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Determination of genes involved in lignification of pomegranate seeds by transcriptome sequencing

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Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruit tree species, originating in Central Asia but with a wide geographical global distribution. Besides using pomegranate as raw fruit, it has been used as herbal remedy. In consumption of pomegranate soft-hard seededness is very important. Soft seededness arises in a reduction of lignin. Lignin topochemistry has ultrastructural aspects and lignification results from the enzyme mediated polymerization. Also lignin has three different monomers (coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol) are synthesized in the cytoplasm. Aim of the present study is to determine initiation time of lignification after pollination and genes involved in lignification mechanism in soft and hard seeded pomegranates known as a hard-seeded Hicaznar and soft-seeded 33N26 varieties were used as plant materials. Fruits from the two defined varieties were taken at intervals after pollination and fertilization at different sizes. Seed samples were used for transcriptome sequencing. Primary sequencing were produced by Illumina HiSeqTM 2000, called as raw reads, was subjected to quality control (QC). After QC, raw reads were filtered into clean reads aligned to the reference sequences. De novo analysis was performed to detect genes expressed in seeds of pomegranate varieties. We performed downstream analysis including gene expression, deep analysis based on gene expression, deep analysis based on DEGs, including Gene Ontology (GO) enrichment analysis. This dataset provides valuable information regarding pomegranate transcriptome changes for mechanism of soft-hard seeded pomegranate and may help guide future identification and functional analysis of genes that are important for lignification.

Biography

Yildiz Aka Kacar has received her MSc on Plant Tissue Culture in 1994 and PhD degree on Molecular Characterization at Horticulture Department of Cukurova University in Adana, Turkey 2001. She has spent a Postdoctoral period at University of California Riverside, USA on genetic mapping in citrus from 2004-2005. She has received the title of an Associate Professor in 2006 and has a full Professor position since 2013 at Cukurova University, Turkey.

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13th Biotechnology Congress

November 28-30, 2016 San Francisco, USA

PNA analysis for authentication of four medicinal Paeonia species based on rDNA-ITS sequence

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Genus *Paeonia* is an important medicinal plant in Asian traditional medicines. Among *Paeonia* species, *P. lactiflora, P. japonica, P. veitchii,* and *P. suffruiticosa* are pharmaceutically defined in different ways in the national pharmacopoeias in Korea, Japan, and China. The roots of three *Paeonia* species, *P. lactiflora,* P. japonica and *P. veitchii,* commonly has been used as *Paeoniae* Radix, and the root bark of *P. suffruiticosa* has been used as Moutan Radicis Cortex in Korean Traditional Herbal Medicine. However, only the roots of *P. lactifloora* and root bark of *P. suffruiticosa* is pharmaceutically described as *Paeoniae* Radix and Moutan Radicis Cortex, respectively, in the pharmacopoeia of China and Japan. Since the morphological similarities of root and aerial part of these species, the identification of accurate species is very difficult. In addition, these herbal medicines bistributed as dried root slices or processed medicinal ingredients in the herbal market. Therefore, it is important to authenticate the different species used in these herbal medicines. So, we analyzed DNA barcode sequence of rDNA-ITS region using 17 samples of four *Paeonia* species and then obtained species-specific marker nucleotides that can be used as genetic markers to identify these four plants at the species levels. Based on rDNA-ITS sequences, peptide nucleic acids (PNA) analysis which is of probe-based fluorescence melting curve analysis was carried out to develop a powerful technique for detecting species-specific point mutations, namely marker nucleotide or single nucleotide polymorphism, capable to discriminate the four *Paeonia* species without sequence analysis. Also, this method can provide rapid and efficient authentication of four *Paeonia* species. Therefore, PNA analysis of four herbaceous *Paeonia* species will help to accurately authenticate each species and standardize the origin and quality of *Paeonia*e Radix and Moutan Radicis Cortex.

Biography

Wook Jin Kim is currently a Senior Research Scientist at the Korea Institute of Oriental Medicine (KIOM), South Korea. He has been working on development of DNA marker for discriminating between authentic medicinal plant species and adulterants since 2012.

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November 28-30, 2016 San Francisco, USA

Biocatalytic asymmetric phosphorylation catalyzed by recombinant glycerate-2-kinase

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D-glycerate-2-phosphate is an important substrate and crucial metabolite of central carbon metabolism, glycolysis/gluconeogenesis and pentose phosphate pathway, glycine, serine and threonine metabolism, methane metabolism, biosynthesis of plant secondary metabolites, phenylpropanoids, terpenoids and steroids, alkaloids derived from shikimate pathway, antibiotics, amino acids. Therefore an efficient, robust and scalable route for the preparation of enantiopure D-glycerate-2-phosphate is needed. A straightforward one-step biocatalytic phosphorylation of glyceric acid catalyzed by a recombinant glycerate 2-kinase heterologously expressed as maltose binding protein fusion has been investigated using racemic and the enantiopure D- and L-glycerate as substrate. The reaction was coupled with the phosphoenolpyruvate/pyruvate-kinase-system for ATP-regeneration and monitored by 31P-NMR spectroscopy. This phosphorylation reaction using recombinant glycerate 2-kinase is highly enantio-selective and sustainable, as it yields enantiomerically pure D-glycerate-2-phosphate in less reaction steps and with higher purity than chemical routes.

Biography

Birhanu Mekuaninte Kinfu has completed his BSc at the University of Gondar in Applied Biology and his MSc in Biotechnology at Addis Ababa University. He has worked as University Lecturer for 2 years. Winning the prestigious DAAD Research Grant Award (German academic exchange service) under its 'young academics and scientists' program, he is currently pursuing PhD at Microbiology and Biotechnology Department, the University of Hamburg, Germany. His main work focuses on biocatalytic phosphorylation of selected metabolites, metagenomics and *in vitro* protein expression systems.

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Development of an automated portable system for extraction of DNA from difficult samples

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DNA extraction from difficult samples (e.g. stool, soil) is problematic, as there are inhibitors that influence further processing of DNA and compounds which are mutagenic and destructive against DNA. Especially fecal DNA has diagnostic relevance because it contains DNA from various sources, like blood or intestinal mucosa. Analysis of fecal DNA might allow conclusions on the presence of intestinal diseases like tumors and inflammations at early stages in a quick and non-invasive manner. Kits for DNA extraction from difficult samples are commercially available but these are neither automated nor quick nor easy to use. Thus, an easy automated portable system for extraction of DNA from difficult samples would be beneficial. It could be used on-site, e.g., bedside in hospitals. After testing different strategies and kits, the gene MAG-RNA/DNA kit from Chemicell using magnetic beads was found to be suitable. Since this kit has been developed for other sample types, it was adapted and scaled down to a portable microchip system. For further evaluations soil was used because of easier availability and handling. Centrifugation was replaced by sedimentation. As a result complete DNA extraction could be performed inside the chip. As pumping is performed by air pressure, most of the tubing is not in contact with liquids and can be reused. The chip itself can be reused as well. Future plans include further automation of the system, testing of different sample types and evaluation of the sedimentation step. Secondly, we test pre-filled chips in order to provide a ready-to-use system.

Biography

Wojciech Hahnel has completed his BSc in Biotechnology in 2011 at Mannheim University of Applied Sciences, Germany, followed by MSc in Biotechnology with focus on bioprocess development in 2013 at the same university. Since 2014, he is pursuing PhD at Hochschule Kaiserslautern University of Applied Sciences, Germany. During his studies he has been working at the Dublin City University (DCU) in Dublin, Ireland and at the University Hospital in Bochum, Germany.

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Strategy to overcome nucleic acid degrading enzymes in insect pests for the RNAi application

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ene silencing through RNA interference as a biotechnological approach for the control of crop insect-pests have been intensively **J** applied in the last few years. dsRNA microinjection and *in vitro* feeding are the most wildly used approaches for administering RNAi in insects. However, RNAi efficiency appears to be variable among different insect groups, especially when applied by feeding, for some insect groups the oral delivery of the dsRNAs has been reported highly ineffective. In initial studies, our gene silencing data for cotton boll weevil (Anthonomus grandis) were unclear when dsRNA administration was done by feeding. The purpose of this work was to assess the possibilities of RNAi as a tool for the control of this insect pest using oral delivery of dsRNAs and to investigate the reason for the low efficiency in gene silencing, aiming to develop a strategy to deal with the efficiency and usage of dsRNA by feeding. Data showed an optimal nucleasic activity of the A. grandis gut nucleases at acid pH, ranging from 5.5 to 6.5 and the A. grandis gut homogenate significative degraded both dsRNA and dsDNA. Three nuclease sequences were found in A. grandis transcriptome, named AgNuc1, AgNuc2, and AgNuc3 in which AgNuc2 and AgNuc3 showed to be highly expressed in the insect gut. The silencing of the three nuclease genes strongly diminished dsRNA degradation when dsRNA were incubated with homogenate from silenced insects. On the other hand, when dsRNAs were protected with a Cell Penetrating Peptide (CPP) fused with a dsRNA Binding Domain (DRBD), no dsRNA degradation was found. Furthermore, dsRNAs complexed with CPP-DRBD were found to enter into A. grandis gut cells. The dsRNA complexed administered in the diet caused a greater gene silencing, compared to naked dsRNA. All data point out to the relevance for overcoming the gut nucleases with/or in parallel with the RNAi applications for the control of crop insectpests.

Biography

Rayssa Almeida Garcia is currently pursuing PhD in Molecular Biology from the Federal University of Brasilia, Brazil. She does her research work in the Plant-Pest Interaction Laboratory at Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil under the supervision of Dr Maria Fatima Grossi-de-Sa. She has published a large number of papers in reputed journals and is a Fellow Member of the World Academy of Science.

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13th Biotechnology Congress

November 28-30, 2016

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Ergothioneine fermentative production in Escherichia coli

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Ergothioneine (ERG) is sulfur-containing amino acid synthesized by certain bacteria and fungi. Recently, findings point to critical functions in human physiology. Human takes ERG from food and concentrates it in specific tissues or cells such as liver, kidney, central nervous system and red blood cells. ERG is marketed as dietary supplement or nutraceutical so that acts as anti-oxidant. It has been recognized that filamentous fungi or actinomycetes produce ERG. However, in 2010, the ERG biosynthetic gene was identified for the first time. Here, we challenged to produce ERG from glucose with our constructed cysteine producer. *E. coli* has a regulation system that synthesized cysteine from energetically-favored thiosulfate, as the assimilation of sulfate spends 2 ATP and 4 NADPH. This cysteine producer produces 16 g/L of cysteine from thiosulfate. Therefore, we established world-first ERG fermentation and challenged production of much cheaper ERG. We cloned ERG biosynthetic genes from *Mycobacterium smegmatis* and performed heterologous expression of cloning ERG genes in *E. coli*. The analysis of the culture medium by LC-MS/MS detected ERG peak. When a plasmid carrying these ERG biosynthetic genes was introduced into cysteine producer with enhanced biosynthesis, weakened degradation and improved export of L-cysteine, the transformants slightly produced ERG into medium from thiosulfate (30 mg/L of ERG). Interestingly, this transformants produced 200 mg/L of ERG from sulfate. We propose that spending of NADPH is important for production of ERG.

Biography

Miyu Nishiguchi is currently pursuing Masters in Applied Microbiology at Nara Institute of Science and Technology, Japan.

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13th Biotechnology Congress

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Development of lipase-sucrose complex to improve of transesterification activity and stability in the organic solvents

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Lipases are one of the most important enzymes as industrial catalysts. When lipases are used in the presence of organic solvents, the reaction rate is remarkably increased by solubilizing the poorly soluble substrates. Furthermore, lipases can catalyze ester synthetic reactions and transesterification reactions in the presence of organic solvents. However, enzymes including lipases are generally inactivated in the presence of organic solvents by direct contact with molecules of organic solvents. Some coordinated water molecules exist around sugars such as sucrose. Thus when lipase was complexed with sucrose and used in the presence of organic solvents, coordinated water molecules around the lipase-sucrose complex might reduce direct contact between lipase and organic solvent molecules. In this study, the transesterification activity of the lipase-sucrose complex in the presence of organic solvents was examined. The lipase-sucrose complex was prepared by freeze-dry of a lipase solution containing 1% (w/v) of sucrose (mass ratio of Lipase & Sucrose is 1:130). The lipase-sucrose complex showed the higher transesterification activity than freeze-dried lipase without sucrose in the presence of n-hexane and n-hexane containing 50% (v/v) of n-octane, 1-propanol, 1-pentanol, or 1-octanol. After incubation in 100% of n-hexane, n-octane or 1-propanol at 30 oC for 24 hours, the residual transesterification activity of lipase-sucrose complex was higher than that of the freeze-dried lipase without sucrose. Furthermore, after incubation in 100% of 1-pentanol or 1-octanol, the transesterification activity of lipase-sucrose complex was also increased. In conclusion, the transesterification activity and organic solvent-stability of the lipase were successfully improved by complexation with sucrose.

Biography

Shota Kajiwara has received his Master of Engineering degree in Osaka Prefecture University, Osaka, Japan in 2016. He is currently a Doctoral student of Osaka Prefecture University. His research interest includes the stabilization of enzyme in the presence of organic solvents.

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November 28-30, 2016 San Francisco, USA

Novel technique to develop transgenic selectable marker free pigeon pea (*Cajanus cajan*) conferring resistance against pod borer *Helicoverpa armigera*

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Pigeon pea is one of the major grain legumes of tropics and subtropics, covering vast regions of developing countries from Africa, Asia to Latin America. It ranks fifth in area among pulses after soybean, common bean, peanut and chickpea. Globally, pigeon pea is cultivated on 4.92 million hectares with an annual production of 3.65 metric tons and productivity of 898 kg/ha2. As they are grown in harsh environments and exposed to a variety of biotic and abiotic stresses, their productivity has not increased conspicuously for the last 50 years. Among many insect pests, the pod borer *Helicoverpa armigera* causes significant damage to this crop. It is the most devastating Lepidopteran pest and causes extensive economic losses to the tune of US\$ 300 million annually. The present study seeks to protect pigeon pea plants from *H. armigera* infestation by incorporating *cry1Ac* and *cry2Aa* genes, through a unique and efficient gene transfer method. An *Agrobacterium tumefaciens*-mediated transformation strategy was implemented using *in vitro* transgenic shoot-grafting technique. *A. tumefaciens* harboring different binary vectors containing *cry1Ac* and *cry2Aa* genes were used for transgenic pigeon pea development. An overall 7-9% of transformation frequency was recorded. After monitoring transgene integration by Southern hybridization, transgenic T1 and T2 lines were further analyzed by western blot, ELISA and insect bioassay. Transgenic lines obtained, exhibited optimum expression of *Cry1Ac* and *Cry2Aa* proteins. This study was further extended to the development of selectable marker (*nptII*) free *cry1Ac* expressing transgenic lines using cre-*lox* mediated marker elimination system.

Biography

Gourab Ghosh is currently pursuing his PhD in Transgenic Crop Science from St. Xavier's College, University of Calcutta, India. He has two publications in reputed, peer-review journals to his credit.

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13th Biotechnology Congress

November 28-30, 2016 San Francisco, USA

Nanoliposome particle possessing protein based CRISPR/Cas9 system for the therapeutic applications

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ene editing (CRISPR/Cas9) technology has been spotlighted as a superior therapeutic tool that is capable of treating the J fundamental causes of disease induced by genetic abnormalities, which to date have been considered as incurable diseases. Thus many researchers have focusing establishment of gene editing moiety delivering, because efficient and safe delivery in the body remains one of the major challenges of biomedical and nano-pharmaceutical research. A plasmid based CRISPR/Cas9 system has shown several critical limitations such as off-targeting, integration of DNA segment and toxicity of transfection agent. To overcome these problem, protein based CRISPR/Cas9 system was recently co-opted to the therapeutic or gene editing method. However, the protein system has still remained the stability problems, especially in *in vivo* system as like degradation by enzymatic reaction or low efficiency. Herein, we elucidate novel method for the delivery system of protein based CRISPR/Cas9 with high efficient and biocompatibility. The CRISPR/Cas9 complex was successfully encapsulated into the nanometer sized liposome (nanoliposome), which was composited with bioapplicable phospholipid chemicals through metal coordination reaction. After chemical surface modification, the nanoliposome with gene editing materials was shown long term solution stability without agglomeration and penetrated well into the cell cytosol. We suggest optimization of preparation for the nanoliposomal protein based CRISPR/Cas9 system as a platform particle to the therapeutic application. As a proof of concept, the nanoliposome with CRISPR/Cas9 system was exploited to apply for the type-2 diabetes therapy and exhibited effectiveness of glucose control without off-targeting, acute toxicity and degradation. More importantly, our particle platform system was shown higher regulation effect than a clinical used chemical drug without various adverse reactions effect including renal disorder or allergic reaction.

Biography

Jee-Yeon Ryu is currently a PhD candidate at Ajou University College of Pharmacy. She is studying synthesis of nanomaterials aimed at biological applications.

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Effect of simulated gastric juice over viability in microencapsulated Lactobacillus casei

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Probiotics must be delivered alive to exert a positive health effects in site of action (the intestine). Once reach the intestine, they should establish themselves and exert a positive health effects. They must survive the stomach acidic, bile acid and others degradative enzyme through intestinal tract. The aim of current study was to evaluate the performance of microencapsulated *Lactobacillus casei* using alginate gel matrix to facilitate a suitable carrier system to enhance this objective and confer protection from the acidity. A solution with lyophilized *L. casei* at 5 g/L (10⁸ CFU/mL) was prepared and mixed (1:1) with sodium alginate solution at 2%. As a hardening solution, calcium chloride at 0.1 M with gently stirring was used. Microencapsulator provide by BUCHI (Encapsulated B-390) was used. Simulated gastric juice (SGJ) with 9 g/L of sodium chloride and 3g/L of pepsin was prepared. Different pH was adjusted with 1 M HCl. 400 mg of microspheres containing *L. casei* were mixed in 20 mL of SGJ with pH adjusted at 2.0; 2.5; 3.0; 3.5 and 4.0 and incubated until 120 min at 37 °C and 50 rpm. Microencapsulated *Lactobacillus* were filtered and dissolved in sodium citrate to released and count CFU in supernatant. The best results were obtained at pH 4.0 (over 70% of survival at 90 minute). At pH 3.5 the survival was over 20% at 60 min, however at pH less than 3.0 there was a rapid loss of viability and the survival was null at 90 min. Then, a microencapsulated is an available technique to maintain viability of probiotics to protect the passage through intestinal tract.

Biography

Araceli Olivares is a Biochemical Engineer at the Pontifical Catholic University of Valparaiso (PUCV), Chile. She is a Researcher of the Center for Studies on Healthy Food (CREAS) since 2011. She has 3 published patents (one in Chilean and two in Unites State Patent Office) and 5 application patents in USPTO.

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November 28-30, 2016 San Francisco, USA

Molecular adaptation of a metagenome-derived mercuric reductase from Kebrit Deep brine environment in the Red Sea to high salinity

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Relevated concentration of heavy metals, no oxygen and high hydrostatic pressure. In order to highlight the structural-functional relationship of enzymes adaptation to such extreme environmental conditions, DNA isolated from the microbial community of Kebrit brine is subjected to 454-pyrosequencing and a metagenomic dataset is established and looked for enzymes involved in mercury detoxifications. An operon containing the genes essential for mercury detoxification was identified in our 454-pyrosequencing metagenomic dataset. A total of 28 *merA* orthologs were identified in Kebrit brine metagenomics library, choosing two *merA* genes: One representing the consensus sequence (K35-NH) and the other (K09-H) have amino acid substitutions replacing non-polar with acidic amino acids. Kinetic parameters were measured at the NaCl concentration that gave maximum activity for the respective enzyme. K09-H maximal activation is observed at 2 Molar NaCl and retains 65% of its activity at 3 Molar. K35-NH showed maximum activity at 0 Molar NaCl which is equivalent to 52% activity of K09-H at 4 M. Both and K35-NH retained 90% of their activity after 10 minutes incubation at 65 oC. Comparing the kinetics of both enzyme suggested that the amino acids difference between the two orthologs are acquired evolutional structural adaptation to confer site-specific level of halophilicity to survive in such extreme environment as Kebrit Deep. This halophilic feature if used properly is a potential for many industrial and bioremediation applications in detoxification of mercury.

Biography

Eman Bellah Aly Ramadan has completed her Bachelor of Medicine, Bachelor of Surgery (MBBC) in 1997 and MSc degree in Pediatrics from Ain Shams University, Faculty of Medicine in 2002. She has received MSc in Biotechnology from American University in Cairo, Egypt in 2011. She is currently a PhD candidate of American University in Cairo, Egypt.

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Functional adaptation of mercuric reductases from the deep brine environment of Atlantis II in the Red Sea to high temperature

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The lower convective layer (LCL) of the Atlantis II brine pool of the Red Sea is a unique environment characterized by high salinity of 4.4 Molar, temperature of 68 °C and very high concentrations of heavy metals. Mercuric reductase enzymes functional in such extreme conditions could be used in the environmental detoxification of mercurial poisoning. This would be of use in the Egyptian Gold mines, where mercury used in the extraction process imposes a serious ecological hazard. We constructed an environmental Atlantis II mercuric reductase library, where we have identified two classes of mercuric reductases. One is the halophilic/thermostable *merA*. It is designated ATII-LCL-*H*. It retains 50% of its activity after 10 minutes incubation at 75 °C. The other is a non-halophilic/ thermostable *merA* designated as ATII-LCL-*NH*. It retains 61% of its activity at 65 oC. The ATII-LCL-*H merA* has two characteristic signature boxes and a short motif composed of 4 aspartic acids ($4D_{414-417}$). In order to understand how two enzymes from the same environment have evolved to withstand heat, we mutated the isoform ATII-LCL-*NH*. Substitution of 2D at positions 415/416 enhanced the thermal stability while other mutants did the opposite effect. The 2D mutant retains 88.6% of its activity at 65 °C. Three-dimensional structure prediction revealed newly formed salt-bridges and H-bonds in the 2D mutant as compared to the parent molecule.

Biography

Mohamad Maged Galal was graduated from the Faculty of Pharmacy, Cairo University in 2007. He has completed his MSc in Biotechnology in 2011 and currently pursuing PhD from the American University in Cairo, Egypt.

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Evaluation of nutrient removal (NO₃-N, NH₃-N and PO₄-P) with *Chlorella vulgaris*, *Pseudomonas putida*, *Bacillus cereus* and the consortium of these microorganisms in the secondary treatment of municipal wastewater effluents

Abril Ivett Priscilla Gomez-Guzman, Sergio Jimenez-Magana, Cesar Gomez-Hermosillo, Victor Perez-Luna, F Javier Parra-Rodriguez, A Suggey Guerra-Renteria, Blanca Rosa Aguilar-Uscanga, Josue Solis-Pacheco and Orfil Gonzalez-Reynoso University of Guadalajara, Mexico

Microalgae and some bacteria offer a promising technology for the removal of nutrients such as Nitrogen and Phosphorus in municipal wastewater. In this investigation the microalgae *Chlorella vulgaris*, the bacteria *Pseudomonas putida*, *Bacillus cereus* and the consortium microalga/bacteria of these microorganism were used as model in the removal of nutrients using a model wastewater. The results of removal of nutrients with *Chlorella vulgaris* was 24%, 80.6% and 4.30% for NO₃-N, NH₃-N and PO₄-P, respectively. For *Bacillus cereus* the removal of nutrients was 8.4%, 28.8% and 3.8% of NO3-N, NH3-N and PO4-P. With *Pseudomonas putida* was 5%, 41.8% and 4.3% of NO₃-N, NH₃-N and PO₄-P (in 168 hours respectively). The consortium of *Chlorella vulgaris*, *Bacillus cereus*, *Pseudomonas putida* were able to remove the 29.4%, 4.2% and 0% of NO₃-N, NH₃-N and PO₄-P in 240 hours. The highest biomass production was found with *Bacillus cereus* 450 mg/L followed by *Pseudomonas putida* 444 mg/L, the consortium 205 mg/L and *Chlorella vulgaris* 88.9 mg/L. This study showed that *Chlorella vulgaris* consume first NH3-N as a source of Nitrogen and when it is exhausted then consume NO₃-N, it happen when both compounds are available in the medium. In the removal of phosphate (PO₄-P) the microorganisms *Chlorella vulgaris* and *Bacillus cereus* were able to remove a higher amount because they can assimilate this nutrient even in stress conditions (like changes in pH) such as happening in the municipal wastewater.

Biography

Abril Ivett Priscilla Gomez-Guzman is currently a graduate student in the University of Guadalajara, Jalisco, Mexico. She is pursuing her PhD studies in the program of Science of Biotechnological Processes. She has completed her Masters studies from the Autonomous University of Baja California and her Bachelor's degree studies from the Technological Institute of Tepic in Biochemical Engineering. She was a Professor of the Autonomous University of Guadalajara from 2011-2013. She has published one paper in reputed journals.

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Development of herbicide resistant potato lines: A step towards transgenics in Turkey

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The significant breakthrough in plant biotechnology is the development of techniques to transform genes from unrelated sources into commercially important crop plants. Modern technologies of genetic transformation have enabled researchers to introduce any trait of economic importance in crops. The herbicide resistant crops were cultivated on an area of approximately 100 million hectares in 2015; mainly expressing EPSPS gene that encode resistance to non selective glyphosate. In order to introduce herbicide resistant trait in four potato cultivars (Lady Olympia, Agria, Desiree and Marabel), we first optimized an efficient, cost effective, reproducible, genotype independent and stable *Agrobacterium* mediated genetic transformation protocol in potato using leaf and internodal explants. *Agrobacterium* strain LBA4404 harboring recombinant binary vector pBin19 containing beta-glucuronidase *uidA* gene under the control of 35S promoter was used for this purpose. Besides that, the optimal concentration of glyphosate was determined using leaf and internodal explants of cultivars in *in vitro* experiments. After developing an efficient transformation protocol, we infected explants cultivars with *Agrobacterium* strains LBA4404 harboring pCAMHE-EPSPS plasmid under the control of 35S promoter. Glyphosate was used at a concentration of 1.5 mM for the selection of primary transformants. The primary transformants were further analyzed for the gene integration and expression analysis. The results revealed the proper integration and expression of introduced gene in putative transgenic plants obtained as a result of different transformation events. The transgenic potato lines are being screened against glyphosate applications in green house conditions. These transgenic lines are expected to an excellent source of germplasm for an efficient potato breeding program.

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Effect of the polycyclic aromatic hydrocarbons exposure on sperm DNA in idiopathic male infertility

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There is an increasing awareness of the potential role of genetic and environmental factors in idiopathic male infertility. However, there is little compelling evidence to date to suggest that the risk of idiopathic male infertility among the general population is influenced by exposure to certain chemicals. Thus the first objective of the present study is to assess the occurrence and distribution of PAHs in mussels of Alex Coast, to identify the origin of PAHs in the Alex Coast, Secondary, to investigate the possible association between exposure to PAHs and male idiopathic infertility through; estimation of urinary metabolites of PAHs, malonaldehyde (MDA), GSH, GST, testosterone, FSH, prolactin, Semen analysis and sperm chromatin dispersion test (Halo sperm). The present results of the study revealed that there were high concentration of many PAHs detected in the tissues of two species of mussels collected from Alex Coast which may supposed to be at big risk for human health. Also, the present results revealed that there was a high level of urinary 1-hydroxy pyrene, 1-hydroxy naphthalene, 2-hydroxy naphthalene in the urine of detected infertile group. In the current study, a high significant increase in the level of MDA in the sera of detected idiopathic infertile group was observed with a significant decrease in glutathione content. Where, the compounds resulting from the oxidation of PAHs have the ability to enter redox cycles, which increased the formation of reactive oxygen species (ROS) and thus caused sperm DNA damage. The data provide strong evidence that semen samples containing a statistical threshold of 30% sperm DNA fragmentation have a reduced level of pregnancy success. The results of the present study elucidated that there were DNA fragmentation from 32-40% in the sperm of some idiopathic infertility subjects.

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