

1856<sup>th</sup> Conference

Annual Biotechnology 2018



# Annual Biotechnology Congress

July 23-24, 2018 | Vancouver, Canada

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## Poster Presentations

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## Genome analysis of bio-control fungus *Trichoderma harzianum* and the biosynthesis of peptaibols alamethicin

Jian Ling, Zhenchuan Mao and Bingyan Xie  
Chinese Academy of Agricultural Sciences, China

*Trichoderma harzianum* has strong ability for encoding secondary metabolites, which contribute a lot for its bio-control function. peptaibols alamethicin is a family of antibiotics isolated from *T. harzianum* and was found significantly suppress against various *Fusarium* fungi. Unfortunately, little was known about its biosynthetic genes and pathway to data. In this study, we assembled the high-quality genome of *Trichoderma harzianum* T29, which consists of 72 scaffolds, with total size of 32 Mb and the 1.5 Mb N50 value. A total of 10773 gene models were predicted in the genome. We predicted 36 secondary metabolite gene clusters in T29 genome and the function of some secondary metabolite related genes were identified by homologous deletion. We further identified a serial of genes from THBJ\_06541 to THBJ\_06568, including core enzymes, modifying enzymes and transporter enzymes coded for the biosynthesis of alamethicin. To verify the biosynthesis pathway of peptaibols alamethicin, we disrupted PKS (paD) in the cluster by homologous recombination and found the peptaibols alamethicin were disappeared in  $\Delta$  paD. These results will provide theoretical guidance for understanding the mechanism of *T. harzianum* against *Fusarium* fungi.

### Biography

Jian Ling has been engaged in the research of plant pathology in Institute of Vegetable and Flower Research in Chinese Academy of Agricultural Sciences (CAAS) since 2008. His main researches are focused in analyzing the molecular mechanism of the interaction between crops and pathogens, using genomics and metabolomics, and developing the safe and efficient biocontrol technology against *Fusarium* wilt and root knot nematode.

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## Hydrolysis of lignocellulose and micro cellulose with novel ulfobetaine–tungstophosphoric acid catalyst

An-Ya Lo<sup>a</sup>, Cheng-Tso Cheng<sup>b</sup>, Wei Wang<sup>c</sup>, Chia-Chia Chang<sup>a</sup>, Jih-Mirn Jehng<sup>b</sup>, Shang-Bin Liu<sup>d</sup> and Wen-Hu Chen<sup>c</sup>

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<sup>d</sup>Institute of Atomic and Molecular Sciences, Academia Sinica, Taiwan

Ionic liquid (IL) and heteropoly acid (HPA) composite catalyst were prepared for the hydrolysis of waste wood powder and microcellulose. Here in this study, the tungstophosphoric acid (H<sub>3</sub>PW<sub>12</sub>O<sub>40</sub>, TPA) and pyridinium propyl sulfobetaine (PPS) were adopted as HPA and IL, respectively. The nuclear magnetic resonance (NMR) spectroscopy was adopted to examine the correlations between acidic strengths vs. water content of both H<sub>2</sub>SO<sub>4</sub> and PPS-TPA solution. On the one hand, the acid strength of the prepared catalyst solution is the same with that of industry adopted sulfured acid (2 wt% in concentration) for glucose and xylose production. On the other hand, is also prepared with higher concentration for non-cellulose preparation. For this purpose, waste wood powder and microcellulose were adopted for hydrolysis. The results showed that PPS-TPA catalyst exhibits higher activity on lignocellulosic hydrolysis. Other than nanocellulose production, PPS-TPA has potential on glucose production yield without producing inhibitor, and exhibit potential for quick xylose production process.

### Biography

An-Ya Lo has completed his PhD eight years ago from Department of Materials Science and Engineering, National Chiao-Tune University, Taiwan. He is the Head of Practicum Career Service Division of R&D office in NCUT. He has published more than 40 papers in reputed journals.

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## Effect of polystyrene surfaces and altered polylactic acid surface chemistries on fibrinogen orientation and endothelialization and its application for coronary stents

**Nupur Dave**

Dulles High School, USA

Coronary Artery Disease (CAD) is a leading cause of death which accounted for around 370,000 deaths in 2016. CAD is caused by a build-up of cholesterol in an artery, blocking oxygen from going to the heart. Current treatments for CAD include placing a drug-eluting stent in the artery in order to promote blood flow to the heart. However, this treatment results in-stent thrombosis, the fatal clotting of blood, in around 1% of all CAD patients. In this study, we investigate the use of biodegradable polymers to prevent fibrinogen proliferation on *in vivo* surfaces, and the effect of soluble fibrinogen on fiber characteristics. Hydrophilic polylactic acid surfaces (UV induced) resulted in less fiber occupancy and smaller fibers, and endothelial cells attachment was successful on hydrophilic PLA surfaces. These results show promise in UV treatment for coronary stents and a suitable biodegradable polymer. Further research on fibrinogen orientation and fiber characteristics was done on polystyrene surfaces for *in vitro* study. We found that a higher concentration of soluble fibrinogen, an essential protein in blood coagulation, resulted in dense fibers on polystyrene (PS) surfaces. This study reveals crucial information for the future development of coronary stents and the treatment of a leading heart complication, coronary artery disease.

### Biography

Nupur Dave is a rising high school senior at Dulles High School in Sugar Land, Texas. She has analyzed and developed this research project at Stony Brook University under the mentorship of Dr Miriam Rafailovich. She has a long founded interest in Biomedical Engineering and wishes to major in some fields of Engineering in the future.

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## Treatment with low-temperature atmospheric pressure plasma enhances cutaneous delivery of epidermal growth factor by regulating E-cadherin-mediated cell junctions

Gyoo-Cheon Kim and Jin-Woo Hong

Pusan National University, Republic of Korea

The barrier system of the skin not only defends against antigens and harmful substances, but also hinders the permeation of medicines and cosmetics into the dermis. Several strategies have been developed to enhance the absorption ability of skin, including the use of chemicals and skin ablation devices. However, the cost and inconvenience of these strategies highlights the need for a novel and safe method for increasing skin absorption. In this study, we examined the effect of low temperature atmospheric pressure plasma (LTAPP) on the efficiency of drug penetration through the skin, as well as its mechanism of action. HaCaT human keratinocytes and hairless mice were exposed to LTAPP treatment, and the cellular and tissue gene expression, and morphological changes were monitored. We found that the LTAPP exposure reduced the expression of E-cadherin in skin cells and led to the loss of cell-cell contacts. The exposure of mouse skin to LTAPP also reduced the expression of E-cadherin and prevented intercellular junction formation within the tissue, leading to enhanced absorption of hydrophilic agents, eosin and epidermal growth factor. The reduction in E-cadherin expression and reduced skin barrier function recovered completely within three hours of LTAPP exposure. Taken together, these data show that LTAPP can induce a temporal decrease in the skin barrier function by regulating E-cadherin-mediated intercellular interactions, leading to the enhanced transdermal delivery of drugs and cosmetics.

### Biography

Gyoo-Cheon Kim is a Professor of Department of Oral Anatomy at School of Dentistry, Pusan National University and a Member of the Board of Directors in Korean Academy of Oral Anatomy. He was educated in various biomedical areas including Molecular Biology, Histology and Human Anatomy. His research interest is to induce apoptosis in oral cancer cells by means of specific bacterial proteins. Currently, he has focused on the area of plasma medicine including induction of selective cancer cell death, tooth whitening, treatment of oral diseases, wound healing, and skin rejuvenation. Since 2014, he has been a CEO of Feagle Corporation, which produces plasma medical devices.

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## Expression analysis of the translocator protein 18 kDa (Tspo) mRNA in various human normal and cancer tissues

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Mangosuthu University of Technology, South Africa  
University of the Witwatersrand, South Africa

The translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor, is a distinct receptor from the central benzodiazepine receptor. It is involved in numerous biological functions, including the regulation of cellular proliferation, apoptosis and mitochondrial functions. Previous studies have shown that the expression of TSPO protein correlated positively with tumor malignancy and negatively with patient survival. The aim of this study was to determine the expression of TSPO mRNA in various cancers. Colorimetric and fluorescent in situ hybridization was performed to localize the TSPO mRNA in various cancer tissues. Quantitative RT-PCR was performed on RNA extracted from various cell lines representing normal lung cell (MRC-5), lung cancer cells, (A549), cervical cancer cells (HeLa) and liver cancer cells (Hep G2), to compare the transcript levels of TSPO in these. There was a change in the distribution of the TSPO mRNA transcription pattern between the cancerous and normal tissues as indicated by in situ hybridization. Staining intensity was used as an indication of the level of TSPO mRNA. There was a significant increase in the level of transcription in liver, prostate, kidney and brain cancers while a significant decrease was observed in lung cancers. The RT-PCR results indicate that the highest transcription levels of TSPO were observed in the normal lung cells and the mRNA levels decreased in lung cancer cells. The widespread presence of the TSPO mRNA and TSPO protein in epithelial cells seen in the different organs suggests that the TSPO protein also plays an important role in non-steroidogenic tissues. Our results suggested that modulation of the regulation of TSPO mRNA transcription in different cancer tissues plays an important role in various cancers and may allow for it to be exploited as a prognostic marker in the different cancers.

### Biography

Zodwa Dlamini is a Professor of Functional Genomics and Molecular Medicine. Her research interests include the omics technologies including the use of bioinformatics to provide unprecedented possibilities to identify the underlying molecular basis of common diseases including cancer and diseases of lifestyle. She obtained her BSc Hons at the University of the Western Cape, MSc from the University of Natal and PhD at the University of Natal. She joined the University of Western Cape as a postdoctoral fellow. She was then appointed as a Lecturer at University of Witwatersrand and was promoted to a Senior Lecturer. She then joined the University of Limpopo as an Associate Professor in Biochemistry and interviewed successfully at University of South Africa in the role of Deputy Executive Dean and Professor in Molecular Genetics. In June 2015, she was appointed by Mangosuthu University of Technology as the Deputy Vice Chancellor of Research, Innovation and Engagements.

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## ***In vitro* propagation response of *Rosmarinus officinalis* L. to biotic and abiotic elicitors on phenolic content and photosynthetic pigments**

**Aisha Abdullah Alayafi**  
Jeddah University, Saudi Arabia

**M**icropropagation protocol was modified to evaluate the best procedure to induce multiple shoots from *Rosmarinus officinalis* L. sterilized seedlings obtained from seeds which vitality is always very low. Aseptic shoots (1-1.5 cm) were cultured on full strength Murashige and Skoog medium modified with several growth regulators (abiotic elicitors): Benzyladenine (BA), Kinetin (Kin) and coconut water (biotic elicitors). Data was indicated that BA at the concentration of 3 mg/L encouraged shoot multiplication. The highest number of leaves and plant length also was obtained with medium modified with 3 mg/L BA and coconut milk at 5 ml/L. All treatments were significantly different from the control. Total phenolics content, anthocyanins, chlorophylls were extracted and spectrophotometrically determined as secondary products. Data was concluded the highest content of phenolic 10.45 (mg/g) and chlorophyll b 0.67 (mg/g) when BA was 3.0 mg/L. Whereas chlorophyll a reached to 0.64 mg/g in presence of 5.0 mg/L BA and 5.0 ml/L coconut milk. Anthocyanin scored high level when BA was 3.0 mg/L combined with 5.0 ml/L coconut milk.

### **Biography**

Aisha Abdullah Alayafi is an assistant professor in Biology department and Faculty of Science from University of Jeddah, Saudi Arabia.

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## Optimization of single cell protein bioproduction by marine fungi using seaweed biomass

Catalina Landeta Salgado<sup>1</sup>, María Elena Lienqueo<sup>1</sup> and Javier Gimpel<sup>2</sup>

<sup>1</sup>University of Chile, Chile

<sup>2</sup>Centre for Biotechnology and Bioengineering, Chile

In the last two decades, interest in biotechnology has focused on obtaining products of commercial importance from low-value residual biomass. Seaweed and waste from the algae industry has a high concentration of alginate, cellulose, and a low content of hemicellulose and lignin [1, 2]. The use of seaweeds to produce biofuels such as biogas and bioethanol has been studied [1, 3]. However, there are no studies that have been carried out to evaluate the feasibility of taking algal industry waste or algae as material to obtain Single Cell Protein (SCP). This SCP would serve for feeding farm animals. The marine fungi are a diverse group of opportunistic and obligate organisms isolated from marine environments. These fungi are often included in screening for new metabolites, and their ability to assimilate complex polymers such as alginate, cellulose, and hemicellulose [4]. In this study, the growth of 10 strains of marine filamentous fungi, in alginate and cellulose, was preliminarily evaluated. The protein concentration of the pre-selected fungi was evaluated in two different wastes from the algal industry (Waste A and B) and *Macrocystis pyrifera*. The highest concentrations of protein were obtained with *Asteromyces cruciatus* and *Dendryphiella salina*. The productivity found for *A. cruciatus* was 12.7, 1.9 and 5.0 mg/g day, using *M. pyrifera*, waste A and B respectively. The productivity found for *D. salina* was 7.9, 3.3 and 2.6 mg/g day using *M. pyrifera*, waste A and B respectively. The Box- Behken design (BBD) allows us to optimize the growth conditions (temperature, salinity and pH) for the treatment of seaweed biomass using *D. salina* and *A. cruciatus*. With the optimal values given by BBD the protein can be increased, 2.1, 2 and 4 fold, using *M. pyrifera* with *A. cruciatus*, *M. pyrifera* with *D. salina* and Waste B with *D. salina* respectively. This indicates that alginate and cellulose, from waste from the algal industry, can be assimilated by filamentous marine fungi. Thus, these microorganisms could be excellent sources of bioproducts, which can be used in the food industry.

### Biography

Catalina Landeta Salgado is a Biologist from the Catholic University of Ecuador, has a magister in Environmental Management and Auditing from the Polytechnic University of Catalonia, Spain, a magister in Energy Engineering, mentioning biofuels from the Catholic University of Chile. She is currently a PhD candidate in Chemical Engineering and Biotechnology at the University of Chile. She has worked for more than four years in nationally recognized research projects in Ecuador. The achievements in their results could help the development of the only bioethanol pilot plant, from agroindustrial waste, in Ecuador.

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## e-Posters

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## Dasatinib sensitizes triple negative breast cancer cells to chemotherapy by targeting breast cancer stem cells

**Jun Tian**

McGill University, Canada

Patients with triple negative breast cancer (TNBC) exhibit poor prognosis and are at high risk of tumor relapse and metastasis due to the resistance to conventional chemotherapy treatments. Tumor recurrence and resistance to chemotherapy have been in part attributed to the presence of breast cancer stem cells (BCSCs), a subpopulation of breast cancer cells that possesses stem-like properties. Therefore, targeting BCSCs is a priority to overcoming chemotherapy failure in TNBCs. We generated chemotherapy-resistant TNBC cells through cyclic treatments with paclitaxel (pac). The pac-resistant cells displayed increased self-renewal potential and higher percentage of BCSC subpopulations compared to the parental TNBC cells. A screen with various kinase inhibitors revealed dasatinib, a Src kinase family inhibitor, as a potent suppressor of BCSC numbers and a blocker of self-renewal ability in chemotherapy-resistant breast cancer cells. We also found dasatinib to block pac-induced BCSC enrichment and Src activation in both parental and pac-resistant TNBC cells. Interestingly, dasatinib induced an epithelial differentiation of the pac-resistant mesenchymal cells, further resulting in their enhanced sensitivity to paclitaxel. The combination treatment of dasatinib and paclitaxel not only decreased the proportion of BCSCs and their self-renewal capacity but also synergistically reduced cell viability of pac-resistant cells. *In vivo* studies, using xenograft mouse preclinical models of breast cancer further demonstrated the efficiency of the dasatinib/paclitaxel combination treatment in inhibiting tumor growth. Together, these results highlight dasatinib as a promising anti-BCSC drug that could be used in combination with paclitaxel to overcome chemotherapy resistance in TNBC.

### Biography

Jun Tian is currently a fifth year PhD student in Department of Medicine, McGill University, Canada. She has received her Bachelor of Science degree from Nankai University, China in 2013. Her research interest focuses on studying the role of breast cancer stem cells in breast cancer initiation, metastasis and chemotherapy resistance. So far, she has published seven co-authored scientific articles in peer-reviewed journals.

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## Optimized extraction and identification of functional *Orthosiphon stamineus* proteins

Kamal Usman<sup>1</sup> and Zaidah Rahmat<sup>2</sup>

<sup>1</sup>Qatar University, Qatar

<sup>2</sup>Universiti Teknologi Malaysia, Malaysia

The plant, *Orthosiphon stamineus* (cat whiskers) is a medicinal herb belonging to the family Lamiaceae, and the plant leaves are commonly used as the herbal tea, popularly known as Java tea. It is used as a diuretic agent and for treatment against heat rheumatism. Currently, there are no protein profile data available for this important plant species. This study focused on the optimization of total protein extraction using four different extraction methods; QB buffer, phenol/sodium dodecyl sulfate with three pre-washed steps, phenol/sodium dodecyl sulfate without pre-wash steps and sigma protein extraction kit, it is aimed at determining the best protein extraction method, determine patterns and theoretically identify proteins. Overall, phenol/sodium dodecyl sulfate with three pre-wash steps result in better protein quality and pattern of separation. A total of 104 functional proteins were identified, each containing at least one unique peptide. Among the distinct proteins, rubisco activase and triosephosphate isomerase correspond to the chloroplastic protein of photosynthesis/carbohydrate metabolism, while phosphoglycerate kinase and glyceraldehyde are cytosolic enzymes of glycolysis pathway which were found to be significant housekeeping proteins in of the leaf tissue. The result of this study will be useful for advanced pharmaceutical research and could serve as a baseline for further proteomics work on *O. stamineus* and similar plant species.

### Biography

Kamal Usman is an advanced level PhD candidate and currently working as a Teaching Assistant in the Department of Biological and Environmental Science at Qatar University. He has a great passion for teaching and research. Since the completion of his undergraduate studies, and before joining Qatar University, not only does he experienced classroom teaching and advanced level research exposure while completing his master's degree at Universiti Teknologi Malaysia, was also privileged to contribute to education's development at the top level. He has served as a Senior Technical Officer on educational policy and development planning to a UK funded project in Nigeria; state programme on accountability, responsiveness and capability (SPARC) between 2012 to 2013. He also briefly taught at Bayero University, Kano preparatory to commence his PhD in 2015. His PhD thesis seeks to elucidate the mechanism of plants heavy metal uptake.

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## Gene polymorphism in XPG and breast cancer risk

Masood N, Malik SS, Mubarak S and Khadim TM  
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**Introduction:** Xeroderma pigmentosum group G (XPG) plays crucial role in excision repair of UV-induced DNA damage in nucleotide excision repair pathway. Single nucleotide polymorphisms in XPG gene have been reported to associate with different cancers. Therefore, current study was designed to evaluate relationship between XPG (rs1047768 T>C) polymorphism and breast cancer risk in Pakistani population.

**Methodology:** A total of 175 individuals were screened for this polymorphism including 100 pathologically confirmed breast cancer cases and age matched 75 controls. Genotyping was carried out with Tetra amplification-refractory mutation system (ARMS) PCR and results were confirmed by gel electrophoresis. Data was analyzed using SPSS version 24.

**Results:** Conditional logistic regression analysis showed significant association between TC genotype (OR: 8.9, CI: 2.0–38.7) and increased breast cancer risk. Although homozygous CC genotype was more frequent in patients as compared to controls but it was statistically non-significant (OR: 3.9, CI: 0.4–35.7).

**Conclusion:** In conclusion, XPG (rs1047768 T>C) polymorphism may contribute towards increased risk of breast cancer.

### Biography

Masood N has completed her PhD from COMSATS Institute of Information Technology, Islamabad, Pakistan. She is currently working as Assistant Professor at Fatima Jinnah Women University, The Mall, Rawalpindi, Pakistan. She has published more than 35 papers in reputed journals and has won many awards for research productivity in her home country. She has been supervising a number of students at BS, MS and PhD levels. She is a member of Quality Enhancement Cell at University.

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## Development of glyphosate-tolerant transgenic potato plants harboring the G2aroA gene

**Arfan Ali**

Four Brothers Group Lahore, Pakistan

Glyphosate weed control is a very effective strategy to minimize cost and improve economic outcomes of world and Pakistan agriculture production. Development of glyphosate -resistant potato holds great promise. A new G2-aoraA gene from *Pseudomonas fluorescence* which encodes 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) was transformed by using an Agrobacterium-mediated transformation into potato cultivate AGB-Red. Transgenic potato plants were generated vis node tissue culture method using kanamycin selection. 10 regenerated potato plants were obtained and allowed to grow normally in pots under normal conditions. The polymerase chain reaction (PCR), southern blotting and western blotting analysis confirmed that the target gene was integrated and expressed effectively into potato chromosomes at the very potential level. The glyphosate tolerance assay showed that transgenic potato had a high resistance level to glyphosate. Furthermore, potato plants treated with 50.0 mili mole of glyphosate could grow slowly and can develop tubers. It was concluded that transgenic potato may be used for cotton breeding research of glyphosate -tolerant potato.

### Biography

Arfan Ali is from Centre for Excellence in Molecular Biology, University of the Punjab and Biotechnology Lab Four Brothers Group, Lahore, Pakistan.

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## Association mapping for morphological traits under saline stress in wheat

**Harpreet Singh**

Maharishi Markandeshwar University, India

**Introduction:** Salinity is a major concern constraint to wheat production affects yield and its related parameters. In the present study association mapping was conducted for different morphological characters using diverse 90 wheat accessions. A total of 135 SSR markers distributed on all chromosomes of wheat were used to map rice genome association analysis was carried out by means of mixed linear model. To manage the spurious associations, population structure with kinship in the given panel was considered during association. Population structure analysis revealed three subgroups in the 90 wheat accessions. The admixture level was in range from 0.9%-23.2%. A total of 13 marker trait associations were identified and out of these five marker trait associations explained phenotypic variation of more than 10%. These identified marker trait associations could enhance breeding effectiveness by significantly reducing the time and labor consuming efforts which otherwise be needed in conventional breeding.

**Findings:** The entire panel was selected carefully by screening material from different geographical locations. The screening was done meticulously. A total of 13 marker trait associations were found and out of these traits panicle length explained phenotypic variance of 16.2%. Some of these QTLs share overlapping regions with other genes when BLAST was performed and shows 100% identity.

**Conclusion & Significance:** Salt tolerance is a posing threat for field crops like wheat with millions of hectares of land are affected by it. There is an urgent need to develop varieties that can withstand such harsh environment. The present study is one of the steps towards developing these varieties. Once the regions are identified, the genes could be introgressed to conventional varieties which can support food requirements of world population.

### Biography

Harpreet Singh is a Ph.D. scholar in Maharishi Markandeshwar University from India.

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## Accepted Abstracts

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## Structural elements of a therapeutic vaccine against hepatitis B virus

**Abdul Hai**

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A 23 kDa molecular weight chimeric protein comprising both the core and surface portions of the Hepatitis B viral envelope has been designed on the premise that if the HBV surface protein is fused with the core protein of the viral envelope, it can produce both B-cell and T-cell immune responses. The engineered protein contains 216 amino acids from both the core and surface regions of the viral envelope protein. NNPREDICT and PSIPRED programs have been used to obtain the secondary structure elements. The tertiary structure of the protein was predicted using 3D-JIGSAW program. In the predicted structure,  $\alpha$ -helices form a helical bundle domain and the  $\beta$ -strands form another separate domain.

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## Cognizance of vitamin D and its deficit in young adults of Dubai, UAE

**Anushka Lydia**

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Vitamin D deficiency [VDD] coupled with an unbalanced diet and sedentary life, owing to the advent of social media, is a major concern worldwide. VDD is the most common dietary deficit and the most common undiagnosed health conditions prevailing in the young adult of Dubai region. Several studies suggest the remarkable increase in the occurrence of VDD in UAE population. Studies have also forged a strong relationship between VDD and infertility in Emirati women, particularly with respect to IVF failure. VDD worsens the hormonal imbalance, making them prone to miscarriage. The awareness about the importance of vitamin D in diet and its resources seem to be lacking in the young adults among Dubai residents. This paper aims to assess awareness, perception and understanding of VDD among young adults residing in Dubai. It will also focus on the current research: past, present and future challenges of various aspects of VDD. The current research draws material from government sources especially those based in the UAE, data is drawn from research papers, media reports, websites (National Center for Biotechnology Information), and information published by *The American Journal of Clinical Nutrition*. Long term strategies to address this deficiency problem will include public education, national health policies for screening and prevention through food fortification, and treatment with vitamin D supplementation.

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## Gene-silencing suppressors for high-level production of the HIV-1 entry inhibitor griffithsin in *Nicotiana benthamiana*

**Peyman Habibi**

Federal University of Parana, Brazil

The exploration of emerging host organisms for the economic and efficient production of protein microbicides against HIV is urgently needed in resource-poor areas worldwide. In this study, the production of the novel HIV entry inhibitor candidate, griffithsin (GRFT), was investigated using *Nicotiana benthamiana* as the expression platform based on a non-viral vector. To increase the yield of recombinant GRFT, the RNA silencing defense mechanism of *N. benthamiana* was abolished by using three viral suppressors. A transient expression system was used by transferring the GRFT gene, which encodes 122 amino acids, under the control of the enhanced CaMV 35S promoter. The presence of correctly assembled GRFT in transgenic leaves was confirmed using immunoglobulin-specific sandwich ELISA. The data demonstrated that the use of three gene silencing suppressors allowed the highest accumulation of GRFT, with a yield of 400  $\mu\text{g g}^{-1}$  fresh weight, and this amount was reduced to 287  $\mu\text{g g}^{-1}$  after purification, representing a recovery of 71.75%. The analysis also showed that the ability of GRFT expressed in *N. benthamiana* to bind to glycoprotein 120 is close to that of the GRFT protein purified from *E. coli*. Whole-cell assays using purified GRFT showed that our purified GRFT was potently active against HIV. This study provides the first high-level production of the HIV-1 entry inhibitor griffithsin with a non-viral expression system and illustrates the robustness of the co-agroinfiltration expression system improved through the use of three gene silencing suppressors.

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## Effect of glycerol, sunflower oil and glucose concentrations on the physical -chemical and mechanical properties of Chitosan / Polyvinyl Alcohol films

**Gezira de Ávila**

University of Cartagena, Colombia

Chitosan / PVA films have attracted considerable attention because they are biocompatible, water soluble, biodegradable and non-toxic. The aim of this work is to evaluate the effect of the addition of 3 plasticizers: glycerol (GL), sunflower oil (SO) and glucose (G), on the physical-chemical, mechanical and barrier properties of films based on chitosan and polyvinyl alcohol. The methodology implemented consisted in the preparation of chitosan / PVA solutions in a ratio of 1: 1 (w / w) by the sol-gel method, then different concentrations of plasticizer were added (20%, 40% and 60%). For the formation of the films, the solutions were placed in plastic Petri dishes and allowed to dry for 72 hours at room temperature. Subsequently, the properties of the films were evaluated as water vapor permeability (WVP), degree of swelling (DS), solubility (S), tensile strength (TS), elongation at break (% E) and biodegradability in the soil. The characterization of the plasticized films was carried out by infrared spectroscopy with Fourier Transform (FT-IR), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). With this research it is expected to obtain a biodegradable film that can be used in the field of food packaging materials and the pharmaceutical industry.

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## The anti-cancer properties of crocin and epirubicin on chemosensitive cervical cancer cells

Reyhane Hoshyar, Arash Ghorbani and Homa Mollaei  
University of Medical Sciences, Iran

Cervical cancer is still one of the leading causes of death amongst women in the developing countries. Although there are many adopted strategies for declining its symptoms such as surgery, radiotherapy, chemotherapy and their combination following tumor resection, tumor recurrence is usually occurring due to cancer cell resistance to chemotherapy. Also, damaged normal cells are another drawback of these therapies. Hence, today many studies have been accomplished to investigate anticancer characteristics of natural agents such as medicinal plants. Among them, the tumoral properties of crocin on different kinds of cancerous cell lines are interested in many scholars. In this study, the sensitive human cervical cancer cell line (OV2008) was exposed to crocin and epirubicin to investigate the molecular mechanism of this combination. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed that proliferation of sensitive cells (OV2008) was declined by crocin and epirubicin combination at a time- and dose-dependent manner (0-24-48 h). Hoechst staining assay also proved these results and showed that antiproliferative effect of crocin and epirubicin might be due to the induction of apoptosis. Furthermore, up-regulating of Bax and down-regulating of Bcl2 expression lead to achieving the genetic mechanism of crocin-epirubicin apoptosis induction in this cell line. Escalating LDH releases announced that crocin combined with epirubicin had an apoptotic effect on this cancerous cell. Regarding these results, the combination of crocin and epirubicin can play proliferative and apoptotic roles against sensitive cervical cancer cells which may be used as an adjuvant agent for cervical cancer treatment to increase the efficiency of therapy.

### Biography

Reyhane Hoshyar is an Associate Professor at Department of Clinical Biochemistry, School of Medicine, Director of Cellular and Molecular Research Center, Director of Health Technology Office, Birjand University of Medical Sciences, Birjand, Iran since 2013. Her field of interest is in Molecular Biology of diseases especially cancer.

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## Engineering carbon-based nanomaterials for cancer therapy and bioimaging

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The significant advances on the development of synthetic processes has allowed the development of many unusual carbon nanomaterials. Since the nineties, with the discovery of fullerenes, several carbon nanomaterials with different structures and properties have been reported, such as nanotubes, nano onions, nano horns and graphene. The singular features of carbon-based materials have been widely explored for the development of new therapeutic approaches on oncology by the design of new smart materials capable of meeting the clinical demands for the therapy and bioimaging of tumors and cancer cells. The development of new multifunctional carbon nanoplatfoms, can offer new possibilities for cancer detection at early stages of the disease and consequently, improve the success of the treatment. Several approaches have been developed for engineering new carbon nanoplatfoms for carrying high payloads of bioimaging agents or drugs that can be released in controlled manner. Besides, the accurate biofunctionalization of the carbon nanoplatfoms surface can prolong their circulation time in biological fluids and improve their targeting efficiency to specific tumors and cancer cells. Recently, the development of carbon nano-capsules, showed the possibility to hermetic seal in their interior payloads with exciting properties for bioimaging. Indeed, this carbon shelters provide the possibility to explore payloads for biological proposes, that are characterized by high level of toxic effects or inability to reach the desire target. Moreover, after filling, carbon nano- capsules offer the possibility to functionalize the external surface with targeting ligands or biocompatible molecules. This discussion intents to covers the recent progress on the development of new synthetic strategies for nanoengineering carbon nanomaterials as a multifunctional nanoplatfoms for cancer therapy and bioimaging. In addition, it is also a main objective of this topic to discuss the most relevant achievements into the *in vitro* and *in vivo* performance of the carbon nanoplatfoms on the detection and treatment of cancer.

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## *In vitro* study of anti-metastatic effect of crocetin on triple negative breast cancer cells

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Breast cancer is considered as the second most malignant cancer in women. Among various types of breast cancer, the triple negative breast cancer (TNBC) is more aggressive and metastatic, with earlier relapse and poorer survival rate than hormone receptor-positive subtypes. Moreover, patients with metastatic TNBC cannot be treated with any standard-of-care therapy. Thus, medicinal herbs and their derivative compounds are being increasingly established as beneficial alternative and complementary treatments for cancer. Crocetin is an important carotenoid present in saffron (the red dried stigma of *Crocus sativus L.*), which exhibits anti-proliferative and apoptotic effects against primary tumors. The present study aims to investigate the anti-metastatic effects of crocetin on the metastatic 4T1 cells (which closely resemble metastatic, triple negative breast cancer in humans). The 4T1 cells with different concentrations of crocetin (0-0.28mM) were incubated for various times (24, 48 and 72h). Cell viability was assessed by MTT. Scratch and trans well chamber assays were performed to investigate the effects of crocetin on cellular migration, mobility and invasion. The relative attachment of the 4T1 cell to immobilized ECM was evaluated by cell-matrix adhesion assay. The mRNA levels of major genes in metastasis (matrix metalloproteinase 9, vascular endothelial growth factor  $\alpha$  and vimentin) were measured using real-time polymerase chain reaction. It was found that crocetin inhibited the growth of 4T1 cells in time- and dose-dependent manner (the IC<sub>50</sub> values were 0.24, 0.17 and 0.16 mM, respectively, at 24, 48 and 72 h). The doses corresponding to approximately 90% and 75% cell viability were opted for subsequent assays. The migration of 4T1 cells was significantly interrupted by crocetin and it reduced cell invasion and mobility. Crocetin also decreased the adhesion of 4T1 cells, in a dose-dependent manner. Crocetin down-regulated the level of MMP-9, VEGF- $\alpha$  and Vim (activated genes in metastasis). These *in vitro* results indicate that crocetin, as a therapeutic herbal product, has anti-metastatic potential, in addition to its proven anti primary-tumor effects. Therefore, it may be an effective candidate for controlling malignant metastatic breast cancer.

### Biography

Laleh Arzi is PhD candidate in biochemistry at Institute of Biochemistry and Biophysics (IBB), University of Tehran. She obtained a BSc degree in Cellular and molecular biology from Kharazmi University and an MSc in biochemistry from (IBB), University of Tehran. She is interested in cancer research and molecular mechanism of herbal medicine, thus she is working on "anti-metastatic mechanism of bioactive compounds of saffron on metastatic breast cancer in vivo and in vitro" for her PhD project. She has several publications in international journals and has participated in several international conferences in Germany (Dresden and Tubingen) Greece (Athens) and Canada (Vancouver). She is also a lecturer in Azad University in Tehran, teaching Biochemistry and Biophysical chemistry.

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### Notes:

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## Transition metal complexes/organometallic compounds as anticancer/anti HIV drugs or in pharmaceutical industry

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Cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health. Cancer patients have burdened by drug induced toxic side effects, and no turned to seek help from the complementary and alternative medicine hoping for a better cure. Research on Platinum based drugs and Non Platinum based drugs is a Multi-Million Dollar Industry in USA and there is every need to produce safe drugs for the cure of this monstrous disease. Flavonoids have a long history of use in traditional medicines in many cultures. The phytochemical, curcumin is one of the major dietary flavonoid, belonging to a group of flavonol, Curcumin is a natural polyphenol. It is highly potential molecule capable of preventing and treating various cancers. Various dietary chemo preventive agents, turmeric powder or its extract are broadly used as therapeutic preparations in Indian System of medicine. We provide a summarized synthesis and structural determination of Curcumin Oxime, Curcumin Thiosemicarbazone derivative of Gold (III) complex. The use of these analogs for prevention of cancer tumor progression and treatments of human malignancies. A pharmacologic agent for treating and/or preventing cancer, among other diseases and conditions, and particularly breast, prostate, and pancreatic cancer, in humans and animals. The novel pharmacologic agent is an isoflavonoid or isoflavonoid mimetic covalently attached to a cytotoxic pharmacophore that, preferably has the ability to conjugate with a metal salt to form a more potent metal complex, particularly a Au (III) complex and other complexes of Platinum, Palladium, Ruthenium, Copper etc.

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## Time dependent degradation of polyphenols from thermal processed berries and their *in vitro* antiproliferative effects against melanoma

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Berries are an important source of bioactive compounds, which have showed to have positive health benefits. Those compounds are namely phytochemicals and among them, anthocyanins contribute in high amount to the nutritional and potential health value. Because of berry seasonal availability and also due to their rapid degradation, people found multiple ways to preserve them. The most common options are: freezing, jellies or jams. The last one is also the most popular way of conservation in Romanian household. The most common fruits used as primary ingredient in jams are: berries, plums, cherries. Starting from this we thought what has all this common? The answer was: that they share many bioactive compounds is polyphenols such as anthocyanins, flavonoids or phenolic acids. Their stability is a continuous challenge for food industry. There are also multiple published data providing that they are sensitive to light, pH or high temperature. All those vectors are present during jam preparation. In this context we started a study regarding phytochemical composition and bioactive compounds degradation after jam preparation. We also monitored their degradation during storage time and their *in vitro* antiproliferative potential. However, to the best of our knowledge, no report exists on the effect of processing on the phenolic compounds content of homemade jams from chokeberry, elderberry, blackcurrant or blackthorn. The obtained results revealed that processed and stored in time, the bioactive compounds from berries jam are degraded, they still exert antioxidant and antiproliferative potential. Prior to LC-MS analysis, polyphenolic compounds were identified as: flavonoids (anthocyanins, flavanols) and non-flavonoid (hydroxycinnamic acids (HCA) and hydroxybenzoic acids (HBA). The most significant decrease was observed for HCA comparing to other classes of the quantified compounds. This variation is expected due to variations in constituents and phenolic types among different analyzed berries.

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## Molecular characterization and monitoring of avian paramyxovirus type 1 in poultry of Libya

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Avian paramyxovirus-1 (APMV-1) is the causative agent of Newcastle disease which affects many species of birds leading to high mortality and heavy economic losses among poultry industry worldwide. On March 2013, the Libyan poultry industry faced severe outbreaks caused by Newcastle disease. APMV-1 was isolated and characterized. Following detection of the virus in allantoic fluid by rRT-PCR, genetic sequencing of the APMV-1/chicken/Libya/13VIR/7225-1/2013 isolate revealed the presence of a velogenic APMV-1 belonging to lineage five (GRRRQKR\*F Lin.5) or genotype VIII in class II, according to the nomenclature in use. The use of live attenuated and inactivated vaccines in commercial poultry had significantly reduced the impact of the disease. Another Newcastle disease outbreak emerged on March 2015 in backyard chickens. Two viruses were detected in cloacal swabs namely APMV-1/Libya/15VIR5368/2015 and APMV-1/Libya/15VIR5371/2015. Genetic sequencing of these viruses revealed the presence of velogenic APMV-1 genetically similar to the virus isolated on 2013. During the same period, neurologic signs and mortality were noticed in pigeons. Samples of brain tissue were tested by rRT-PCR which revealed presence of velogenic APMV-1 belonging to lineage 4A (GKKRKR\*F Lin.4A). To the best of our knowledge, this is the first report about the detection and molecular characterization of APMV-1 in pigeon in Libya. The phylogenetic analysis of the F gene showed 86% identity to isolates from Iran and Egypt. This study may indicate the circulation of APMV-1 within backyard birds and pigeons which may present a threat to commercial poultry. Considering these findings, vaccination of backyard birds and pigeons is strongly recommended.

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## Nanosystems formed by amphiphilic antimony(v) complexes incorporating amphotericin B for the treatment leishmaniasis

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This work aimed to develop an oral formulation of amphotericin B (AmB) for treatment of leishmaniasis. AmB is an antifungal and antibacterial macrolide polyene derived from *Streptomyces nodosus* strain, that belongs to the group of second generation anti-leishmanial drugs and is extensively used in case of failures in the treatment with antimonial compounds. AmB was incorporated into nano-systems formed by amphiphilic antimony (V) complexes with ligands of alkyl methyl glucamide series (L8 and L10, with 8 and 10 carbon chain, respectively). Incorporation rate of 0.2% AmB into SbL8 and SbL10 dispersions was determined using an HPLC-based technique and was found to be  $84\pm 1\%$  and  $74\pm 1\%$ , respectively. The characterization of SbL10-AmB and SbL8-AmB by circular dichroism and UV-visible spectroscopies showed that AmB is present predominantly under the monomeric form in both SbL8 and Sb10 nanosystems, which is the least toxic form to the host and potentially most bioavailable. The potential for the oral treatment of visceral (VL) and cutaneous leishmaniasis (CL) was evaluated in murine models in comparison to the standard drug Anforicin B<sup>®</sup> or Glucantime<sup>®</sup> administered intraperitoneally or orally. In Balb/c mice infected with *Leishmania amazonensis*, the SbL10-AmB mixed formulation (170 mg Sb/kg and 14 mg AmB/kg, each two days by oral route) resulted in a significant decrease of the lesion size, when compared to orally administered Glucantime<sup>®</sup> and SbL10 (170 mg Sb/kg, each two days), Anforicin B<sup>®</sup> (>1mg/kg/each 5th day, by intraperitoneal route) and control saline group. In Balb/c mice infected with *Leishmania infantum*, both the SbL10-AmB and SbL8-AmB mixed formulations given orally (170 mg Sb/kg and 14 mg AmB/kg per day) reduced significantly the parasite load in the liver compared to the untreated control, to a similar level as AmB given intraperitoneally (0.9 mg/kg/day). This study established for the first time the potential of mixed SbL10-AmB and SbL8-AmB formulations for the oral treatment of both cutaneous and visceral leishmaniasis, indicating their potential for further development and applications.

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## Drought-induce variation in growth rate, dry matter, flavonoid and phenolic content of soybean cultivars

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Soybean (*Glycine max* L Leguminosae) is an important grain legume that is not only a valuable oil seed crop but also used as feed for livestock and aquaculture. Soybean genotypes viz., CO-1 and JS 335 were used for the study of drought-induced variations in growth. The present investigation was undertaken to study the effect of two concentrations of PEG on callus induction of soybean genotypes. Seeds of soybean were inoculated on MS medium supplemented with two different concentrations of PEG (2% and 4%) cultures were incubated at  $26\pm 2^{\circ}\text{C}$  under 16 h photo periods. The effect of different concentration of PEG on callus induction was investigated. *In vitro* callus cultures of both genotypes (CO-1 and JS 335) showed a reduction in callus growth during PEG treatment as compared with the control. The presence of PEG in the medium elevated dry matter content in all treatments compared with the control. Similarly flavonoid levels and phenolic contents were higher in the PEG treatments in comparison to control. Our results can be used for *in vitro* screening and manipulations of soybean cultivars for improvement of drought tolerance.

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