotic susceptibility test was performed according to CLSI-2019 using disc diffusion method for 26 antibiotics and the results revealed that, all isolates were resist to Amoxicillin, Ceftazidime and Cefotaxime. Less resistance were displayed to Cefepime (58%), Ceftriaxone (54%) Cefixime (52%) and Cefoxitin (42%). Low resistance were exhibited to rest of antibiotics: Kanamycin (42%), Nitrofurantion (38%), Streptomycin (36%), Trimethprime (32%), Nalidixic acid (26%), Ciprofloxacin (26%), Aztreonam (20%), Tobramycin (20%), Piperacillin (14%), Ofloxacine (14%), Amikacin (14%), Levofloxacin (12%), Azithromycin (10%), Doxycycline (8%), Meropenem (6%), Gentamicin (4%), Netilmicin (2%) while all isolates were sensitive to Imipenem. Concern antibiotic resistance patterns (MDR, XDR and PDR), the results revealed that 19 (38%) of E. coli isolates were non-MDR while 31 (62%) were MDR with variable no. of classes resistance: 2 (4%), 6 (12%), 1 (2%), 12 (24%), 10 (20%) for MDR-7 classes, -6 classes, -5classeses, -4 classes and -3 classes respectively. Results of molecular investigation of efflux pumps in E. coli revealed that, class RND AcrAB-TolC, AcrAD-TolC and AcrFE-TolC genes were distributed as follow: acrA 50 (100%), acrB 43 (86%), acrD 48 (96%), acrF 33 (66%), acre 50 (100%) and tolC 50 (100%). Three class MFS pumps (EmrAB-TolC, EmrD and MdfA) were also investigated for E. coli and the results: emrA 50 (100%), emrB

50 (100%), emrD 50 (100%) and mdfA 49 (98%). Three class SMR pumps (EmrE, YnfA and TehA) genes were distributed as follow: emrE 48 (96%), ynfA 50 (100%) and tehA 49 (98%). Two class ABC pumps (MacAB-TolC and MdlAB-TolC) genes were investigated for E. coli and the results: macA 50 (100%), macB 49 (98%), mdlA 50 (100%) and mdlB 49 (98%). Two MATE pumps (MdtK and DinF) genes were studies and the results revealed that: mdtK and dinF genes were present in all E. coli isolates. Concern results of coexisted pumps in same E. coli isolates the results showed that: 32 (64%) of isolates have genotype AcrAB-TolC/ AcrAD-TolC/ AcrFE-TolC/ MdfA/ EmrD/ EmrAB-TolC/ EmrE/ YnfA/ TehA/ MacAB-TolC/ MdlAB-TolC/ Mdtk/ DinF while 16 (32%) have genotype AcrAB-TolC/ AcrAD-TolC/ MdfA/ EmrD/ EmrAB-TolC/ EmrE/ YnfA/ TehA/ MacAB-TolC/ MdlAB-TolC/ Mdtk/ DinF. Results of biofilm formation revealed that, 98% of isolates were biofilm former while 2% were non-biofilm. 60% was weak biofilm, 24% was moderate and 14% was strong biofilm former. Conclusion: The current study conclude that, all efflux pumps may be highly associated with resistance to amoxicillin, cefotaxime and ceftazidime and moderately associated with Cefepime, ceftriaxone and Cefixime and may be unrelated to resistance of other studied antibiotics or the concentration of theses antibiotics inadequate to induce the expression of these pumps. Additionally biofilm formation were highly related to presence of studies pumps.

Keyword: UPEC, AcrA, AcrB, TolC, AcrD, AcrF, AcrE, MdfA, EmrD, EmrA, EmrB, EmrE, YnfA, TehA, MacA, MacB, MdlA, MdlB, Mdtk, DinF

Molecular Investigation of Outer Membrane Channel Genes Among Multidrug Resistance Clinical Pseudomonas aeruginosa isolates

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Abstract

Multidrug resistance *Pseudomonas aeruginosa* (MDRPA) is most important issue in healthcare setting. It can secrete many virulence effector proteins via its secretion system type I through type VI T1SS-T6SS) Use it as conductor for delivering the effector proteins outside to begins harmfull effect on host cell increasing pathogenicity, competition against other microorganism and nutrient acquisition. The study include investigation of 50 isolates of MDRPA for transport secretion system and resistance for antibiotics. Molecular diagnosis using P. aeruginosa specific primer pairs, investigation of *AprF, HasF, XcpQ, HxcQ, PscC, CdrB, CupB3, Hcp* using specific primer pairs by PCR were also performed. The results revealed high resistance to beta lactam antibiotics (78% for ceftazidime, 78% for cefepime and 46% for piperacillin) can indicate possessing of isolates for beta lactamases and this confirmed by dropping resistance to piperacillin to 16% when combined with tazobactam. Also the results shown the ability of MDRPA for pyocyanin biosynthesis using the system of genes. The current study conclude that all isolated of P. aeruginosa were highly virulent due to their possessing of all transport secretion system and beta lactamases make using of piperacillin-tazobactam and meropenem a good choice to kill bacteria along with impairment of effector proteins production reducing the possible harmful effects of these proteins.

Keyword: TSS, *AprF, HasF, XcpQ, HxcQ, PscC, CdrB, CupB*, *HcP*, piperacillin-tazobactam

Genetic Comparative Study of Staphylococcus aureus isolated from infections and Rural water

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Abstract

One hundred specimens from wounds, burns, and Rural water were collected, wounds and burns isolates were collected from patients laying in hospital from different age and gender. It was found that 50 isolates belong to *Staphylococcus spp*., 38 isolates were identified as *S. aureus* from infections isolates and 12 isolates were identified as *S. aureus* from rural water according to microscopic, cultural and biochemical testing. The study of seven extracellular enzyme as virulence factors including the enzymes: urease, lipase, DNase, haemolysin, coagulase, β-lactamase, and lecithinase. Revealed that 100% of *S.aureus* which isolated from infections had the ability to produce these enzymes, while the isolates of rural water were unable to produce the enzymes DNase, lipase, β-lactamase, but they were capable to produce haemolysin, urease, lecithinase, and coagulase the range for production of these factors were 50 %, 82, %, 7, %, and 43% respectively. 14 *Staphylococcus* isolates from infections isolates and 4 isolates from rural water isolates were selected according of their ability for production most of studies virulence enzymes for detection of genes encoding for the enzymes heamolysine (hly) and (coa) by using of polymerase chain reaction (PCR) technique. The Gene hIy was detected in all isolates from infections and rural water and coa was detected in infections isolates only.

Keyword: *Staphylococcus aureus*, PCR, virulence factor, heamolysine (hly), coagulase (coa), rural water.

Correlation between *IL-13*rs20541 (A>G) Gene Polymorphism and Bronchial Asthma among Iraqi patients

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Abstract

Bronchial asthma has a complicated genetic history. Changes in gene expression may be caused by gene polymorphism, cytokines play a central role. IL-13 is an interleukin that has been shown to play a role in the disease's immunopathogenesis. The current case-control study investigated the relationship between rs20541 of the *IL-13* gene and Bronchial Asthma in Iraqi patients. Seventy-five patient and fifty healthy individuals as a control. The results indicated a highly significant elevation in the levels of the IgE, and IL-13 in the patients compared to control at (P -value ≤ 0.01), (456.45 ± 290.106 vs. 30.08 ± 24.414), (59.5980 ± 20.93750vs.6.7034 ±4.10547) pg./ml respectively. The DNA was extracted from blood samples. Detection of genotype *IL-13*SNP (rs20541) were achieved by RFLP-PCR. The result shows no significant differences in the frequency distributions of *IL-13* SNP (rs20541) for all genotypes in cases and controls. A protective role of asthma, (O. R: 0.62; C.I.95%: 0.23 - 1.6) and (O. R 0.89: C.I.95%: 0.42 - 1.89) were observed for wild type homozygous and heterozygous genotype respectively. Whereas the AA genotype (42.7%) in cases and (34.0%) in control, that (O.R: 1.44; CI.95%: (0.66 - 3.07) mutant homozygous were risk factors of asthma among individuals. Results revealed that the presence of IgE and IL-13 were significantly associated with genotypes of *IL-13* rs20541 (GG, AG, AA) among patients and control at (P ≤ 0.05). In conclusion, the AA genotype in case and control mutant homozygous were risk factors of asthma among individuals. It's possible that this has a predisposing impact on the development of asthma.

Keyword: Bronchial Asthma, RFLP, IL-13, SNP.

Evaluation of superoxide dismutase enzyme in some Iraqi plant waste

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Abstract

Superoxide dismutase plays an important role in pharmaceutical, nutraceutical, and cosmeceutical industries as it can eliminate toxic superoxide radicals. In this investigation, waste of five-different plants were screened for their content of SOD enzyme. These waste were peels of watermelon, melon, banana, bitter orange, and potato. Peels of the melon, watermelon, and bitter orange were found to contain higher SOD activity than peels of potato and banana. The results also showed that this SOD activity was stable for six months. A specific variety of melon named Ananas was found to contain the highest SOD activity and specific activity than peels of other local melon varieties. Among the different buffers that have been used to extract this enzyme from the melon peel, potassium phosphate buffer (50 mM KH_2PO_4, pH 7.8) containing EDTA (0.1 mM) gave the highest specific activity. Furthermore, polyacrylamide gel electrophoresis in the absence and presence of either KCN or H_2O_2; as selective inhibitors of specific SOD isoenzymes, was used to detect the different SOD isoenzymes present in peels of the studies plants. The results indicated the presence of different isoenzymes in these peels.

Keyword: Peels of plants, Superoxide Dismutase Activity, Superoxide Dismutase Specific Activity, Thermal Stability, Storage Stability, Superoxide Dismutase Inhibitors.

Expression profile of CAPZA3 and TR-KIT genes in Men with Globozoospermia & Asthenoteratospermia that undergo ICSI protocol

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Abstract

Objective (s): Sperm-mediated oocyte activation depends upon suitable expression and assembly of sperm-borne oocyte-activating factors (SOAFs) during spermiogenesis. Several factors have been considered as candidates for oocyte activation in recent years. Globozoospermia is a severe sperm morphology disorder that is a rare type of teratozoospermia with an incidence of 0.1% among infertile individuals. testis-specific genes including CAPZA3[capping protein (actin filament) muscle Z-line, alpha, which is considered as a nominee for sperm associated oocyte activating factors, an actin-capping protein controlling actin polymerization during spermiogenesis. They contain a common bidirectional promoter.Another gene TR-KIT (a truncated form of the KIT receptor) which is a major sperm-associated oocyte-activating factor. The objective of this study was to investigate the expression profile of CAPZA3 and TR-KIT mRNA, in men with total globozoospermia, Asthenoteratospermia, and fertile individuals.

Materials and Methods: Semen samples were collected from three groups including 25 fertile men, 20 Asthenoteratospermia and 12 Globozoospermia that undergo intra-cytoplasmic sperm injection (ICSI), Expression of CAPZA3 and TR-KIT were assessed by Real time PCR.

Results: Individuals with Globozoospermia have presented significantly lower expression of CAPZA3 and TR-KIT mRNA when compared with fertile men. Asthenoteratospermia (male factor) showed significantly lower expression of CAPZA3 mRNA, whereas non-significantly of TR-KIT mRNA. Levels of CAPZA3 and TR-KIT mRNA in the spermatozoa of fertile men were significantly higher than the corresponding values of the globozoospermic and Asthenoteratospermia subjects.

Conclusion: Analysis mRNA of CAPZA3 gene maybe assist the researcher to identify individuals with a lack of ability to induce oocyte activation and make them a candidate for artificial oocyte activation and help researcher to identify genetic defects associated with failed fertilization.whereas, mRNA of TR-KIT gene appears inability to induce oocyte activation.

Biosynthesis, antibacterial potential and burns healing effect of silver nanoparticle loaded on *Glycyrrhiza glabra* on balb-c albino male mice

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Abstract

Background and objective: *Glycyrrhiza glabra* is one of the useful medicinal plants with increasing demand for (herbal medicines, health products, pharmaceuticals). *Glycyrrhiza glabra* used in traditional medicine across the world for its ethnopharmacological value. Materials and Method: this study was included the evaluation of the effects of green synthetic nanoparticles of *Glycyrrhiza glabra* aqueous extract loaded with silver nitrate on antibacterial activity and anti-inflammatory activity through burn healing effect. The antibacterial activity of green synthetic silver nanoparticles was studied against one type of Gram-negative (*E.coli*) and Gram-positive (*staphylococcus aureus*). Also, the effects of burns healing effect of green synthetic silver nanoparticle was evaluated using albino male mice. The activity of green synthetic nanoparticle at (1.5mM) was investigated in compared with sliver sulfadiazine as positive control and negative control mice (without any treatment) by determining days require for healing. Results: the result showed that different nanoparticles concentrations (1, 1.5, 1.75, 2 mM) can inhibit the bacterial isolate with varying zones of (17, 20, 12, 17mm) for Gram-positive *Staphylococcus aureus*) and (10, 22, 10, 22mm) for Gram-negative (*E.coli*) at (1, 1.5, 1.75, 2 mM) respectively. Also, the results showed that green synthetic nanoparticle could heal burns in 12 day compared to 14 days for sliver sulfadiazine and 18 days for negative control. Conclusion: green synthetic nanoparticle possessed antibacterial and anti-inflammatory activity due to active constituents of plant.

Keyword: nanoparticle, anti-inflammatory, antibacterial, *Glycyrrhiza glabra*, anti-bacterial, Gram-negative, Gram-positive, burn, heal.

Ecological and Health risk assessment of some heavy metals in baby milk and their foods available for consumption in Kut city – Iraq

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Abstract

This study focused on estimating lead and cadmium levels in 108 samples dedicated to feeding children, including buffaloes milk, cows milk, infant dried powder milk, liquid pasteurized milk, condensed milk, and food consisting of rice, wheat, cereal and fruits, from different regions and local markets from Kut city, which is considered one of the most important components of the basic meals for children was determined using atomic absorption spectrometer. The results showed accumulations of lead and cadmium elements in the studied samples with a concentration rate of 0.95, 0.244 and 0.65, 0.146 and 0.25, 0.073 and 0.387, 0.251 and 0.499, 0.295 mg / kg in buffaloes milk, cows, infant dried powder milk, liquid pasteurized milk and condensed milk respectively. The concentration rate for foods consisting of rice 0.279, 0.0815 mg / kg, wheat 0.227, 0.092 mg / kg, cereal 0.1965, 0.0595 mg / kg and fruits 0.317, 0.091 mg / kg for lead and cadmium, respectively. On the other hand, the results of the chemical analyzes of this study showed that there is contamination with lead and cadmium elements in the studied samples and it was more than the permissible limits globally and at an insecure level for human consumption.

Keyword: Baby foods, Heavy metals, Lead, Cadmium, Milk.

Assessment of serum vitamin D level in hypertensive patients a case control study

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Abstract

Introduction: Vitamin D has numerous effects due to its role in bone health and calcium metabolism. Vitamin D deficiency nowadays may be due to low sunlight exposure. A close relationship is between hypertension and Vitamin D deficiency, this due to that 1, 25 (OH) 2D is a potent inhibitor of Renin Aldosterone Angiotensin System (RAAS) which is the chief way responsible for occurrence of hypertension. 25 ,1 (OH) 2D improves activity of the NO system, and reduces inflammatory and atherosclerotic parameters by acting as a vascular protecting agent via decreasing the deleterious effect of advanced glycation end products on the endothelium.

Purpose: To assess serum level of vitamin D3 in hypertensive patients versus normotensive control and to determine the correlation between serum level of vitamin D3 and SBP and DBP in hypertensive patients.

Methods/Materials: The present case control study involved 150 (81 male and 69 female) hypertensive patients and 76 (30 male ande 46 female) normotensive age- and sex-matched collected by stratified random sampling. All Individuals included in the study were submitted to: Complete history and physical exam to evaluate exclusion criteria, renal and liver function tests, lipid profile, fasting blood glucose, and serum calcium. All patients and controls were subjected to blood pressure measurements and vitamin D tests.

Results: The results are typical for hypertensive patients where there is a significant increase in SBP and DBP (both $p < 0.001$) and the level of vitamin D3 is significantly decreased ($p < 0.001$) in hypertensive patients. There is no significant difference between male and female in vitamin D level in patient group. Among the hypertensive individuals, 11.3% were Vitamin D deficient and 82.7% had insufficiency and 6% sufficiency. No significant correlation was observed between vit.D3 with SBP ($p=0.645$) and DBP ($P=0.962$).

Conclusion: There is a decrease in serum vitamin D3 level in most hypertensive participants in comparison with normotensive, but serum 25 (OH) D was not correlated with diastolic or systolic blood pressure in hypertensive group.

Keyword: vitamin D3 deficiency, hypertention, 1, 25 (OH) 2D.

Novel electrochemical behaviour based on estimated pH level of SARS-COR2 viral transport media prepared in Basrah/Iraq

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Abstract

In this work, the electrochemical scan of viral transport media (VTM) on a screen printed gold electrode was studied using cyclic voltammetry technique. The dependence of the anodic and cathodic currents on VTM and screen printed gold electrodes was investigated to optimize the experimental conditions of pH values determination. The oxidation and reduction mechanism in this study were proposed. Under the optimum pH conditions, the oxidation and reduction peak current were linearly proportional to the pH value of VTM in the range from 6.5 to 9.0 with a standard behaviour of control sample as reference. The novel proposed method to optimum pH level determination of real VTM samples was successfully applied. Three pH conditions of viral transport media (VTM) were compared to the standard reference sample. The pH value of VTM was investigated using unique electrochemical sensing assay. All VTM were prepared in Basrah University-Faculty of Pharmacy Lab and the pH level was determined by pH meter within 24h and compared with DropSens technique at the same time. Overall, the pH value of VTM which identified rapidly was checked electrochemically within 10 second as a routine test. The electrochemical behaviour of VTM pH and its value was studied using (C.V) cyclic voltammetry carried out at a screen printed gold electrode which was a novel method. The oxidized and reductized reaction peak at the surface of screen printed gold electrode were determined the value of pH according to the specific electrochemical behaviour which depended. Both peaks on cyclic voltammograms corresponded to electrooxidation and electroreduction were used to detect pH level of VTM and which increase the current dramatically with pH value, respectively. The aim of the current study was to evaluate the performance characteristics of the electrochemical pH estimated sensing assay in comparison to conventual pH meter tool of VTM solution. These electron transformations resulted in increased C.V oxidation and reduction currents as recorded using a platinum working electrode it was suggested that the higher oxidation and reduction peak currents were due to an increase in the pH value of VTM solution.

Keyword: Electrochemical, SARS-COR2 VTM, Basrah-Iraq, pH VTM, C.V Estimation.

Mechanical and physical methods for DNA extraction from coagulated blood samples

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Abstract

In this study, mechanical and physical extraction methods were applied to facilitate clot dispersing by using mortar and pistil along with microwave respectively. Blood samples were collected from healthy people, randomly non- selectively by sex and age. Mortar and pistil plus extraction buffer were used to grind dried clotted blood mass previously dried by microwave. Following clot dispersing, normal (organic) methods were used to complete the DNA extraction. DNA has been monitored spectrophotometrically by using different wave lengths (230, 260, and 280) nm. DNA concentrations for different blood samples were varied between 13.05 and 52.5 ng/ µl. all gDNA samples have been showed ratios in the accepted range (1.69-1.88) at 260/280 nm and (1.66- 1.91) at 260/2030 nm of purity. PCR technique was used to amplify the target sequence of the *LEP* gene from the extracted gDNA and the product were analysed by running in 2% agarose gel electrophoresis to ensure the amplification eligibility of the extracted DNA.

Influence of Dietary Vitamin E and Selenium on Eggs' Fertility and Hatchability in Japanese Quail

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Abstract

This study was conducted at the Sulaimani Polytechnic University, Kalar Technical College, Veterinary Techniques Department and animal management laboratory. The aim of the present research was to evaluate the effect of dietary supplementation with different levels of vitamin E and inorganic selenium (Sodium selenite) on the fertility and hatchability rate of Japanese quail, from 15-04 -2021 to 15-07- 2021. A total number of (128) at 12 weeks old Japanese quail (*Coturnix Coturnix Japonica*) were used (96 females and 32 males). These birds were randomly distributed equally into four dietary treatment groups, and each treatment was equally subdivided into 8 replicates per group each replicate box constitutes of 1 male (8 males per group), hens (female) were also distributed on 32 boxes of 8 replicates per group; each replicate constitutes of 3 hens (24 hens per treatment group). Birds housed on vertical boxes and were raised under similar environmental, managerial, and veterinarian conditions. The four experimental diets (for males and females) were: 0 = the basal diet (control) with no any additions (0 Vit.E+ Selenium), T1 = 1 g Vit. E +Selenium / kg diet, T2 = 1.5 g Vit. E+ Selenium / kg diet, T3 = 2 g Vit. E+ Selenium / kg diet. The results showed that the quail birds that were fed supplemented diet with Vit. E+ Selenium had significantly ($P<0.05$) higher results of fertility and hatchability characteristics and the study showed a lower significant effect ($P<0.05$) in embryonic mortality of eggs and higher significant increments ($P<0.05$) in hatchability rate of Vit E+ Selenium supplemented in T2 with hens fed as compared with other treatments and control groups. In conclusion, Supplementation of Vit E +Selenium to the diets of quail act as an ameliorative tool in fertility and hatchability traits of Japanese quail.

Keyword: quail, fertility, hatchability, vitamin E, Selenium

Challenges of cancer chemotherapy (local survey)

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Abstract

Globally, cancer is the second leading cause of death after the heart disease. Disease of highest incidence include lung, breast, colorectal, and prostate cancers. The cancer problem is assessed to have increased up to 18.1 million new cases globally, with about 9.6 million death cases in 2018. 1 in 6 women and 1 in 5 men developed cancer throughout their lifespan worldwide, and about 1 in 11 women and 1 in 8 men were dying with cancer.

Aim: To assess the problems of cancer chemotherapy and try to find the solutions.

Methods: We conducted an electronic questionnaire in the fourth month of 2021, and the other questionnaire was in the Middle Euphrates Cancer Center during the fourth month also over a period of two weeks. Here, patients were met and all the information required of them was taken such as age, gender, place of residence, smoking status, type of cancer The treatment been received, the efficiency and the price of the treatment.

Results: After the questionnaire results were counted, we found that the ratio of females to males was 65: 35, while the ages of the disease were higher between the ages of 40-70, and cases were more frequent in the city compared to rural area at a ratio of 69: 31, as well as the percentage of non-smokers in the questionnaire was higher compared to smokers and percentage was 60% of non-smokers and 20% for both smokers and those who live in a place where smoking is frequent. The predominant type of cancer was breast cancer 22%, followed by lymphoma 9% and lung cancer 9%, and the percentage of people who had cancer in the family was 22% compared to those who They do not have it by 78%, the treatment that was most used was chemotherapy with 60%, followed by surgery, combined with it by 14%, and the percentages are dwindling by 1% for drugs used to treat cancer. The treatment efficacy ratio between effective, good and ineffective was 36%, 41%, and 21%, respectively. As for the response of our patients, it was high, with a response rate of

79%, compared to 21% of non-response. Finally, the price of the treatment was very expensive by 46%, who said it was acceptable by 34%, and cheap by 20%.

Conclusions:

1.cancer is a deadly disease and the traditional treatments for cancer are very costly and have severe side effects

2. Drug repurposing is a good substitution to the traditional therapy regarding the cost, time taking in invention new therapy and known pharmacokinetic and pharmacodynamic of repurposed drugs

Keyword: cancer, chemotherapy, questionnaire

Molecular Basis of Angiotensin Converting Enzyme-2 Receptors in Severe Iraqi Patients with Covid-19 Pandemic and its Relations with Smoking

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Abstract

Background: The virus that causes COVID-19 is thought to have originated in bats and then spread to snakes and pangolins and hence to humans. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes pulmonary injury or multiple-organ injury by various pathological pathways. The incidence of SARS-CoV-2 infection seen most often in adult male patients. SARS-CoV-2 has been shown to share the functional receptor, angiotensin-converting enzyme 2 (ACE2), with severe acute respiratory syndrome coronavirus (SARS-CoV). ACE2 converts the hormone angiotensin I to the active vasoconstrictor angiotensin II and then indirectly increases blood pressure by causing blood vessels to constrict. ACE mediates angiotensin (Ang II) production to activate RAS that plays a key role in cardiovascular diseases, especially hypertension. Thus, ACE inhibitor (ACEI) is used widely for treatment of hypertension, which reduces Ang II levels. Since ACE2 is a homologue of ACE, disputes have arisen about whether ACEI can up regulate ACE2 and thus the risk and severity of coronavirus infection increase.

Aim: To study the association between various biomarkers including angiotensin-converting enzyme and its receptor gene polymorphism with severity of COVID-19 infection in smokers' Iraqi pandemic of Kerbala province: Iraq.

Materials and Methods: A case-control study was conducted on 113 subjects infected with COVID-19 which were divided into two groups according to severity of the disease: 59 patients of them with severe COVID-19 and 54 patients of them with moderate infected covid-19. The study was compared with another 63 samples obtained from apparently healthy individuals as control group whose attended the hospital for checkup. Severe and moderate patients were collected from Al-Hussein Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorates, Kerbala – Iraq during April, 2020- March, 2021 with matched age ranged between (23-88) years. Five milliliters of venous blood

was drawn from each subject which was divided into two parts: The first part (3 ml) was used for serum ferritin, C-reactive protein (CRP), lactate dehydrogenase LDH, alanine aminotransferase ALT, aspartate aminotransferase AST and alkaline phosphatase ALP activities investigations and the level of angiotensin-converting enzyme (ACE) was determined by ELISA Technique. The second part (2 ml) of blood was collected in EDTA tube and stored at -20 °C until DNA extraction. The ACE-2 receptor gene polymorphism was performed by Applied Biosystem–Thermo fisher Scientific Co. thermo cycler. The insertion/deletion amplification polymerase chain reaction method was employed and then 1.5% agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria in the presence of ethidium bromide. The obtained bands were visualized using gel imaging system.

Results: Three types of allele were observed: [ACE-1 deletion/deletion polymorphisms (D/D), ACE-1 insertion/deletion polymorphisms (I/D) and ACE-1 insertion/insertion polymorphism (I/I)] in all of 176 sample studied (severe, moderate and control). The observed data indicated that (59/176, 33.52%) of infected samples were diagnosed as severe cases of COVID-19 and the ACE-2 gene polymorphism was (D/D) genotype (59/59) 100%, The percentage of diagnosed moderate cases of COVID-19 were (54/176, 30.68 %) and (46/54, 85.18 %) indicate the ACE-2 gene polymorphism was (I/D) genotype, and remaining (8/54, 14.1 %) of moderate were (I/I) genotype, while in (27/63, 42.85%) of healthy control the ACE2 genotype were (D/D), and (34/63, 53.96%) of healthy control were (I/D) genotype and the remaining (2/63, 3.17 %) of healthy control were (I/I) genotype. The observed data indicate that only (5/59, 8.5%) of patients with severe COVID-19 were heavy smokers and (4/54, 7.4) of moderate COVID-19 patients were found to be heavy smokers also..

The ACE-2 activity levels was increased in severe COVID-19 cases more than in the moderate cases, and its activity levels in healthy control with (D/D) genotypes were higher than those observed in (I/D) and (I/I) genotypes of moderate cases which confirm that the higher activity of ACE-2 was association with severe COVID-19 cases and also confirms the susceptibility of controls that carry (D/D) polymorphism to infected with severe state if infected with COVID-19 in the future.

Conclusion: The observed data indicated that the (D/D) genotype is severe infection with COVID-19 while moderate infection are either (I/D) or (I/I) genotype. Patients with the D/D allele genotype are at higher risk of morbidity and mortality. Most severe patients (91.5%) are non-smoker while (92.6%) of moderate infection were also non-smoker.

Keyword: ACE-2 Receptor, Gene Polymorphism Covid-19 Pandemic, Allele.

Hepato-defensive Effect of Cysteine, Ursodeoxycholic acid, and Silymarin on Histopathological and Some Biochemical Parameters of Wister Albino Rat Intoxicated by Carbon Tetrachloride

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Abstract

Background: The liver is the largest and the essential organ responsible for the digestive and excretory function, nutrition storage, and the synthesis of new substances. The liver is essential in charge of the detoxification of the exogenous xenobiotics, drugs, harmful chemical substances, and alcohol.

Objective: The present study was designed to investigate the hepatoprotective effects of L-Cysteine, Ursodeoxycholic acid (Ursoflor), and Silymarin against carbon tetrachloride-induced liver injury in rats.

Materials and Methods: Twenty adult male rats were used in this work; they were randomly divided into four groups (4 rats/group). The first group served as control group and received 0.5 ml normal saline orally daily, the second group injected intraperitoneally with carbon tetrachloride (CCl_4) (1.5 ml/kg body weight twice a week), the third group were treated orally with the plant extracted at dose of (200 mg/kg body weight in normal saline orally + CCl_4 daily, the fourth and last group were treated with Ursoflor drug at dose of (50 mg/kg body weight in 0.5 normal saline orally + CCl_4 daily, then some biochemical parameters were determined to investigate the live injury.

Results: At the end of this study, the biochemical assessment showed alterations in enzyme activity which indicates the hepatocyte injury due to CCl_4 toxicity, The generation of reactive oxygen species indicated by elevated levels in MDA and peroxynitrite which was noticed. This improved partially by antioxidant effect of L- cysteine, ursodeoxycholic acid, and Silymarin. These results further backed by histological examination of the liver showed that CCl_4 caused severe injury to the liver including high number of fat droplets deposition in the cytoplasm of the hepat-

ocyte, clear vascularization appeared and disorganization of the liver cells were determined, on the other hand our plant has been showed well protection against these injurious effects of CCl_4.

Conclusion: This study demonstrated that CCl_4 when metabolized in the body is changed into very reactive free radicals that then induce hepatic damage marked by alteration observed in enzyme activities and elevated levels in MDA and peroxynitrite. On the other hand, the treatment L- Cysteine, Ursodeoxycholic acid, and Silymarin minimized the hepatocellular damage induced by CCl_4 due to their antioxidant activity.

Keyword: L-Cysteine, Ursoflor, Silybum marianum, carbon tetrachloride, hepatotoxicity

Alteration of pro-inflammatory cytokines in human lung epithelial cells in response to *Klebsiella pneumoniae* infection

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Abstract

Background: *Klebsiella pneumoniae* are commensal flora in human mouth, skin and intestines but its behave as an opportunistic pathogen in the lower respiratory tract. This pathogenic bacterium can penetrate into epithelial cells, and stimulate innate immune responses in the adjacent epithelial alveolar cells. However, the mechanisms of *Klebsiella pneumoniae-associated pneumonia* is not fully understood, but host cell factors as well as known bacterial virulence determinants are likely to contribute to induce some immunological parameters.

Aims: The present research tested whether *Klebsiella pneumoniae* is colonized in the lower respiratory tract of patients who were admitted in hospital with pneumonia. In this study we also assessed the levels of pro-inflammatory cytokines in both *K. peumoniae* infected to cell line, A549

Methodology and results: We performed the VITIK$_2$ and manually identification methods to isolate and identify bacterial strains from 101 patient's sputum specimens collected from three tertiary hospitals in Al-Najaf city. Among them Approximately 29% patients specimens were *K. pneumoniae* positive and rest of the specimens were *Burkhoderia cepacia*, *P. aeruginosa* and *Entrobacter* spp bacterial positive. Besides, the reverse transcriptase-qPCR assay was carried out to assess the expression levels of pro-inflammatory cytokines in A549 epithelial cell after challenged with *K. pneumoniae* at 4hr. *K. pneumoniae* exposed human lung epithelial cell line (A549) has significantly upregulated the expression of eight pro-inflammatory cytokines, including IL-1α, IL-1β, TNF-α, IL-6, IL-8, MCAF INF-γ and GM-CSF at the mRNA levels. The time-lapse bacterial exposure on A549 cell shows that the INF-γ TNFa, IL-8, and IL-6 were up-regulated, while IL-1b was down-regulated at mRNA levels after 4 hours post-bacterial challenge. Therefore, it may be a good strategy to synthesise peptides or using antibodies against selected pro-inflammatory cytokines to treat *K. pneumoniae* associated pneumonia.

Keyword: A549 cells, *Klebsiella pneumoniae* and IL-1α, IL-1β, INF-γ, TNF-α, IL-6

Antibacterial Activity of Graphene Oxide Nanoparticles against *Streptococcus mutans* and their Effect on *gtf*B and *cnm* Virulence Genes

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Abstract

In the area of dental caries and periodontal disease, the high rate of microbes and their biological activity in the patient's mouth is a concern. The study aimed to shedding light on a relationship between the antimicrobial properties of graphene oxide (GO) and the growth of dental pathogenic bacteria. The swab samples were collected from the patient's cavity mouth between November 2019 and January 2020, then the *cnm* and *gtf S. mutans* genes were identified using PCR before and after exposure to the graphene oxide nanoparticles prepared in different pulse laser energy (500, 600 and 700mJ) in the presence and absence of the magnetic field, and the data were analyzed.After counting the colony forming units (CFU), the graphene oxide shows the high effectiveness on inhibiting the growth of *S. mutans*. This study offers conclusive answers to concerns about the relationship between graphene oxide, antibacterial caries, and periodontal disease.

Keyword: *GO nanoparticles; gtfB; cnm; Streptococcus mutans*. Laser ablation ; Plasma plume

Physiological study about the consequences of the use of Acyclovir in Rabbits

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Abstract

Aim of the study. The aim of this study was to evaluate the anticipated effects of acyclovir in rabbits particularly on some hematological and biochemical aspects.

Materials and methods. Twenty-four male adult albino rabbits of 1300-1500 gram weight were adopted and allocated randomly into three groups of eight rabbits to each. The period of the experiment was one month, during which the control group animals were daily injected intraperitoneally with 1 ml of normal saline. The first acyclovir group animals were daily intraperitoneally injected with 1 ml solution contains 75 mg of acyclovir sodium. The second acyclovir group animals were daily intraperitoneally injected with 1 ml solution contains 90 mg of acyclovir sodium.

Results. The results elucidated that acyclovir could cause significant declination in red blood corpuscles count (R.B.C.), total white blood cells count (W.B.C.), and packed cells volume (P.C.V.). Besides, acyclovir caused significant elevation in total serum cholesterol (TCH), triglycerides (TGS), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) with a significant declination in high density lipoprotein (HDL). Acyclovir also caused significant elevation in hepatic enzymes; the aminotransferases (ALT, AST) and the alkaline phosphatase (ALP). These effects of acyclovir were more significant in the higher dosed group animals; the second acyclovir group as compared with the other treatment and control groups at ($P \leq 0.05$).

Molecular study of prevalence of *H.capsulatum* in pneumonic patients

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AL-Qadysia AL-Furat AL-Awsat, Technical University/ Iraq

Abstract

study aimed to show prevalence of H. capsulatum in pneumonic patients An aggregate of (100) sputum samples were evaluated for this investigation samples cultured directly on SAD agar and staining to diagnose of fungi after that detect the fungi with RT-PCR, results showed that 7 (7%) of samples were positive for molecular test and 5 (5%) of samples were positive for cultured.

Green synthesis of iron nanoparticles loaded on bovine lactoferrine nanoparticles incorporated into whey protein films in food applications

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Abstract

Since nanotechnology offers a "different stage" followed by fresh or altered properties imposed on many existing products, it is commonly used in the manufacture of drug formulations of a new generation and is also used in the food industry as well as in many types of nutritional supplements. The synthesized iron oxide Fe_2O_3-NPs (IONPs) and bLf nanoparticles (0.2 percent, w / v) were successfully generated by thermal gelation (75 ° C for 20 min). The NPs were examined by UV / Vis absorption spectroscopy, transmission electron microscopy TEM. A typical absorption peak of iron oxide nanoparticles that synthases (by using CONCARPERES extract as reducing agent) appear at range of 200-300 nm and 200-400 nm of blfNPs and IONPs carried on blfNPs respectively which observed by color transformed from transparent yellow to black, demonstrating the synthesis of iron nanoparticles. The total particle size of prepared IONPs, blfNPs and IONPs carried on blfNPs was 80 nm, 60 nm and 200 nm, respectively. Mechanical test of prepared films appeared that increasing NPs ratio resulted in the decreases in the film thickness, tensile strength (TS), and elongation at break (EAB), O2 permeability, water vapor permeability and decreased the film solubility.The synthesized nanoparticles combined with prepared whey protein films had antibacterial activity against pathogenic bacteria like Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli and Salmonella enterica that studied by well diffusion method. Antioxidant activity of extracts and prepared films 5%, 10%, 15% of blfNP loaded IONPs were expressed as percentage of DPPH radicals inhibition percentage. Values in percentage ranged from 70.1, 74.59, 76.41, 79 %. The total phenolic content ranged from (6.6, 7.1, 7.6, 7.9 mg/g of sample) for plant extract and prepared films5%, 10%, 15% of blfNP loaded IONPs respectively, expressed as gallic acid equivalents. This approach to biosynthesis for applications has been found to be cost-effective, environmentally sustainable and promising in different fields. Overall, findings indicated that bLf nanoparticles loaded IONPs

combined with whey protein film may be used for potential food applications.

Keyword: Concarpers leaves, Iron oxide nanoparticles, Lactoferrine nanoparticles, Whey protein films.

Enterobacter Cloacae: the association of antibiotic resistance, integron class I and carbapenemase genes

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Abstract

The opportunistic pathogen, *E. cloacae* has been reported to carry carbapenemas genes worldwide. Our objective was assessing the association of antibiotic resistance, integron class I and carbapenemase genes among *E. cloacae*. Herein, 200 *E. cloacae* were collected and identified. The antibiotic resistance of them was evaluated using Kirby Bauer method. the existence of class I integron, carbapenemase genes was investigated using polymerase chain reaction (PCR). Of the 200 *E. cloacae* isolates collected, 120 isolates (60%) were from male and 80 isolates (40%) were from females. Of them, 110 isolates (55%) showed a pattern of MDR phenotype. Of these, 18 isolates (9%) showed resistance to imipenem. Based on PCR test, 134 isolates (67%) had class I integrons. Also, out of 110 MDR isolates, 52 isolates (72%) were positive in terms of the presence of class I integrons. Isolates with integrons were mostly from urinary (61%) and blood (44%) and from ICU settings (46%) and inpatients (38%). A significant relationship was observed between the presence of integron and resistance to ciprofloxacin, imipenem, meropenem, and norfloxacin antibiotics. The prevalence of bla_{IMP}, bla_{OXA-48} were 18% and 4%, respectively, but none of other carbapenemase genes were detected. The existence of class I integron was high among *E. cloacae* from Baghdad city. The carriage of genes resistance to carbapenems were significantly associated to the class I integron.

Keyword: *Enterobacter Cloacae*, Antibiotic resistance, integron class I and carbapenemase genes

CORONAVIRUS-2 "CELL CYCLE AND POSSIBLE THERAPEUTIC TARGETS"

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Abstract

Novel coronavirus appeared in Wuhan, Hubei province, China, where a pneumonia cases of unknown cause was detected in December 2019 from patients considered as hallmark of COVID-19. Patients infected with this virus suffer from potential damage to vital organs, such as gastrointestinal, cardiac, liver, renal, respiratory and nervous systems…etc.

Coronavirus-2 is an enveloped, positive-sense, single-stranded RNA virus belongs to the genus Beta-coronavirus. The positive-sense genome can act as messenger RNA (mRNA) and directly translated into viral proteins by the host cell's ribosomes. Spike S protein is the main antigen and pathogenic component of the virus. It composed of two subunits, S1 and S2, S1 is mainly responsible for binding of the virus to the host receptor (ACE2), while the S2 domain related to virus fusion and entry to the host cell. The virus genome is the main pharmacological target due to its critical function in viral RNA transcription and replication. Recently, different forms and variants of the virus have been reported. The multiform of coronavirus 2 (COVID-19) has resulted many challenges and difficulties to the healthcare and researchers around the world to develop broad-spectrum antiviral drugs and vaccines against SARS-CoV-2. The implications of these mutations on the detection, transmission, vaccination, and severity of disease are yet to be studied and understood. All the proteases, protein binding molecules and pathways for the entry, cell cycle and virus replication could represent possible and valuable targets for effective antiviral therapies and drug discoveries. Emphasizing on the cell cycle, in this review we analyse the current available and the possible feature cure of covid-19. In the conclusion, this review could provide beneficial information about the potential future treatment strategies for the treatment the pandemic COVID-19 disease.

Keyword: Coronaviruse-2, Cell cycle, Current and feature treatment

Functional Association of TNF Gene Polymorphisms with Autoimmune Diseases

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Abstract

Tumor Necrosis Factor (TNF) polymorphisms have been associated with susceptibility, progression, development and therapies of autoimmune diseases. However, inconsistencies have been observed in previous studies.

Purpose: The study was designed to evaluate the association of TNF gene polymorphisms with autoimmune diseases.

Methodology: This case-control study involved analysis of TNF polymorphisms; rs1800630, rs1799724, rs1800629 and rs361525 in four groups of autoimmune disease patients including autoimmune thyroid disease (AITD; n=54), rheumatoid arthritis (RA; n=51), type 1 diabetes (T1D; 56) and vitiligo (n=51). Healthy individuals were selected as controls (n=52). The participants >30 years of age and any comorbidity were excluded from the study. The genotyping was performed through direct sequencing and PCR-RFLP method, followed by odds ratio and chi square analysis. Plasma analysis of TNF-α, IL-2 and IL-10 levels was performed using ELISA followed by one way ANOVA.

Results: On comparison of each group of patients with controls, significant association of rs361525 with AITD, RA and T1D (after adjustment for Bonferroni correction) was observed. The chi-square values were obtained as, AITD (χ^2; 7.712, p; 0.005), RA (χ^2; 8.939, p; 0.003) and T1D (χ^2; 16.347, p; 0.000). The homozygous rs1800629 (GG) was significantly associated with plasma TNF-α (p=0.038; AA vs GG, 0.009; GA vs GG). However, heterozygous rs1800629 (GA) was associated with IL-2 (p=0.006; AA vs GA, 0.024; GA vs GG). On comparison of heterozygous rs1799724 (CT) with IL-10, significant association was observed (p=0.007; CC vs TT, 0.005; CT vs TT).

Discussion: The TNF polymorphisms may be functionally associated with susceptibility of autoimmune diseases.

Keyword: Association, autoimmune, polymorphism, tumor necrosis factor, genotype

AIR pollution -covid-19 and forensic implications

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Abstract

Covid-19 and the new variant are a classic example of viral and environmental toxicology link. Observing literature related spread velocity and diffusion of this respiratory virus it is clear the role played by AIR POLLUTION. The high rate of this environmental pollutant produced a worsening factors that increased mortality rate also. Two major effect was observed: a proinflammatory effect on the lungs of patient due by the air pollutants like NO2, Particulate matter and many other typical substantie A chronic exposition to this toxic produce an inflammatory status as seen in other respiratory classic disease as ASTMA, BCPO and other. Another great contribute is played by the role of CARRIER that PM produces: this particulate matter Carry on respiratory tract bioaeresols with viral particle (it seem in a level not able to produce a clinical infection) but also other dangerous substantie like BENZO -A-PYRENE. This substantie contribute il proinflamatory effect and also are able to provide a MUTANT AGENT environment involved also in VARIANT production. (see literature reported). Many world zone with air pollution was involved in first wave of covid -19 like wu -han but also in north Italy and also involved in VARIANT explosion (MANAUS and other). So we can consider this pathology not only an infectious disease but also an environmental toxicological problem. Climate change, humidity level, air pollution, UV irradiation, PM, high industrializated regions and other environmental factor are involved in this pathology. Now are produced vaccines but the emerging of the new variant can be a real problem. But what it is relevant is to recognize also in international world organization that air pollution play a non secondary role.

Keyword: covid-19 diffusion, air pollution, environmental toxicology, mortality

rate, Worsening factors, carriers, mutant agent, variant, social responsibility, climate change.

Asprosin and diabetic nephropathy

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Abstract

Diabetic nephropathy (DN) is one of the most common microvascular consequences of diabetes, affecting up to 20% to 40% of T2DM patients and potentially leading to end-stage renal disease (ESRD), increasing T2DM patients' mortality and morbidity. this study have been done to investigate the possible role of Asprosin in T2DM patients with early-stage renal diseases. The current study was performed on (60) patients with T2DM. These patients were divided into two equal groups according to their urinary albumin to creatinine ratio (ACR), including patients with normoalbuminuria (No.=30) (ACR less than 30 mg/g creatinine) and those with microalbuminuria (No.=30) (ACR= 30 – 299 mg/g creatinine). Thirty healthy persons matching the same age and sociodemographic status with diabetics subjects were selected as a control group. The concentration of glucose, HbA1c, urea, and creatinine by colorimetric methods were measured for all participants, turbidimetric method for the quantitative determination of microalbumin in human urine, and Asprosin by enzyme linked immunosorbent assay method.. The serum levels of Asprosin were significantly elevated in patients with T2DM with microalbuminuria compared to those with normoalbuminuria and control groups with statistically significant difference (P-value0.002). In addition, serum Asprosin showed a positive correlation with urea and creatinine and a negative correlation with eGFR. Receiver operating characteristic curve (ROC) was used to assess the diagnostic value of Asprosin in identifying microalbuminuric from normoalbuminuric diabetic patients. The area under the curve (AUC) was 0.658, 95%CI= (0.520 - 0.796), p-value=0.035. The sensitivity and specificity of the test at the cut-off value of Asprosin=9.81 ng/ml were 60.0% and 63.3%, respectively. The study concluded that serum Asprosin level positively correlated with blood urea and creatinine, and negatively correlated with eGFR in T2DM patients, which implies circulating Asprosin may participate in the pathogenesis of DN due to affecting blood glucose concentration and insulin resistance, as well as inflammatory responses in the kidney.

Keyword: Type 2 diabetes mellitus, albuminuria, Asprosin

Phylogenetic tree and genetic relationship of some date palm cultivars using microsatellite

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Abstract

This study done the aim of determining the phylogenetic tree of date palm *Phoenix dactylifera*. L six cultivars, scarce and planted in four different locations of Basra, southern Iraq. During planting season 2019-2020. In this paper, we used a simple sequence repeat (SSR) technique with six primers, to estimate genetic relationships and determine the phylogenetic tree. The results of amplification of DNA samples showed the presence of 86 bunds, of which 47 polymorphism, all primers also gave a unique band distinct to cultivar, amounting to 24 bands. The polymorphism percentage was 98.15%. The results of the phylogenetic tree analysis showed relationship of cultivars according to genetic proximity and genetic dimension, two cultivars (Sakri, Abd-alhadi) were associated with each other and recorded a genetic proximity of 0.353. While a genetic dimension of 0.032 was recorded between cultivars (Ashger, Swadani).The results of the Principle Components Analysis gave five components that participated in the total variation, and the highest percentage of the first component was 34.30 of the cumulative total variation. This technique can be adopted to study the genetic relationships between the cultivars resulting from the same origin and to know the genetic differences and the common characteristics between these cultivars.

Keyword: SSR, Date Palm, phylogenetic tree.

Immunohistochemical Detection of Cyclin D1, CDK4, and CDK6 Cell Surface Markers in Tissues from Breast Tumors Infected with Kaposi Sarcoma-Associated Herpes Virus (Human Herpes Virus-8)

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Abstract

This research designed as a retrospective case-control study. A total number of 100 formalin-fixed, paraffin embedded breast tissues included. These tissues were including 40 malignant breast tumors, 40 benign breast tumors and 20 of apparently healthy breast tissue sections were included as baseline control. They were collected from the archives of histopathology laboratories of different general hospitals as well as many private laboratories in Mid-Euphrates Governorates of Iraq, during the period from July 2010 to March 2019. The mean age of the patients with breast carcinoma was higher (46.80 + 12.517 years) than the mean age of the benign tumor (34.70 + 14.4 years), while the mean age of those females in the group of apparently healthy control (34.70 + 14.4 years). The positive results of Kaposi Sarcoma-Associated Herpes Virus (Human Herpes Virus-8 / *HHV-8)* -CISH detection in malignant breast tumors, where 32.5% (13 out of 40 tissues) showed positive signals, while in the benign breast tumors group were 15 % (6 out of 40 tissues) and none were detected in the 20 apparently healthy breast control tissues. The results of immunohistochemistry revealed that Cyclin D1 cells were observed in 47.5% (19 out of 40) of breast cancer group, 32.5% (13 out of 40) of benign breast tumor group, and 15% (3 out of 20) of breast healthy (control) tissues group. Regarding CD4cells, the percentage of detection was 35% (14 out of 40) in breast cancer group, 30 % (12 out of 40) in benign breast tumor group and 10% (2 out of 20) in control group. The CDK6 detected in 30% (12 out of 40) of breast cancer group, in 25% (10 out of 40) of benign breast tumor group, and in 5% (1 out of 20) of tissues of control group.

It could be concluded from this study that a possible role for *HHV-8* in the de-

velopment of about half of our Iraqi patients with breast tumors. Also, the positive Cyclin D1, CDK4, and CDK6 cell surface markers signals in the malignant and benign tissues could share a role in the pathogenesis and / or carcinogenesis of these breast tumors.

Geneticaly Molecular identification with Polymerase Chain Reaction (PCR) of wild and mutanted by Ultra Violet rays of phosphate dissolving bacteria (Bacillus Polymyxa)

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Abstract

Ten isolates of phosphate dissolved bacteria as Bacillus spp. Which were isolated from rhizosphere of various soils and crops at Basrah province, Iraq. Ten isolates were exposed to UV-ray at periods 15, 30, 45 and 60 minutes in addition to ten isolates were nonmutant (wild) then genetically identified with PCR technique in First BASE Laboratories Selangor Malaysia, all isolates sequence genetic by Extracting DNA with bioformatic programs.Results showed that all isolates either wild or mutant were belong to Bacillus polymyxa at ratio A260\A280 was 1.8 and mulucular weight was 308pb, also at Acceession number U32191 so from alteration amino acid and bacterial isolates recorded at gene bank under sequence numbers:

LC545926, LC545927, LC545928 LC545929, LC545930, LC545931, LC545932 LC545933, LC545934, LC545935.

Keyword: Agricultural Biotechnology, Bacillus sp., PCR technique, UV-ray.

Study of Antibacterial Activity of an Aqueous Extract of *Boswellia Carterii* and Its Effect in Phagocytosis *in vitro*

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Abstract

A total of 125 samples were collected from various clinical sources, including (urine, sputum, wounds, Otitis media, Blood) isolated from people with different clinical infections of both gender at different ages, and 59 samples were obtained that had bacterial growth. The results showed that urine samples had the highest infection (68%), followed by wounds, sputum, Otitis media (60%), (44%) and (40%) respectively; Blood samples were the percentage of lowest infection than (28%) of the total, Microscopic diagnostic results, biochemical tests, API Staph System, API 20E System, and Vitek 2 Compact showed that highest infection was *Staphylococcus aureus* (32.20%), *Pseudomonas aeruginosa* (25.33%), *Escherichia coli* (22.03%) and the lowest *Klebsiella pneumonia* (20.33%). Boswellia carterii had an antibacterial activity for all bacteria used in the study, which was proportionally proportional to the increase in concentration with 10.8, 10.4, 7 and 10 mm diameter respectively for *S. aureus*, *E. coli*, *P aeruginosa*, *K. pneumonia* (25 mg /ml), and 24, 22, 18.4, and 20 mm. respectively for the previous bacteria with concentration (200 mg/ml). The results showed a significant increase in the phagocytosis coefficient, with the phagocytosis of blood samples treated with *Boswellia carterii* extract (79.7%) compared to control samples (57.75%).

Keyword: Antibacterial Activity, Aqueous Extract, *Boswellia Carterii*, Phagocytosis, in vitro

Calprotectin and Cystatin C Levels as Predicting Biomarkers of Severe Covid-19 of Iraqi Pandemic and their Correlations with Kidney Injury Molecule-1

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Abstract

Introduction: Coronavirus disease or (COVID-19) causes severe acute respiratory syndrome, coronavirus 2 (SARS-CoV-2) was spread to hundreds of countries and has been declared a global pandemic. Severe COVID-19 results in death due to progressive hypoxemia, acute respiratory distress syndrome (ARDS), and multi-organ failure. COVID-19 has several clinical signs and symptoms such as dry cough, high fever, vomiting, myalgia, sputum production, headache, haemoptysis and diarrhea. Human pathogenic coronaviruses (severe acute respiratory syndrome coronavirus (SARS-CoV) and (SARS-CoV-2) bind to their target cells through angiotensin-converting enzyme 2 (ACE2), which is expressed by epithelial cells of the lung, intestine, kidney, and blood vessels (5).

COVID-19 causes kidney involvement in about 3-9% of the patients and several studies reported that in-hospital mortality of COVID-19 patients who developed acute kidney injury (AKI) is significantly higher (5.3 times higher in AKI than 1.5 times in chronic illnesses). Firstly, COVID-19 exploits the ACE II as a receptor to entry the cells which is present much higher in kidney than lungs. Hence, lungs contamination with SARS-CoV-2 may be paralleled in kidneys. Measurement of serum cystatin C levels is gaining a greater role in the estimation of kidney function.

Calprotectin, a heterodimeric protein, is a member of the calcium-binding S100 protein family located in the cytoplasm of neutrophils and monocytes, both of which play important roles in the inflammatory response in the human body. In COVID-19 patients, an early biomarker of neutrophil activation such as calprotectin could be

used to early identify patients with bacterial co-infections and patients at risk to develop severe events. Kidney injury molecule 1 (KIM-1) is a transmembrane glycoprotein that is up-regulated after acute ischemic kidney injury in the proximal tubule of renal tissue.

Objective: To study the association between Calprotectin, cystatin C and kidney injuury molecule-1 with severity of COVID-19 infection and their correlation with routine COVID-19 biomarkers in Iraqi pandemic of Kerbala province: Iraq.

Materials and Methods: This cross-sectional study was conducted on 91 sample patients with covid-19 divided into two group, 50 patients of them with severe COVID-19 and 41 patients of them with moderate infected COVID-19. Severe and moderate patients were collected from Al-Hussein Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorates, Kerbala – Iraq during March, 2020- April, 2021 with matched age ranged between (26-88) years. Five ml of whole blood will be withdrawn from each participant and then centrifuge to obtain serum and store at -20°C until the time of use. The bio-markers determined include (Ferritin, C-reactive protein, total lactate dehydrogenase, creatinine, urea, cystatin C, calprotectin, and kidney injury molecule-1) and complete blood count test was measured.

Result: Of 91 COVID-19 patients, 50 cases (54.9%) were classified into the severe group and 41 cases were classified into moderate group (45.1%). With mean of age 60.16 11.8 year. There was no significant value in levels of CLP, cystatin C and KIM-1 with the severity of COVID-19 patients. This study showed positive correlation between KIM-1 with CLP and Cys-C in moderate patients group and with CLP in severe patients group. There is negative correlation between Cys-C with serum creatinine and serum urea in severe and moderate patients groups but positive correlation between KIM-1 and serum creatinine and serum urea only in severe patients group.

Conclusion: There was positive correlation between KIM-1 with cystatin C, CLP, serum creatinine and urea. A non-significant increase in CLP, cystatin C and KIM-1 with severity of COVID-19 patients was observed.

Keyword: Severe acute respiratory syndrome coronavirus 2, Acute respiratory distress syndrome, Angiotensin-converting enzyme 2, Cystatin C, Calprotectin, Kidney injury molecule 1.

Serum Level of Vitamin K as Predicts Mortality in Iraqi COVID-19 Patients

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Abstract

Background: The ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was initiated at Wuhan, Hubei, China, and there was a rapid spread of novel SARS-CoV-2 and the disease COVID-19 in late 2019. COVID-19 has made a serious public health threat worldwide with millions of people at risk in a growing number of countries. COVID-19 is regarded as an independent risk factor for cardiovascular diseases due to the induction of endothelial dysfunction, coagulopathy, cytokine storm, and plaque instability. The entire world is now experiencing the challenge of COVID-19 infection. However, still very few evidence-based treatment options are available for the prevention and treatment of COVID-19 disease.

One of the predominant theories favors the concept of a "cytokine storm" in which the immune response is exacerbated through the induction of an excessive pro-inflammatory cytokine response driving lung injury. It was reported that presence of a high viral load causes massive destruction of lung tissues, in turn leading to hyper inflammation causing acute respiratory distress syndrome (ARDS). In addition to respiratory symptoms, a growing body of evidence also shows that the virus can specifically infects endothelial cells affecting thus the normal process of coagulation. Severe COVID-19 patients were found to possess coagulopathy characterized by abnormal coagulation parameters widespread presence of blood clots as well as arterial and venous thromboembolism. Furthermore, preliminary data from several studies seem to indicate that anticoagulant therapy is associated with lower mortality

in COVID-19 patients.

Prevalent coagulopathy and thromboembolism are observed in severe COVID-19 patients with 40% of COVID-19 mortality being associated with cardiovascular complications. Abnormal coagulation parameters are related to poor prognosis in COVID-19 patients. Also, displayed presence of extensive thrombosis in infected lungs. Vitamin K is well-known to play an essential role in the coagulation system. Latest study revealed an existing correlation between vitamin K deficiency and COVID-19 severity, highlighting a role of vitamin K, probably via coagulation modulation. In agreement, other recent studies also indicated that anti-coagulant treatments can reduce mortality in severe cases. Altogether, potential mechanisms linking COVID-19 with coagulopathy in which vitamin K may exert its modulating role in coagulation related with disease pathogenesis. In this study, we illustrate the recent evidence supporting COVID-19 as a vascular disease and explore the potential benefits of using vitamin K against COVID-19 to improve disease outcomes. Vitamin K is an essential component preventing blood clotting and a major player of the coagulation system of which a link between vitamin K deficiency and the worst COVID-19 outcomes was recently revealed.

Materials and Methods: In this case-control study, the participated in the study included 60 patients min-max. Ages (30-50 years), which were diagnosed with quantitative RT-PCR and chest X-ray or CT scan at the 7-13 days from symptoms on set. COVID-19 patients were divided according to disease severity were assessed using Murray scores. To compare the results, (60) apparently healthy persons of the same ages and gender were included in this study as a control group. All of the patients and healthy persons were subjected to the estimation of serum vitamin K, D-Dimer, CRP, ferritin, lipid profiles, and anthropometric data were analyzed.

Results: Serum vitamin K level was lower in COVID-19 patients group compared to healthy control group (612.32 ± 106.76 vs. 1198.95 ± 151.59 ng/ml, $p = 0.0001$). Ferritin, CRP, and D-dimer serum levels were higher in covid-19 patients compared to control group ($p = 0.0001$), as well as TC, LDL-C, and VLDL-C in COVID-19 patients showed a significant decrease in the levels included in this study when compared with healthy controls. We also found that serum levels of vitamin K are negative associated with the serum ferritin, D- dimer, and positive correlated with serum levels of TC, LDL-C, and CRP levels.

Conclusion: The present results revealed that vitamin K level is lower in patients with COVID-19 especially in severe cases as compared with healthy controls and

this low levels indicate a useful tool to predicts higher mortality among patients with COVID-19. These findings suggest that vitamin K could play a role in the disease mechanisms in COVID-19.

Keyword: COVID-19, Vitamin K, D-Dimer, Lipid profiles, Severity, Mortality.

Morphological and Histological Study on The Female Reproductive System in Local Duck (*Anas Platyrhynchos*)

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Abstract

The current research aimed at obtaining principal data on the morphological and histological structure in the female genital system of the local duck (*Anas platythynchos*) and conducted that nine (mature) ducks were used gross findings morphometrically parameters including length diameter and weight. microscopic structures have revealed that the left ovary was large irregular in shape and showed numerous follicles of different developmental stages. The mature laying left oviduct was fully developed into five well distinguished regions (infundibulum, magnum, isthmus, uterus and vagina) extended from ovary to cloacae the present study showed that the ovary of adult duck is covered with simple cuboidal epithelium (germinal epithelium) internally two distinct regions can be distinguished cortex and medulla. The cortex peripheral region contains a numerous follicles in different stages of the development that are classified as primordial, primary, secondary and tertiary follicles and the microscopic structures of oviduct lining epithelium was pseudo stratified columnar ciliated and non-ciliated secretory and lamina propria was filled with glands, blood vessels and two layers of tunica muscular is smooth muscle with inner circular and outer longitudinal layer increasing gradually in thickness. The tunica serosa of duck oviduct formed by loose connective tissue covered by mesothelium.

Keyword: Local Duck, Female, Genital system, Histology.

Studies on Streptococcus mutanse, Lactobacillus spp. and E.coli from dental carries and antimicrobial studies using Arirthrospirolina platen-.sis extract

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Abstract

Dental caries is a multifactorial pathological condition which involves a susceptible host, a cariogenic biota and a cariogenic diet. The aim of the present study was to isolate the *Streptococcal* mutanse, Lactobacillus sp. and E.coli from various age groups within 11.40 affected by dental caries and the use of antimicrobial tests using spirolina extracts. were identified from decayed tooth samples. Which were predominantly present in mouth and decaying teeth. This might be due to the secretion of acids. *Arithrosprolina platensis was* selected to conduct antimicrobial tests against the isolated species. These tests revealed that the root extract of *A. platensis* against *Lactobacillus spp.* was more effective than the other bacterial species.

Preparation of a highly effective and stable surface disinfectant of chemical origin

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Abstract

Preparation a biologically effective disinfectant and disinfectant for surfaces to kill microorganisms, bacteria, viruses, and fungi, to clean surfaces and floors, as there is no harm to health. Sodium carbonate salt was used, which is the main material in the preparation of the preparation, which is an effective alternative to disinfectant products manufactured from materials that may harm the environment. Salts can be used in the manufacture of disinfectants, sterilizers and cosmetics because they are a strong anti-fungal, a killer of bacteria and germs, and have no side effects and deodorize. Dissolve 50% of the sodium carbonate salt in distilled water and add to it 3% pine oil or 3% laurel oil as an active ingredient in the composition. The oils used are considered to be of limited effectiveness as an antimicrobial and bacterial spores, as a natural plant extract, after that mix polyacrylamide 0.91%. Examination of the active substance and the acidity function according to the Iraqi specification No. (60) and the Iraqi specification No. 3826, where it gave an effectiveness of 16.4% and the values of the acidity function (7.5) were within the limits of the specification. Through the results of examining the effectiveness of disinfectants against pathogenic bacteria rapidly spreading in the atmosphere and on the human body, the prepared saline is effective against human pathogenic bacteria (Staph.aureus and Ecoli) and Candida fungi, which was conducted in the laboratories of Al-Razi Center for Research and Medical Kits Diagnostic Production.

Keyword: environmentally friendly disinfectants, sodium carbonate, pine oil, laurel oil, polyacrylamide