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22<sup>nd</sup> Global Congress on **Biotechnology**  
&

5<sup>th</sup> International Conference on  
**Enzymology and Protein Chemistry**

February 28-March 02, 2019 | Berlin, Germany

# Posters

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**The fabrication of self-shrinking multilayer bio-capsules templated on CaCO<sub>3</sub> vaterite crystals**J Campbell<sup>1</sup>, R Ali<sup>1</sup>, V Vigneswaran<sup>1</sup>, D Volodkin<sup>1</sup> and A Vikulina<sup>1,2</sup><sup>1</sup>Nottingham Trent University, UK<sup>2</sup>Fraunhofer Institute for Cell Therapy and Immunology, Germany

Biopolymer based multilayer capsules are novel vectors for advanced drug delivery. Capsules assembled using decomposable and mesoporous CaCO<sub>3</sub> vaterite crystals can host enormous amounts of biomolecules (such as proteins and peptides, small drugs, nucleic acids, etc1) and release them in a controlled manner. Protection and controlled release of biomolecules are the main advantages of the capsules; this can be achieved by adjusting the capsule structure by varying the number of layers, polymer nature and distribution into capsules. Loading of biomolecules into capsules at physiologically relevant and mild conditions is indispensable for bio-applications. This work aims to fabricate capsules from a variety of biopolymers and assess their stability and the encapsulation performance. The following biopolymers have been tested for polyanions (chondroitin sulfate, hyaluronic acid, dextran sulfate, and heparin) and polycations (poly-L-lysine, dextran amine, collagen, and protamine). The most attractive pairs of biopolymers, in-terms of capsule integrity are identified and retention of biomolecules within the capsules is considered. Shrinkage of capsules at room temperature during CaCO<sub>3</sub> removal is used for capture of biomolecules into capsules and is discussed by taking into account the charge compensation in cooperative interpolymer complexation. Interestingly, occupation of the vaterite crystal pores with polymer during capsule fabrication is also responsible for the observed capsule shrinkage and fusion phenomena.

**Biography**

A Vikulina has completed her PhD in the field of Biological Science in Lomonosov Moscow State University, Russia. Currently, she is Marie-Curie Fellow in Fraunhofer Institute for Cell Therapy and Immunology, Potsdam, Germany. Her research is focused on the development of drug delivery carriers for controlled drug delivery and testing as well as for deciphering the pathways of biological action and transport of drugs. She has been awarded by prestigious Alexander Von Humboldt and Marie-Curie Fellowships, served as a member of organizing committees at international conferences and scientific olympiads. She is also a Guest Editor in Micromachines Journal.

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## Cellulase production with *Penicillium verruculosum* strain in lab and pilot scale

**Martin Schomber**

University Leipzig, Germany

Bioethanol is produced in general by starch fermentation from corn or sugar beets. An alternative is the use of lignocellulose by digesting cellulose to glucose. This digestion is an enzymatic process in which endocellulases, cellobiohydrolases and  $\beta$ -glucosidase, named as cellulase complex, are interacting together. The most common used fungal strain for cellulase production, *Trichoderma reesei*, has been studied intensively and optimized in recent years. The metabolism of cellulase production has been described in different literatures for fungus but is not exactly understood so far. The problem of cellulase production by *Trichoderma reesei* is based on a not well balanced enzyme complex. There is a low production rate of  $\beta$ -glucosidase in this fungus that leads to the addition of the minor enzyme in industrial scale fermentation. Our studies focus on a cellulase production with *Penicillium verruculosum* mutants that has a more balanced cellulase complex. Beech wood is used as lignocellulose substrate in this study which is pretreated by organosolv process technology for separation of hemicellulose, lignin and cellulose. This poster will present first fermentation results by using different substrates and varying fermentation methods to optimize the enzyme production in both, lab and pilot scale.

### Biography

Martin Schomber has completed his Master studies in pharmaceutical biotechnology at the age of 25 years from Technische Hochschule Mittelhessen. In his studies, he has been a part of different research work groups in molecular, enzymatical and industrial parts of biotechnology. During a research stay at the University of Auckland (New Zealand), he was involved in a methodical study to identify  $\beta$ -1,3 glucan in wood for specific identification of different kinds of wood for industrial application.

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## How to convert industrial dairy effluent into high quality proteins with microalgae

**Marion Champeaud**

Université de Poitiers, France

Utilization of whey or whey permeate is one of major concerns of the dairy industry nowadays, especially the acid whey, which mostly remains untreated prior to disposal. In 2010, 734 million tons of milk, and 160-180 million tons of whey per year were produced worldwide. In 2014, milk production was higher than 800 million tons and is constantly increasing over the years. Despite the different strategies considered by the industrials to valorize whey: lactose crystallization, food applications in bakery products, dry mixes, snack, and milk replacer, alcoholic fermentation, or biogas conversion, only 50% of this whey is processed. In this study we present an industrial fermentation model to valorize these dairy by-products to obtain added-value bio-products at the same time. We demonstrate that the microalgae *Galdieria sulphuraria* is able to consume 100% lactose, 98% of lactate and 79% of the citrate present in whey permeate. Specific transport experiments show that lactose uptake by *Galdieria sulphuraria* involves the induction of a specific low affinity transport system ( $K_m=53\pm 2.9$ ). The biomass production is whey permeate specific and range from 30 to more than 110 g/l of dry matter. In addition to direct the bio-remediation of industrial dairy waste, the algae biomass produced show a real nutritional interest due to its high protein content (>50%) and is naturally rich in essential amino acid.

### Biography

Marion Champeaud graduated with a Bachelor of Biology five years ago and a Master's degree in Agro-Industry three years ago from Bordeaux University in France and she is currently doing her PhD in partnership between a biotechnology company Fermentalg and Poitiers University in France (UMR CNRS 7267). Her research studies in engineering process and microalgae culture area resulted in 2 patents-pending and 2 publications in progress.

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**Peroxiredoxin I as a candidate biomarker of male fertility****Do Yeal Ryu, Saehan Kang, Won Ki Pang, Won Hee Song, Md. Saidur Rahman and Myung Geol Pang**  
Chung Ang University, Republic of South Korea

Conventional semen analysis has limitations to predict male fertility in livestock industry. Although several studies have been performed to overcome the limitation, the current solution to prediction is still unsatisfactory. It is generally accepted that peroxiredoxin I (PRDX I) have a critical role in the regulation of male fertility. Therefore, the current study was designed to investigate the correlation between PRDX I and male fertility. PRDX I was examined on spermatozoa collected from 14 individual boars with different litter size (10.3-14.2). 530 sows were artificially inseminated to determine the litter size. Subsequently, several sperm function were evaluated. Our study showed that there is a significant positive correlation between litter size and PRDX I ( $r=0.686$  and  $p = 0.007$ ) as well as hyperactivity ( $r=0.5769$  and  $p=0.031$ ). Subsequently, the prediction accuracy of boar fertility was determined by the receiver operating characteristic (ROC) curves. The ROC analysis showed that PRDX I can predict litter size with overall accuracy 92.86% (sensitivity 90%, specificity 100%, negative predictive value 80%, and positive predictive value 100%). In addition, hyperactivity also showed 80% overall accuracy to predict litter size (sensitivity 70%, specificity 75%, negative predictive value 50%, and positive predictive value 87.5%). As a biomarker, PRDX I and hyperactivity are expected to increase pups than average litter size (0.54 and 0.45, respectively). As far we know no other authors have found the correlation between PRDX I and litter size. Consequently, PRDX I might be the novel candidate biomarker for diagnosing male fertility and litter size in livestock industry.

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**Patent protection of monoclonal antibodies useful to combat multidrug-resistant bacteria**Katia dos Reis<sup>1</sup>, José Procopio Moreno Senna<sup>1</sup> and Gisele Rodrigues Atayde<sup>2</sup><sup>1</sup>Institute of Immunobiological Technology, Brazil<sup>2</sup>University of São Paulo at São Carlos School of Engineering, Brazil

Over the past 10 years, there has been an increasing demand for biopharmaceuticals, especially monoclonal antibodies. The rate of these biological products was significantly higher than the rate of traditional pharmaceuticals. Biopharmaceuticals now account for more than 15% of the total market and there is a forecast that sales of biopharmaceuticals should exceed \$250,000,000,000 by 2020. The growth in demand of these products can be justified by the high specificity of monoclonal antibodies and the need for researchers and the market to develop new products, new technologies that could be used to combat various diseases. Multidrug-resistant bacteria strains are a global problem and the use of monoclonal antibodies to combat hospital infections may be a valuable alternative to public health. Another important aspect is the patent protection of these new assets, especially in the Institute of Immunobiological Technology (Bio-Manguinhos), a unit of the Oswaldo Cruz Foundation (Fiocruz) responsible for research, innovation, technological development and production of vaccines, rapid test devices and biopharmaceuticals aimed at meeting the demands of national public health.

**Biography**

Katia dos Reis completed her Bachelor of Chemistry and Master of Biochemistry at the Federal University of Rio de Janeiro. She is a Professor of Chemistry in the State Department of Rio de Janeiro and did her PhD Graduate Program in Technology of Chemical and Biochemical Processes of the School of Chemistry at the Federal University of Rio de Janeiro. Currently, she has been a Patent Analyst at the Bio-Manguinhos Technological Transfer Office (TTO-BIO), advising researchers on the protection of products and processes from Bio-Manguinhos/Fiocruz Technological Development.

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**Engineered Erwinase with possible fewer adverse effects for treatment of acute lymphoblastic leukemia**Iris Munhoz Costa, Débora Custódio Moura, Adalberto Pessoa Jr. and Gisele Monteiro  
University of Sao Paulo, Brazil

Acute lymphoblastic leukemia (ALL) is the most frequent neoplasm in children and adolescents. Treatment of the disease is performed with L-asparaginase (ASNase), an enzyme obtained from the bacteria *Escherichia coli* and *Erwinia chrysanthemi* (Erwinase). ASNase hydrolyzes L-asparagine and prevents tumor cells from obtaining this amino acid from the bloodstream for protein synthesis, leading to ALL cell death by apoptosis. However, both formulations are associated with a high rate of adverse effects as the production of anti-asparaginase antibodies and hypersensitivity, which compromise the efficacy of the treatment. The development of mutant proteoforms from commercially available bacterial enzymes may contribute to the development of an enzyme with lower adverse effects. Therefore, we created a mutant library using the ASNase of *E. chrysanthemi* by error prone PCR, and a double mutant proteoform (DM) presented higher specific activity for L-asparagine and a 30% increase in the *k<sub>cat</sub>* in relation to the wild-type (WT) enzyme. In addition, DM enzyme showed less recognition by anti-asparaginase antibodies and is able to kill the same amount of ALL cell line MOLT-4 than WT enzyme, using a smaller amount of protein. The results indicated that the DM enzyme has cytotoxic potential and may have fewer adverse effects.

**Biography**

Iris Munhoz Costa is a PhD student in the graduate program in Pharmaceutical Technology-Biochemistry in the School of Pharmaceutical Sciences at University of São Paulo. She works with biopharmaceutical research for the treatment of acute lymphoblastic leukemia. She has obtained her Master's degree in Pharmaceutical Technology-Biochemistry at University of São Paulo in 2015; Graduation in Pharmacy and Biochemistry at Universidade Paulista in 2012. She was a student of scientific initiation in the Department of Pharmaceutical Biochemical Technology in the Faculty of Pharmaceutical Sciences at the University of São Paulo in the area of molecular biology and antioxidant response in 2011.

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**Bioprospecting and rational engineering of new L-asparaginase to present a better biopharmaceutical for blood cancer treatment**Tales A Costa-Silva<sup>1</sup>, I M Costa<sup>1</sup>, G S Agamez-Montalvo<sup>2</sup>, A Pessoa<sup>1</sup> and G Monteiro<sup>1</sup><sup>1</sup>University of Sao Paulo, Brazil<sup>2</sup>Federal University of Ceara, Brazil

L-asparaginase (E.C.3.5.1.1) produced by bacteria is used in the treatment of acute lymphocytic leukemia (ALL). However, Linnumerable side effects were registered by the usage of bacterial L-ASNase during ALL treatment. Other drawbacks associated with prokaryotic L-asparaginase treatment are hypersensitivity reactions, low thermal stability, human proteases degradation and rapid clearance. Some techniques have been used to overcome these downsides such as bioprospecting eukaryotic sources or modification of commercial bacterial L-asparaginases by site-directed mutagenesis. In order to find eukaryotic sources of L-ASNase, 20 filamentous fungi were used in this study, which were isolated from the microbiome of the jellyfish *Olindias sambaquiensis*. Six fungi samples isolated from jellyfish tentacles (brown structures in jelly fish responsible to toxin production) showed L-asparaginase production by submerged fermentation process. The highest activity was shown by Strain OS02 with 2.7 U/g. This strain was selected for optimization of L-asparaginase production by central composite design of response surface methodology. For maximum enzyme production (11.45 U/g), the best condition was modified Czapek-Dox medium supplemented with L-asparagine and adjusted to pH 7.4 at 32.5 °C and 190 rpm. Regarding protein engineering of commercial bacterial L-asparaginases we used site-directed mutagenesis to obtain L-asparaginase protease-resistance: a new *Escherichia coli* L-asparaginase (EcAII) variant, triple mutant. The preliminary results showed that mutant enzyme was expressed in *E. coli* BL21 (DE3) and preserved original L-asparaginase activity. These L-asparaginases proteoforms may be alternative biopharmaceuticals with the potential of further improving outcome in ALL treatment.

**Biography**

Tales A Costa-Silva has completed his Graduation in Biological Sciences at Federal University of Alfenas, Brazil and is pursuing his PhD at Sao Paulo University, Brazil. He has experience in Microbiology, focusing on Industrial Microbiology, acting on the following subjects: Industrial Enzymology (Production, Purification, Immobilization, Characterization and Application) and Pharmaceutical Biotechnology.

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**Effect of vanadium stress on physiological and anthocyanin genes in Brassica genotypes with different photosynthetic activity**Javeria Ejaz<sup>1</sup>, Muhammad Imtiaz<sup>2</sup> and Raina Ijaz<sup>1</sup><sup>1</sup>University of Poonch Rawalakot, Pakistan<sup>2</sup>Guangzhou University, China

Heavy metals are major environmental concern when they present in high concentration in soil. Heavy metals in agricultural land and products are of great attention throughout the world as they are toxic to both plants and human health. The present study aimed to elucidate the photosynthetic performance, antioxidant enzymatic activities, anthocyanin contents, anthocyanin biosynthetic genes expression and vanadium uptake in two mustard genotypes purple and green differing in photosynthetic capacity under vanadium stress. The results indicated that vanadium significantly reduced photosynthetic activity and protein contents in both genotypes. The activities of antioxidant enzymes were increased significantly in response to vanadium stress; however, the purple mustard genotypes had significantly higher antioxidant enzymatic activities. The anthocyanin contents were also significantly reduced under vanadium stress. The anthocyanin biosynthetic genes were highly expressed in the purple genotype, especially the genes TT8, F3H, and MYBL2 under vanadium. The expression of all biosynthesis genes were higher in vanadium-treated purple mustard genotype than green mustard, however with the increase in vanadium concentrations these expression level decreases consecutively. These results indicate that induction of TT8, F3H, and MYBL2 genes was associated with upregulation of biosynthetic genes for higher anthocyanin biosynthesis in purple mustard as compare to green mustard. The vanadium uptake by the roots was always higher than the shoots in the both mustard genotypes. The results showed that the purple had higher vanadium tolerance than the green genotype. Future work should be directed to unveil the mechanistic explanations of genes expression and inter-relationship of vanadium and plants that are currently being unravelled.

**Biography**

Javeria Ejaz is an emerging young scholar, currently doing PhD in Plant Biotechnology at University of Poonch, Faculty of Agriculture, Rawalakot (Azad Kashmir), and focusing on the fate of Biotechnology in Plant Breeding & Molecular Genetics. She has participated in many national and international conferences and seminars, and has given presentation about her research work. She has good scientific publications as well.

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**Biocontrol efficacy of two *Bacillus* species against chilli pepper anthracnose causal agent *Colletotrichum truncatum***H B P Sandani<sup>1</sup>, N P Ranathunge<sup>1</sup>, P L N Lakshman<sup>1</sup>, G Degrassi<sup>2</sup> and W M W Weerakoon<sup>3</sup><sup>1</sup>University of Ruhuna, Sri Lanka<sup>2</sup>ICGEB Laboratory, Buenos Aires, Argentina<sup>3</sup>Field Crop Research Institute, Maha Iluppallama, Sri Lanka

Applicability of two *Bacillus* strains isolated from standard compost was assessed as a reliable alternative in the management of the anthracnose in chilli pepper. Out of twenty promising antagonistic bacterial isolates, two were genotypically characterized as *Bacillus* spp. based on 16S rRNA analysis. Selected two antagonistic *Bacillus* strains significantly suppressed the mycelial growth and the spore germination of chilli pepper anthracnose causal agent *Colletotrichum truncatum* ( $p < 0.05$ ). Malformation of the fungal hyphae was dominant in the area subjected to antagonism. One of the major antagonistic mechanisms extended towards the anthracnose pathogen *C. truncatum* by these two *Bacillus* strains was detected as antibiosis in the cellophane overlay technique. They produced antifungal peptides, lipopeptides and also minute amount of organic solvent soluble molecules as antifungal compounds against *C. truncatum*. These antifungal compounds showed minimum inhibitory concentrations of 3200 ppm and 2460 ppm on *C. truncatum* and also these metabolites are thermally stable up to 120°C while they could retain the antifungal properties more than 10 weeks under cold conditions and up to eight weeks at room temperature. One of the *Bacillus* strains produces a bacteriocin like small peptide inhibitory against *C. truncatum*. Induction of latent host defense mechanisms is another antagonistic mechanism employed by this *Bacillus* sp. upon *C. truncatum* infection in chilli pepper. The tested *Bacillus* sp. could enhance the expression of defense related PAL and PO enzymes in chilli pepper plants upon *C. truncatum* infection. Also these antagonistic *Bacillus* strains promote chilli pepper plants' growth through the production of significant amounts of Indole Acetic Acid (IAA) which is a growth hormone as an indirect mechanism against *C. truncatum*. They also showed a prominent swimming and swarming ability indicating their high potentiality in colonizing plant tissues paving the way for successful green house and field applications. These two strains effectively managed the seed borne infection of *C. truncatum* resulting healthy chilli pepper seedlings with good vigor. Also they effectively managed the fruit decay caused by *C. truncatum* at color breaking stage and green stage suggesting the suitability of *Bacillus* spp. as a potential candidate to be used in the development of a biocontrol agent against chilli pepper anthracnose causal agent *C. truncatum*.

**Biography**

H B P Sandani is a PhD candidate from Ruhuna University, Sri Lanka. She conducted her PhD research project in Biotechnology and Crop Protection. She has got 18 research publications including three full length research articles in referred journals, extended abstracts and abstracts. Her ambition is to do a great contribution for the world food production through a green approach with the help of her research findings.

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# Accepted Abstracts

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**Treatment of gamma radiation damage in male rats by using of stem cells and silymarin****Ashraf Z M**

Mansoura University, Egypt

Therapeutic effect of mesenchymal stem cells transplantation (MSCs) and an antioxidant such as silymarin have been postulated as hepatoprotectors against ionizing radiation induced injury. The present study was undertaken to evaluate the protective effect of MSCs and silymarin to ameliorate damage caused by gamma radiation. Bratton-Marshall Reagent was given by intravenous injection to male rats, one day post gamma irradiation at the dose level of 4 Gy. Rats were orally administrated silymarin at dose (70 mg/kg dissolved in distilled water) before irradiated three days and continued for 21 days post irradiation. After one and three weeks post irradiation results revealed that irradiated animals receiving MSCs and silymarin separately or with each other exhibited a pronounced elevation in liver antioxidant such as glutathione (GSH) superoxide dismutase (SOD), glutathione-S-transferase (GST), total antioxidant capacity (TAC), catalase (CAT) and glucose-6-phosphate dehydrogenase (G-6-PDase) activity accompanied with significant decline in lipid peroxidation and hydrogen peroxide levels in comparing with irradiated rats. Moreover, RAPD-PCR with primers OP-B10 and OP-B14 exhibited different banding patterns in all treated rats compared to untreated control rats after one and three weeks of treatment. In conclusion, treatment with MSCs and silymarin possess a radio protective capacity against ionizing-radiation induced oxidative stress and organ injury.

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**Isolation and characterization of phenol-degrading yeasts from industrial effluent**Atena Alirezaei Dizicheh<sup>1</sup>, Mansour Bayat<sup>1</sup>, Mahmood Alimohmmadi<sup>1</sup> and Mohammad Ghayyomi Jazeh<sup>2</sup><sup>1</sup>Islamic Azad University, Tehran, Iran<sup>2</sup>Islamic Azad University, Tonekabon, Iran

Now-a-days environmental pollutants are one of the problems the industrial world is facing. Among these compounds, phenolic compounds are toxic pollutants to which chlorophenols belongs are known as xenobiotic chemicals. 4-chlorophenol (4-CP) is one of the chlorophenols with a high solubility in water, so that it is most detected in waste water and also can accumulate in their bodies biologically. In present study 13 strains of bacteria and 6 strains of yeast and mold phenol degradation was purified from Shahid Tondgooyan Petrochemical wastewater treatment unit was first carried out within 15 days. Then capability of the isolated microorganisms in biodegradation of 100 ppm 4-chlorophenol in presence of 2 g/l glucose as a growth substrate was examined. Two microorganisms, selected as superior species. The strains were designated as TY1 and TY2 and strains were identified by molecular method using amplification of ITS gene region. The phenol degradation was determined by the spectrophotometric method 4-amino antipyrine. The results showed that 100% removal of 100 ppm 4-chlorophenol by TY1 in 45 hrs, TY2 in 21 hrs and mixed culture of TY1TY2:50/50 in presence of 2 gr/l glucose within 18 hrs. Percentage of pure cultures in mixed culture had no significant effect on 4-CP removal efficiency. Furthermore, the results of the sequencing showed that the isolates with the genus *Trichosporon* sp. The significance and impact of the study is the utilization of native yeast strains isolated from the wastewater itself having potential for environmental bioremediation in petroleum refinery and petrochemical industries.

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Applicability of two *Bacillus* strains isolated from standard compost was assessed as a reliable alternative in the management of the anthracnose in chilli pepper. Out of twenty promising antagonistic bacterial isolates, two were genotypically characterized as *Bacillus* spp. based on 16S rRNA analysis. Selected two antagonistic *Bacillus* strains significantly suppressed the mycelial growth and the spore germination of chilli pepper anthracnose causal agent *Colletotrichum truncatum* ( $p < 0.05$ ). Malformation of the fungal hyphae was dominant in the area subjected to antagonism. One of the major antagonistic mechanisms extended towards the anthracnose pathogen *C. truncatum* by these two *Bacillus* strains was detected as antibiosis in the cellophane overlay technique. They produced antifungal peptides, lipopeptides and also minute amount of organic solvent soluble molecules as antifungal compounds against *C. truncatum*. These antifungal compounds showed minimum inhibitory concentrations of 3200 ppm and 2460 ppm on *C. truncatum* and also these metabolites are thermally stable up to 120°C while they could retain the antifungal properties more than 10 weeks under cold conditions and up to eight weeks at room temperature. One of the *Bacillus* strains produces a bacteriocin like small peptide inhibitory against *C. truncatum*. Induction of latent host defense mechanisms is another antagonistic mechanism employed by this *Bacillus* sp. upon *C. truncatum* infection in chilli pepper. The tested *Bacillus* sp. could enhance the expression of defense related PAL and PO enzymes in chilli pepper plants upon *C. truncatum* infection. Also these antagonistic *Bacillus* strains promote chilli pepper plants' growth through the production of significant amounts of Indole Acetic Acid (IAA) which is a growth hormone as an indirect mechanism against *C. truncatum*. They also showed a prominent swimming and swarming ability indicating their high potentiality in colonizing plant tissues paving the way for successful green house and field applications. These two strains effectively managed the seed borne infection of *C. truncatum* resulting healthy chilli pepper seedlings with good vigor. Also they effectively managed the fruit decay caused by *C. truncatum* at color breaking stage and green stage suggesting the suitability of *Bacillus* spp. as a potential candidate to be used in the development of a biocontrol agent against chilli pepper anthracnose causal agent *C. truncatum*.

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**Human papillomavirus (HPV): Systemic treatment with Gene-Eden-VIR/Novirin safely and effectively clears virus****Hanan Polansky, Edan Itzkovitz and Adrian Javaherian**  
The Center for the Biology of Chronic Disease, USA

This paper reports the results of a clinical study that tested the effect of systemic treatment with the botanical product Gene-Eden-VIR/Novirin on the clearance rate (also called time to clearance) of the human papillomavirus (HPV). The study compared the clearance rate in treated and untreated individuals suffering from a symptomatic HPV infection. The mean time to clearance in Gene-Eden-VIR/Novirin treated individuals was 5.1 months or 151.5 days (95% CI: 4.2-5.9 months or 95% CI: 125.7-177.3 days respectively). The median time to clearance was 3.5 months. The mean time to clearance in the five untreated groups ranged from 6.9 to 20.0 months ( $P < 0.0001$  for the difference between treatment group and each untreated group). Also, 100% of the participants in the treatment group were HPV free at the end of 12 months vs., 53%, 52%, 65%, 20%, and 77% in the untreated control groups. The treated participants reported no adverse experiences. This clinical study has two major contributions. First, it showed that systemic treatment with the natural Gene-Eden-VIR/Novirin decreased the time of HPV clearance, increased the percentage of HPV free individuals and caused no adverse experiences in individuals suffering from a symptomatic HPV infection. Since, there are no other systemic treatments for symptomatic HPV infections, this study presents highly valuable information on the clinical effects of the first treatment in this category. Secondly, the study presents a new method for conducting clinical studies that addresses one of the major deficiencies associated with the practice of the randomized controlled trial method.

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**Utilization of garlic, tiger nut, amaranthus and baobab leaves as nutraceuticals to boost Nera black performance and egg quality****Ari M M, Idahor K O, Mohammed M S, Ehiedu B N, Ogah D M and Adgidzi E A**  
Nasarawa State University, Nigeria

**Introduction:** Herbs with bioactive potencies could be used as feed additives in order to achieve optimum laying capacity in avian species. Hence fresh garlic bulbs, tiger nut, amaranthus and baobab leaves were processed into powder and added to 100 kg commercial feed at a rate of 0.5, 2.0, 2.0, 2.0 kg respectively designated as D2, D3, D4 and D5 with D1 purely commercial feed as Control.

**Methodology & Theoretical Orientation:** Two hundred and fifty Nera black layers at 22 weeks old were randomly allotted to the treatments, each had 48 layers with two replicates of 24 housed individually in battery cages and were fed the diets Ad libitum. Feed intake, weight gain, feed conversion ratio (FCR), hen day production, egg weight, eggshell thickness, Haugh unit, albumen and yolk pH were monitored.

**Results:** There were significant differences ( $P < 0.05$ ) in all the parameters measured except, eggshell thickness that ranged from 6.7 mm in D2 to 7.1 mm in Control. Highest feed intake (4.37 kg) was recorded in D4, slightly followed by D5 (4.25 kg) and the least value (3.66 kg) was recorded in D3. Layers in D5 gained more weight (1.9 g) compared to 1.33 g observed in D3. Feed conversion ratio values ranged from 1.23 in Control to 1.4 in D4, hen day production (16.8% to 20.7%) and egg weight value did not differ statistically ( $P > 0.05$ ) among D2, D3, D4, D5 (47.7 to 49.9 g) but were statistically higher than 43.9 g recorded in Control. Haugh unit, albumen and yolk pH values were statistically inferior ( $P < 0.05$ ) in Control.

**Conclusions:** All the parameter values recorded were within the normal range reported in healthy layers thus, garlic bulbs, tiger nuts, amaranthus and baobab leaves maybe used as nutraceuticals to boost Nera black performance and egg quality.

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**Susceptibility profile of Salmonella Enteritidis ATCC (D) 13076 to the essential oil of thyme and technological process in pasta with eggs****Jasmina Stojiljkovic**

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Salmonella is a common contaminant of egg and can present a health hazard to consumers. Egg and egg products are an important part of the human diet. Since eggs are used for the production of egg pasta and due to an insufficient thermal treatment during pasta drying they can be a potential risk for the consumer's health. Different essential oils of herbs can be used in order to reduce potentially present pathogenic microorganisms. The aim of this paper is to describe the impact of the thyme and technological process of production of pasta with eggs on a decrease of the number of Salmonella enterica serotype Enteritidis (D) ATCC 13076. There is not a significant differences in the effects of the concentration of thyme on Salmonella Enteritidis ATCC (D) 13076 in the production of pasta ( $p>0.05$ ). There is a significant impact of the process against Salmonella Enteritidis ATCC (D) 13076 ( $p<0.05$ ).

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**Effect of ultrasound and thermal treatment on pectin methylesterase activity in papaya (Carica papaya) juice****Juan Villanueva**

National University Hermilio Valdizan, Perú

**A**mong the pectic enzymes present in fruits and vegetables, pectin methylesterase (PME) is usually related to the loss of quality and it causes adverse effects on finished products. In this research, the kinetic of ultrasound and thermal treatments are evaluated in the PME activity in papaya juice. The results showed that the ultrasound treatment caused an increase in the catalytic activity up to 52%. After a while, the catalytic activity decreased in 27% indicating that the ultrasound was not effective in the enzymatic inactivation, whereas the thermal treatment inactivated 71% of the PME. However, these results open perspectives to evaluate the effect of ultrasound and enhance the catalytic activity of enzymes of industrial interest.

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**Recombinant L-asparaginase induction process for pharmaceutical application****Larissa Pereira Brumano, Flaviana da Silva Chaves and Adalberto Pessoa Junior**  
University of Sao Paulo, Brazil

L-asparaginase (ASNase) is an important biopharmaceutical used in the treatment of lymphatic system oncological diseases, mainly acute lymphoblastic leukemia (LLA). LLA is the most common type of cancer in childhood, but it can also occur in adults, and expected to afflict over 53,000 people worldwide by 2020. The treatment with ASNase presents several side effects, mainly allergic reactions and inactivation. Aiming to overcome these problems, a recombinant *E. coli* BL21(DE3) able to overexpress protease resistant ASNase was constructed. ASNase protease resistance is promising since higher immune response occurs when the enzyme is hydrolyzed and protease activity leads to its inactivation and the half-life decrease. To improve this ASNase production, induction conditions studies were performed. *E. coli* was cultivated in defined medium with 5g/L of glucose and the induction was carried using IPTG in different stages of exponential growth phase (0.2, 0.5 and 0.8 gcell/L). Also, IPTG concentration per cell mass was evaluated (1.3, and 3.0 mMol/gcell). Enzyme activity (U/mL and U/gcell) was used as response. Results showed that the induction in advanced stage of exponential phase (0.8 gcell/L) was more advantageous, resulting in a maximum of 1.04 U/mL (527 U/gcell) and a higher cell concentration (1.97 gcell/L) in the end of the cultivation (18 h). When the induction was performed with 0.2 gcell/L (beginning of exponential phase) the glucose was not totally consumed. No statistic difference was observed among the IPTG concentrations, indicating that lower IPTG concentration could be used. The study of the induction process is necessary not only to obtain a high protein expression, but also to try to reduce production costs.

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**Epidemiology of duck as reservoir of avian influenza virus in Bangladesh****Md. Zakir Hassan**<sup>1,2</sup><sup>1</sup>National Reference Laboratory for Avian Influenza (OIE Reference Laboratory), Bangladesh<sup>2</sup>Livestock Research Institute, Savar, Dhaka, Bangladesh

This research was conducted to detect the prevalence and incidence of avian influenza virus (AIV) in duck at hoar area of Kishorganj in Bangladesh in 2013. A total of 736 serum and 150 cloacal samples were collected from asymptomatic semi scavenging duck above four months of age. The serum sample was tested for AIV specific antibody through cELISA and cloacal sample was tested to detect the incidence of AIV through RT-PCR in CDIL, Dhaka. Total of 684 is positive (prevalence) (+ve) of Avian Influenza type A antibody. The prevalence rate was 92.93%. The tested result shown that prevalence rate was at 4-6 months of age as 93.96% and 7-9 month of age as 92.92 % and in 10-12 month of age as 91.91%. A total number of 15 pooling samples from 75 cloacal samples was conducted for detection of AIV that shed in environment. After calculating the result through RT-PCR, it was shown that two pooling samples was positive (incidence) (13.33%) for AIV. Epidemiology of this hoar duck can be shown that they can transmit the AIV in the surrounding poultry population and clinical outbreaks may occur. To sum-up the result of ELISA and PCR, it was illustrated that duck act as a natural reservoir of AIV in Bangladesh.

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**Genotypic variation in gene expression under environmental perturbation: A case study of wheat under salinity****Nidal A Odat**

Al Balqa Applied University, Jordan

The expression patterns and transcript levels of four candidate genes (CAT, GAD, ASN and SOD) were analyzed in leaf of five durum wheat varieties from Jordan with under different conditions of salinity stress (50, 100, 150). Only ASN gene was found to show an up-regulation pattern of expression in response to salinity stress in all varieties while CAT, GAD and SOD showed significant down-regulation of expression under varied salt conditions. The increase in salinity levels cause a significant increase in the expression levels of both GAD and SOD genes, while the expression of CAT was decreasing with salinity. Accordingly, it seems that GAD and SOD have varied responses to different levels of salinity. The results presented here suggest that GAD and SOD are more responsive to NaCl stress compared with CAT and ASN.

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**Genetic diversity analysis of lentil (*Lens culinaris* Medikus *culinaris*) accessions having high concentration of iron (Fe) and zinc (Zn) using by simple sequence repeat (SSR) markers****Rajendra Darai<sup>1</sup>, Ashutosh Sarker<sup>2</sup>, Krishna Hari Dhakal<sup>3</sup>, Madhav Prasad Pandey<sup>3</sup>, Surya Kant Ghimire<sup>3</sup>, Shiv Kumar<sup>4</sup>, Tara B Ghimire<sup>5</sup>, Jitendra Maharjan<sup>5</sup> and Laxman Aryal<sup>1</sup>**<sup>1</sup>Nepal Agricultural Research Council, Nepal<sup>2</sup>ICARDA, India<sup>3</sup>Agriculture and Forestry University, Nepal<sup>4</sup>International Center for Agricultural Research in the Dry Areas, Morocco<sup>5</sup>Seed Science Research and Technology Division- NARI, Nepal

Nepalese lentils are comparatively rich in iron (Fe) and zinc (Zn) in South Asian countries, making lentil a potential crop of whole food solution to aid in the global battle against the micronutrient malnutrition. Knowledge of genetics underlying the uptake of grain Fe and Zn from soils is required to increase their stable concentrations in lentils. Therefore, in present study, 25 accessions of lentil were characterized using 40 simple sequence repeat (SSR) markers in order to characterize genetic variation available among genotypes having high Fe and Zn concentrations. Out of the 40 SSR markers, 23 markers were found polymorphic while 12 markers were monomorphic and 5 markers were null. These 23 polymorphic markers produced a total of 584 alleles, of which total number of polymorphic alleles were 52 and average alleles per locus was 11.49. The allele number for each SSR locus varied between two to four with an average of 2.97 alleles per marker. Markers PLC 16, SSR 124, SSR 156, SSR 113, SSR 28 and SSR 107 showed higher level of polymorphism indicating the power and higher resolution of those marker systems in detecting molecular diversity. The polymorphic information content (PIC) values for the SSRs loci ranged from 0.14 to 0.57. The pair wise genetic similarity among 25 lentil accessions varied from 0.16 to 0.83. The dendrogram constructed based on genetic similarities among 25 lentil accessions identified five major clusters. Maximum seven accessions were grouped in cluster II followed by six in cluster III while cluster IV contained lowest number of accessions i.e. three accessions indicating their higher genetic similarity. Our result showed that significant genetic variability at molecular level on the basis of SSR markers that can be used towards the development of lentil cultivars having high concentration of Fe and Zn.

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**Molecular imprint: An efficient marker towards sustainability assesment for some degrading mangroves of Indian Sundarbans****Sauren Das, Nirjhar Dasgpta and Anjan Hazra**  
Indian Statistical Institute, India

Mangroves, the distinctive plant populations of tropical and sub-tropical coastlines, have attracted considerable scientific attention during the last few decades. High salinity, periodical tidal influence, strong winds, high temperatures, high precipitation and extremely anaerobic soils are the typical physiognomies of this vegetation. They possess unique morphological and physiological adaptive features to cope with these extreme conditions. Mangrove forest provides support significantly to the coastal inhabitants both productive and protective ways. Since industrial revolution, due to elevated salinity, caused by several environmental and anthropogenic liabilities, it suffers much throughout the world. As the mangroves are assemblage of heterogeneous group of taxa, they exhibit differential magnitude of adaptability in relation to sustainability. Apart from the different morphological and physiological adaptive traits, wide genetic plasticity complies as a vital role towards sustainability. Present work describes the molecular (enzymes and genetic polymorphism) validation of four mangroves (*Bruguiera gymnorrhiza*, *Excoecaria agallocha*, *Heritiera fomes* and *Xylocarpus grnatum*) from Indian Sundarbans, of which first two are well-growing and rest suffer much from enhanced substrate salinity since last three decades. Peroxidase and Superoxide Dismutase (in different isoforms) are antioxidant enzymes subsidizing the combating forces against ROS-damaged crisis of pant cell in traumatic substrate. In the present work, it was revealed that, both the enzymes show excess isoforms in *Bruguiera* and *Excoecaria* than the other two. It also presumed that genetic diversity is allied to morphological variance and survival of the plants. DNA polymorphic experiments with molecular markers (RAPD and ISSR) also revealed that percent DNA polymorphism are higher in the first two taxa over *Heritiera* and *Xylocarpus*. Enzyme and marker assisted molecular study might be pointed out towards the differential sustainability among the studied taxa in the presently elevated saline regime of Sundarbans mangrove swamps.

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**Genetic and transcriptomic analyses of disease response to *Botrytis cinerea* in *Arabidopsis thaliana*****Synan AbuQamar**

United Arab Emirates University, UAE

Transcriptional reprogramming forms a major part of a plant's response to environmental stress. We investigated the effects of combinations of biotic and abiotic stresses on the transcriptome level of *Arabidopsis* genome using comparative microarrays. We showed a unique program of gene expression was activated in response to each biotic and abiotic stress. In addition, abiotic stress-induced genes were commonly regulated with *Botrytis cinerea* infection. The *Arabidopsis* cell wall expansin-like A2 (EXLA2) gene was identified based on its down-regulation in response to infection by the necrotrophic pathogen *B. cinerea*, and on the reduced susceptibility of its mutants to the same pathogen. The *exla2* mutants also enhanced tolerance to the phytoprostane-A1 (PPA1). Our results suggest that the absence or down-regulation of EXLA2 leads to increased resistance to *B. cinerea* in a COI1-dependent manner, and this down-regulation can be achieved by PPA1 treatment. The EXLA2 is significantly induced by salinity and cold, and exogenous application of abscisic acid (ABA). The *exla2* mutant also showed hypersensitivity towards increased salt and cold, and this hypersensitivity required a functional ABA pathway. Overall, EXLA2 appears to be important in response to environmental stress, particularly in the pathogenesis of necrotrophic pathogens and tolerance to abiotic stress. Future directions to further analyze the functions of commonly expressed genes in response to environmental stress will increase our understanding of the plant stress response.

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**A multiparametric analysis of the synergistic impact of anti-Parkinson's drugs on the fibrillation of human serum albumin****Tajalli Ilm Chandel<sup>1</sup>, Nida Zaidi<sup>1</sup>, Masihuz Zaman<sup>1</sup>, Ishrat Jahan<sup>3</sup>, Aiman Masroor<sup>1</sup>, Ibrar Ahmad Siddique<sup>2</sup>, Shahid M Nayeem<sup>3</sup>, Maroof Ali<sup>4</sup>, Vladimir N Uversky<sup>5,6,7</sup>, Rizwan Hasan Khan<sup>1</sup>**<sup>1</sup>Interdisciplinary Biotechnology Unit-Aligarh Muslim University, India<sup>2</sup>National Institute of Immunology, India<sup>3</sup>Aligarh Muslim University, India<sup>4</sup>Moradabad Institutes of Technology, India<sup>5</sup>Institute for Biological Instrumentation-RAS, Russia<sup>6</sup>King Abdulaziz University, Saudi Arabia<sup>7</sup>USF Health Byrd Alzheimer's Research Institute-University of South Florida, USA

Protein aggregation has been associated with several human neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. There are several small molecules that can reduce aggregation of proteins. The present study aimed to test the hypothesis that the application of more than one inhibitor either simultaneously or consecutively may result in more efficient inhibition of protein aggregation. To this end, the anti-amyloidogenic behaviour of benserazide hydrochloride (BH) and levodopa (LD) individually and in combination (BH + LD) was investigated using various biophysical, microscopic and computational techniques. BH, LD, and BH+LD treatments showed inhibitory effects on protein aggregation and had the ability to minimize the amyloid-induced cytotoxicity in human neuroblastoma cell line (SH-SY5Y). The two drugs in combination showed synergism (combination index, CI < 1) between them. These drugs also destabilized the preformed fibrils of human serum albumin (HSA). Our studies consistently showed that the BH+LD treatment showed highest efficacy towards inhibition and disaggregation of amyloid fibrils in comparison to treatment with BH and LD individually. Therefore, application of drugs in combination against fibrillogenesis may represent a new route for the development of means for prevention or delaying of the aggregation-related diseases.

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