International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

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Optimization and Transformation of Garden Balsam, *Impatiens balsamina*, Mediated by Microprojectile Bombardment

A.M. Taha, A. Wagiran, H. Ghazali, F. Huyop and G.K.A. Parveez

In this study, a transformation system was developed by initially optimizing the microprojectile bombardment parameters for cotyledonous explants. The parameters optimized were combination of distance from stopping screen to target tissue and helium pressure, number of bombardment, preculture duration prior to bombardment, DNA amount, osmoticum treatment and post-bombardment incubation time. Determination of minimal inhibitory concentration for efficient selection of transformants was also carried out for hygromycin. Different concentrations of hygromycin (25, 50, 75 and 100 mg L^{-1}) were tested against different ages of explants (0, 1, 3 and 5 weeks) for efficient selection of transformants. Using the optimized parameters, transformation of cotyledons was carried out followed by selection on 75 mg L^{-1} hygromycin at different weeks of post-bombardment. Transgenic plants were successfully regenerated from bombarded explants and confirmed via histochemical GUS assay and PCR analysis. *(Biotechnology 8 (1): 1-12, 2009;* ***doi:*** *10.3923/biotech.2009.1.12)*

Candidate Molecules Identified by Proteomic Study of Synovial Cells in Experimental Post-Traumatic Arthritis of Knee in Swine

O. Baatartsogt, K. Choi, P.K. Mandal, H.K. Lim, J.D. Oh and S.H. Chang

This research was done to study the protein expression profile of synovial cells of knee using proteome analysis during the development of experimental Post-Traumatic Arthritis (PTA) in swine. PTA was induced by transection of the Anterior Cruciate Ligament (ACL) of left knee in three piglets. Articular cartilage and synovial tissues were obtained after 0, 2, 5 and 8 weeks for histopathologic examination. After sacrificing the piglets at 8 weeks, synovial tissues were collected for 2-dimensional electrophoresis and mass spectrometric analysis. Histopathologic examination at 8 weeks showed overt chronic inflammation indicating the development of ETA. Through proteome analyses more than 1,500 protein spots were identified in which 7 differentially expressed protein spots were observed in ACL-transacted synovial tissue. Five proteins were down-regulated (cytoskeletal β actin, cofilin-1, destrin, Rho GDP dissociation inhibitor α and an unnamed protein product) and two proteins were up-regulated (α-B crystalline and Smooth Muscle Protein (SMP) 22α). These results showed

that proteins that are related to cellular organization and signal transduction are down-regulated and those that are related to cell rescue, defense and stress are up-regulated. Therefore, the proteome analysis of synovial tissue provided us new candidate molecules which may be useful to understand the pathogenesis for diagnostic and therapeutic studies of Post Traumatic Arthritis (PTA). *(Biotechnology 8 (1): 13-23, 2009; **doi:** 10.3923/biotech.2009.13.23)*

Genetic Diversity of Pistachio Tree using Inter-Simple Sequence Repeat Markers ISSR Supported by Morphological and Chemical Markers

K. Farès, F. Guasmi, L. Touil, T. Triki and A. Ferchichi

The identification and the characterization of some Pistachio cultivars trees in South of Tunisia revealed a remarkable diversity between different cultivars, thanks to molecular markers (ISSR) supported by morphological markers (length sheets, leaf area, length of fruit, forms of final leaflet, their length and by chemical markers (content of vitamins). For the whole of the studied cultivars, the length of fruits is between 16.3 and 20.4 cm, the length of the final leaflet is 5.88 to 8.42 cm its form oscillates from elliptic to round, the average leaf area varies between 17.13 to 34.05 cm^2 in all varieties. The analysis of chemical variability allows to distinguish in different cultivars studied a variability on the level of the composition of vitamin B1, B2, B6 and VC. The content of vitamin B1 is going from 0 to 1 mg g^{-1} in the varieties Kermizi, Meknessy 1, Mumtez, Mateur 2 and Mateur 3 and 0.019 mg g^{-1} in Lybie rouge then the range of B2 is going from 0.001 mg g^{-1} at the variety Mateur 3, with 0.07 mg g^{-1} in Lybie rouge, the content of vitamin B6 is very variable from 0. 0016 mg g^{-1} at Mateur 3, with 0.01643 mg g^{-1} in Red Aleppo and the VC is going from 0.013 mg g^{-1} in Meknessy 1 to 0.09 mg g^{-1} in Lybie blanc. The PCR amplification of Pistachio varieties revealed a high percentage of the polymorphic fragments (26 fragments are polymorphs), the varieties Mumtez, El Guettar and Mateur 1 are characterized by a high percentage of the polymorphic locus (38.46%) whereas the varieties Lybie blanc, Kermizi, Lovy, Meknessy 1, Lybie rouge, Brise vent, Mateur 2, Mateur 3, Mateur 4, Meknessy 2, Kerman, Red Aleppo have an average polymorphism (between 26.92 and 34.61%). The variety Meknessy I (rate = 3.84%) and Lybie blanc (rate = 15.38%) have a low polymorphism. Several varieties have a coefficient of similarity (ds) close to 1 they are considered genetically very similar. Among these varieties Brise vent and Mateur 3 whose a coefficient of similarity equal to 0.667 (dg = 0.333), Lovy and Mumtez whose coefficient is equal to 0.632 (dg = 0.368), Lybie rouge and El Guettar whose coefficient is equal to 0.632 (dg = 0.368), Kermezi and Kerman whose coefficient of similarity is equal

to 0.750 (dg = 0.250), Meknessy 2 and Red Aleppo whose coefficient of similarity is about 0.857 (dg = 0.143). Certain varieties are considered genetically very distant between them, the genetic distance which separate them is large (dg = 1) it's the case of the variety Mateur 4 with Meknessy1 (dg = 1) and Meknessy 1 with Mumtez and Mateur 3. Other varieties can be considered distant like Lybie Blanc with Brise vent and Mateur 3 (dg = 0.846), Meknessy 1 and El Guettar (dg = 0.82). The use of ISSR markers for amplification PCR of different Pistachio cultivars revealed a high percentage of the polymorphic fragments (26 bands). *(Biotechnology 8 (1): 24-34, 2009;* ***doi:*** *10.3923/biotech.2009.24.34)*

Molecular Characterization and Batch Fermentation of *Bacillus subtilis* as Biocontrol Agent, II

S.M. Matar, S.A. El-Kazzaz, E.E. Wagih, A.I. El-Diwany, M.A. El-Saadani, E.E. Hafez, H.E. Moustafa, H.E. Abd-Elsalam, G.A. Abo-Zaid and E.A. Serour

Fourteen *Bacillus subtilis* isolates (B1 to B14) obtained from different Egyptian sites had different antagonistic and inhibitory effect against six fungal isolates belonging to four different genera, *Rhizoctonia solani*, *Helminthosporium* sp., *Alternaria* sp. and *Fusarium oxysporum*. Random Amplified Polymorphic DNA (RAPD), differential display technique and partial sequencing of 16S rRNA gene were used for characterization of isolates. While RAPD technology, using 4 different random 20-mer primers, revealed different levels of molecular variation among twelve out of the fourteen isolates tested, the remaining two isolates B3 and B5, demonstrated identical profiles. Because of the high degree of homology (93%) between isolates B4 and B7 and the no variation observed between isolates B3 and B5, the differential display technique was used to test the reliability of these results. Isolate B7 was selected for batch cultivation in bioreactor, the biomass achieved was 3.2 g L^{-1}. Inhibitory activity of supernatant was increased near the end of the stationary phase. The activity reached its highest value of 1.7 cm in death phase. *(Biotechnology 8 (1): 35-43, 2009;* ***doi:*** *10.3923/biotech.2009.35.43)*

In vitro Regeneration of Garden Balsam, *Impatiens balsamina* Using Cotyledons Derived from Seedlings

A. Taha, A. Wagiran, H. Ghazali, F. Huyop and G.K.A. Parveez

In this study, an effort to develop a reliable tissue culture system for *Impatiens* species was carried out for eventually to genetically engineer this plant for useful

traits. Parts of cotyledons and hypocotyl derived from different age of seedlings, from surface sterilized seeds, were subjected to shoot induction on MS media containing BAP, TDZ or combination of BAP and NAA in different ratios. Roots were developed from the above shoots on full-or half-strength MS media containing IAA, IBA or NAA of different concentrations. Finally fully-developed plantlets were produced on hormone free MS media or full- or half-strength MS media containing specific hormones. It was observed that the most efficient shoot induction (93%) was obtained from proximal part of cotyledon derived from 7 days old seedlings on MS media supplemented with 1 mg L^{-1} BAP. Roots were most efficiently produced (92%), in term of percentage and morphology, on half-strength MS media supplemented with 0.1 mg L^{-1} IAA. Further regeneration of plantlets, approximately 8-10 cm in height, was achieved after 3 weeks on hormone-free MS media. In conclusion, full regeneration system of *Impatiens balsamina* was developed, using proximal part of cotyledon derived from 7 days old seedlings on MS media supplemented with 1 mg L^{-1} BAP for shoot regeneration and 0.1 mg L^{-1} IAA for roots regeneration. Full regeneration system has been achieved within 8 weeks in culture. *(Biotechnology 8 (1): 44-52, 2009;* ***doi:*** *10.3923/biotech.2009.44.52)*

Antagonistic and Inhibitory Effect of *Bacillus subtilis* Against Certain Plant Pathogenic Fungi, I

S.M. Matar, S.A. El-Kazzaz, E.E. Wagih, A.I. El-Diwany, H.E. Moustafa, G.A. Abo-Zaid, H.E. Abd-Elsalam and E.E. Hafez

The antagonistic and inhibitory activity of fourteen *Bacillus subtilis* isolates (B1 to B14) obtained from different Egyptian sites, were tested against six fungal isolates belonging to four different genera, *Rhizoctonia solani*, *Helminthosporium* spp., *Alternaria* spp. and *Fusarium oxysporum*. Cultural, morphological and physiological characteristics of these isolates were found to be identical to *Bacillus subtilis*. When the fourteen *B. subtilis* isolates were tested as biological control agents for their antagonistic effect on the *in vitro* growth of the fungal isolates, four *B. subtilis* isolates B1, B4, B7, B8 had more antagonistic effect on all fungal isolates. Supernatant of *B. subtilis* isolate B7 had antagonistic effect on 6 fungal isolates but it was more effective on *Helminthosporium* sp., *Alternaria* spp. and *F. oxysporum*. *B. subtilis* as well as, isolate B7 showed effectiveness in reducing disease incidence and severity levels of tomato plants when added to the *F. oxysporum* and *R. solani*-infested soil. Also, it stimulated the growth of tomato plants compared to the other. HPLC analysis of the HCl precipitate of *B. subtilis* isolate B7 culture supernatant revealed that an identical

pattern of five peaks to that of a purified preparation of iturin A was obtained. *(Biotechnology 8 (1): 53-61, 2009; **doi:** 10.3923/biotech.2009.53.61)*

Screening of Variables Influencing the Production of HPV E7 Oncoproteins by Recombinant *Escherichia coli*

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E7 proteins are major oncoproteins of Human Papilloma Viruses (HPVs) which play a key role in virus-associated cervical carcinogenesis. Aiming at future optimization of the production of these proteins by a recombinant strain of *Escherichia coli*, this preliminary study has been addressed to the influence of the main process variables on their expression in three culture media, specifically LB, TB and LBG media. A 2^{5-2} experimental design utilizing multiple factorial levels was employed to identify single factors and interactions exerting significant impacts on protein expression. Temperature, agitation intensity, IPTG and kanamycin concentrations and inoculum size were selected as the five independent variables, while the specific growth rate of the microorganism and the concentration of insoluble proteins as well as those of soluble proteins in two successive extracts were selected as the four responses. The highest specific growth rate (1.06 h^{-1}) was obtained during run B7 performed in the TB medium using 40°C, 170 rpm, 0.25 mM IPTG, 40 μg mL^{-1} kanamycin and OD_{600} = 0.6, while the maximum total protein production was achieved during run C9 (5.60 mg mL^{-1}) performed in the LBG medium at 37°C, 140 rpm, 0.5 mM IPTG, 30 μg mL^{-1} kanamycin and OD_{600} = 0.5. The statistical approach used in this study revealed to be a powerful tool for the optimization of protein expression. *(Biotechnology 8 (1): 62-69, 2009; **doi:** 10.3923/biotech.2009.62.69)*

Gene Expression Profile of Synovial Cells in Experimental Post-Traumatic Arthritis of Knee in Swine

O. Baatartsogt, K. Choi, P.K. Mandal, Hee-kyong Lim, Guan-Hao Li, Hong-Sik Kong, Dae-Hyun Hahm, Chi-Ho Lee and Jun-Heon Lee

To get insights into pathological pathways of Post Traumatic Arthritis (PTA) this study was done on gene expression profile of synovial cells of knee using gene chip analysis developing experimental post traumatic arthritis (ETA) in swine. ETA was induced by transection of the Anterior Cruciate Ligament (ACL) of left knee in 3 piglets. Articular cartilage and synovial tissues were obtained after 0, 2, 5 and

8 weeks for histopathologic examination. Synovial cells collected after sacrificing the piglet at 8 weeks, were used at 5 passage for gene expression profiling using Affimetrix Gene Chip. Histopathologic examination showed overt chronic inflammation indicating the development of ETA. Through genome analyses it was observed that 87 known genes were up-regulated and 76 known genes were down regulated. By analyzing, it was found that many genes with differential expression are related to inflammation, immune response, lipid binding, cell adhesion, growth activity and muscle development. The present study provided an insight into the TA related gene expression pattern. The genome analysis of synovial cells provided us new candidate molecules which may be useful to understand the pathogenesis of Post Traumatic Arthritis (PTA). The established porcine model may serve as *in vivo* disease model for further research on traumatic arthritis to elucidate molecular pathogenesis. *(Biotechnology 8 (1): 70-77, 2009; **doi:** 10.3923/biotech.2009.70.77)*

Bacterial Community Structure Change Induced by Gamma Irradiation in Hydrocarbon Contaminated and Uncontaminated Soils Revealed by PCR-Denaturing Gradient Gel Electrophoresis

Wael S. El-Sayed and S. Ghanem

The effect of gamma irradiation on bacterial community structure in garden clay, uncultivated clay and hydrocarbon contaminated soils were investigated by exposing soils to various doses of ionizing radiation using a Co-60 source. Bacterial community structure in irradiated soils was examined 30 days after irradiation at 1, 2, 3, 4, 5 and 10 kGy doses. Gamma irradiation was found to have a selective impact on the microbial community structure in certain soils. Sensitivity to irradiation varies among microbial species and is affected by the properties of the soil. Denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA gene fragments amplified from the three soils were performed. Bacterial population in garden clay soil was stimulated by low irradiation doses and sharply decreased at higher doses. Phylogenetic analysis revealed predominance of only cloroflexi bacterium at elevated doses of irradiation in garden clay soil. In uncultivated clay soil, much of the bacterial populations were affected by irradiation treatment, however, two representatives of gammaproteobacteria, *Enterobacter* sp. and *Pseudomonas* sp. showed some resistance to irradiation and were present at all irradiation doses. DGGE profile suggested that bacteria in hydrocarbon contaminated soil were more resistant to irradiation than garden and/or clay soils. The bacterial diversity in polluted soil

remained intact throughout all radiation treatments. Phylogenetic analysis showed that members belonging to three taxonomic groups, alphaproteobacteria, gammaproteobacteria and actinobacteria were present in hydrocarbon polluted soil that were not affected by all radiation treatments. Gamma irradiation had a greater effect on bacterial community in both garden and uncultivated clay soils. *(Biotechnology 8 (1): 78-85, 2009;* ***doi:*** *10.3923/biotech.2009.78.85)*

Antagonistic Effects of *Streptomyces* sp. SRM1 on *Colletotrichum musae*

T. Taechowisan, N. Chuaychot, S. Chanaphat, A. Wanbanjob and P. Tantiwachwutikul

In an attempt to understand the mode of action of this antagonist in nature, the interaction between *Streptomyces* sp. SRM1 and *C. musae* was studied by dual culture on agar plates. Evidence for the antibiosis of *Streptomyces* sp. SRM1 was demonstrated by inhibition zones in *in vitro* plate assay. The crude extract from the culture of *Streptomyces* sp. SRM1 also produced antifungal activity, which showed antagonistic effects against *C. musae* such as swelling, distortion and excessive branching of hyphae and inhibition of spore germination. An indirect method was used to show antagonistic effect of *Streptomyces* sp. SRM1 against *C. musae* in soil. This study suggests the potential of developing *Streptomyces* sp. SRM1 for the biological control of anthracnose disease of banana caused by *C. musae. (Biotechnology 8 (1): 86-92, 2009;* ***doi:*** *10.3923/biotech.2009.86.92)*

Optimization of Cultural Conditions for Production of Chitinase by a Soil Isolate of *Massilia timonae*

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The aim of this study was to characterize chitinase-producing bacteria isolated from environmental samples and to investigate conditions affecting chitinase production by these bacteria. Ninety-eight isolates recovered from 20 soil samples were screened for chitinase production. Eighteen isolates showed chitinolytic activity, among which isolate U2 was selected for further study based on dinitrosalisylic acid assay results. The isolate U2 was identified as *Massilia timonae* through phenotypic characterization and 16S rDNA sequencing and the

optimal conditions for chitinase production were determined to be 25-30°C, initial pH 6.0-6.5 and chitin concentration of 1% (w/v). The maximum chitinolytic activity was achieved after 36 h of incubation. The addition of different nitrogen sources to the production medium had no significant effect on chitinase production. Among various carbon sources tested, N-acetylglucosamine (GlcNAc), fructose, lactose, maltose and glucose showed modest inhibitory effect while arabinose did not affect enzyme production by *M. timonae* isolate U2. The addition of Triton X100 increased chitinase production by 12.4%. The enzyme was reasonably stable in the pH range 5-7 and at temperatures up to 50°C. These results indicate that *M. timonae* is capable of producing chitinase in relatively simple media containing colloidal chitin as the sole carbon and nitrogen source. *(Biotechnology 8 (1): 93-99, 2009; **doi:** 10.3923/biotech.2009.93.99)*

Molecular Characterization of Some Novel Marine *Alicyclobacillus* Strains, Capable of Removing Lead from a Heavy Metal Contaminated Sea Spot

Eman A.H. Mohamed and Nermeen A. ElSersy

Sea water from heavy metal contaminated area in the Mediterranean, was analyzed for its heavy metal contents and their concentrations. It was observed that lead has the highest concentration (0.48 ppm) among the remaining heavy metal concentrations. Four different Gram-positive, rod-shaped and spore forming *Alicyclobacillus* (formally *Bacillus*) isolates were isolated from the same sea spot. Phenotypic characterization of pure cultures were examined for motility, Gram reaction, spore morphology, catalase and oxidase production. Scanning electron micrograph showed that cells of both strains were occurring singly or in short chains. Randomly Amplified Polymorphic DNA (RAPD) analysis showed a great deal of differentiation among the isolates, revealing that each of them has its own DNA fingerprint. A dendrogram showing the genetic similarity among the sea isolates, clustered them into two main groups at 30% of genetic similarity. Partial sequencing of the 16S rDNA of 2, representative isolates revealed that both of them are novel *Alicyclobacillus* strains S2 and S4. The isolates had the ability to remove lead from contaminated solutions. A promising strain, S4, showed a valuable uptake levels, 64 and 65.3% at 0.5 and 0.9 ppm of pb^{2+}, respectively, after only 2 h of exposure to lead. This strain can be later used efficiently for the bioremediation of lead in contaminated water bodies. *(Biotechnology 8 (1): 100-106, 2009; **doi:** 10.3923/biotech.2009.100.106)*

Influence of Nutrients Utilization and Cultivation Conditions on the Production of Lactic Acid by Homolactic Fermenters

S.T. Ogunbanwo and B.M. Okanlawon

Homofermentative lactic acid bacteria isolated from retted cassava ware screened for the production of lactic acid, pH survival and influence of nutrients utilization and cultivation conditions on the production of lactic acid by fermentation. All the *Lactobacillus* species isolated produced little quantity of lactic acid when grown at 30°C in normal De Man Rogosa Sharpe (MRS) broth. However, a temperature of 40°C at initial pH of 5.5 in constituted MRS medium with 6% (w/v) carbon concentration of D-glucose and 4% (w/v) nitrogen concentration of yeast extract fermented for 48 h supported lactic acid production optimally with *Lactobacillus acidophilus* producing 18.4±0.01 g L^{-1} of lactic acid. *Lactobacillus casei* had the highest percentage cell destruction (53.93%) in phosphate buffered saline pH 3.0 while *L. acidophilus* had the least (18.87%). Lactic acid produced by all the *Lactobacillus* species inhibited at least two or more food spoilage and/or pathogenic microorganisms and can be used in the food industry for decontamination of meat and poultry carcasses. *(Biotechnology 8 (1): 107-113, 2009;* ***doi:*** *10.3923/biotech.2009.107.113)*

Influence of the Molasses and Office Paper as Carbonic Amendments in Municipal Compost Production

Ali Mohammadi Torkashvand

The aim of this study was to investigate the effect of cane molasses and office paper (carbohydrate and cellulose sources, respectively) on total nitrogen and C/N ratio of municipal wastes compost. Each of treatments with 20 kg fresh organic wastes (decomposable municipal) in three replicates as a completely randomized design was done. Treatments including different amounts of molasses and office paper with municipal decomposable wastes that were added to organic wastes in 2 and 4 weeks after composting start (first and second stages). Treatments in free space weekly twice to turn upside down for aeration, while exercising some treatments and adding water for adjusting moisture of organic wastes were also done. After 50 days, a 100 g sample of every treatment was taken to measure total nitrogen, organic carbon, C/N ratio, EC and pH in 1:6 dry organic matter/water. Results indicated that the molasses is a suitable amendment for reclamation of compost quality properties. It held nitrogen in compost caused to reduce C/N ratio. The best time for the use of molasses was 4 weeks after

composting process (first stage) that is accompanied with increase in microorganisms' activity and temperature. In this stage, using 8% molasses had a more effect in increasing total nitrogen; on the contrary, the most effect of 4% office paper was at the first stage. Application of 4% paper at the second stage because of the increase in C/N ration than control amounted 2.64 times is not proposed. *(Biotechnology 8 (1): 114-119, 2009; **doi:** 10.3923/biotech.2009.114.119)*

Medium Optimisation of Chitinase Enzyme Production from Shrimp Waste Using *Bacillus licheniformis* TH-1 by Response Surface Methods

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The optimization of fermentation medium for the production of chitinase by *Bacillus licheniformis* TH-1 was carried out using Response Surface Methodology (RSM) based on the two level factorial design. This procedure limited the number of actual experiments performed while allowing for possible interactions between 5 components. RSM was adopted to derive a statistical model for the effect of chitin, Yeast Extract (YE), peptone, $NaNO_3$ and K_2HPO_4 on chitinase production. The p-value of the coefficient for linear effects of chitin, peptone and YE was 0.0001, suggesting that this was the principal experiment variable, having the greatest effect on the production of chitinase. The optimal combinations of media constituent for maximum chitinase production are determined as 10 g L^{-1} chitin, 0.5 g L^{-1} YE, 0.5 g L^{-1} peptone, 2.55 g L^{-1} $NaNO_3$ and 1.55 g L^{-1} K_2HPO_4. The optimization of the fermentation medium resulted not only in a 5.4 fold increase of enzyme activity compared to unoptimized medium but also a reduced amount of the required medium constituents. The response surface analysis provided a useful tool for the optimization of a low cost enzyme producing medium for potential use on an industrial scale. *(Biotechnology 8 (1): 120-125, 2009; **doi:** 10.3923/biotech.2009.120.125)*

Molecular Phylogeny of Qatari Date Palm Genotypes Using Simple Sequence Repeats Markers

Talaat A. Ahmed and Asmaa Y. Al-Qaradawi

The objectives of the present study is to analyze the genetic diversity among 15 different cultivars of date palm at the experimental farm of Qatar University

using Simple Sequence Repeat (SSR) markers and find out the genetic similarity and/or diversity among the well known Qatari date palm cultivars. DNAs were extracted from the young fresh leaves. Among 16 primer pairs tested for their ability to generate expected SSR banding patterns in Qatari date-palm genotypes, 10 primers successfully produced clear single bands in most of the studied genotypes. So, far, six SSR primers did not amplify clear bands in our genetic materials even using different PCR conditions. The amplified SSR band sizes ranged from 100-300 bp. A total of 40 alleles with an average of 4 alleles per locus were scored. Similarity coefficient matrix was computed to cluster the data and to draw precise relationships among the fifteen studied Qatari date palm genotypes. *(Biotechnology 8 (1): 126-131, 2009;* ***doi:*** *10.3923/biotech.2009.126.131)*

In vitro and *in vivo* Screening of Potato Cultivars Against Water Stress by Polyethylene Glycol and Potassium Humate

Davoud Hassanpanah

Plantlets produced from meristem culture of four cultivars (Agria, Savalan, Satina and Caesar) propagated by single node cuttings, in May of 2008. Experimental design was factorial on the basis of completely randomized design with two factors in three replication. Factor A was plantlets of four cultivars and factor B was four treatment (One concentrations of polyethylene glycol as -1.5 bar $1L^{-1}$ MS medium, second concentrations of potassium humate (1 mL 1L MS medium) and third concentrations of PEG (-1.5 bar) with potassium humate (1 mL 1L MS medium) and other without them as control. Chlorophyll fluorescence leaves measured by chlorophyll flurometer (OS-30p) after 30 days planting. Nitrate reductase activity (NRA) measured 30 and 40 days after planting in all of organs (leaf, stem and root). Five plantlets from each cultivar cultured in a greenhouse. The highest rate of Fv/m to Caesar under normal and normal with potassium humate and Savalan stress conditions. Consequently, cultivars were ranked in order of deficit tolerance based on reductions in Fv/Fm values. Based on Fv/Fm ranking, Agria, Caesar and Savalan cultivars were identified as water deficit tolerance. Satina and Caesar cultivars had the highest rate of NRA and Savalan and Agria the lowest in all of organs. The maximum minituber average weight, number and weight per plant for was under normal with potassium humate condition. The high value of number minituber per plant were found in Savalan and Caesar under normal, normal with potassium humate and stress with potassium humate and Caesar under stress conditions. *(Biotechnology 8 (1): 132-137, 2009;* ***doi:*** *10.3923/biotech.2009.132.137)*

Bioprocessing and Scaling-up Cultivation of *Bacillus subtilis* as a Potential Antagonist to Certain Plant Pathogenic Fungi, III

S.M. Matar, S.A. El-Kazzaz, E.E. Wagih, A.I. El-Diwany, H.E. Moustafa, M.A. El-Saadani, G.A. Abo-Zaid and E.E. Hafez

The antagonistic and inhibitory activity of *Bacillus subtilis* isolate G-GANA7 (GenBank accession No. EF583053) obtained from Abo-Homos in Egypt, was tested against six fungal isolates belonging to four different genera, *Rhizoctonia solani*, *Helminthosporium* sp., *Alternaria* sp. and *Fusarium oxysporum*. The processing of *B. subtilis* isolate G-GANA7 was cultured in 3 L bench-top New Brunswick Scientific BioFlow III bioreactor for producing the maximum yield of biomass and antifungal compound. Fed-batch processes were automated through a computer aided data bioprocessing system AFS-BioCommand multi-process management program to regulate the cell growth rate by controlling interactively the nutrient feed rate, temperature, pH and agitation speed based on dissolved oxygen. In batch cultivation, the process suffered from low yield of cell mass (3.2 g L^{-1}) and antifungal activity because of high initial glucose concentration followed by acetate formation which the causal agent for inhibition of cell growth. Constant and exponential fed-batch strategies were adopted to circumvent this potential problem. Fed-batch cultivation of *B. subtilis* was conducted at the specific growth rate of 0.13 and 0.1 h^{-1} for constant and exponential strategies, respectively. High cell density of 12.8 and 14.6 g L^{-1} for both operations, with an overall biomass yield of 0.45 g g^{-1} was achieved. The inhibitory activity of antifungal in supernatant reached its maximum value of 2 and 2.2 cm for constant and exponential fed-batch cultivations. *(Biotechnology 8 (1): 138-143, 2009;* ***doi:*** *10.3923/biotech.2009.138.143)*

Toward the Mapping of Agronomic Characters on a Rice Genetic Map: Quantitative Trait Loci Analysis under Saline Condition

H. Sabouri and A. Biabani

Improvement of rice (*Oryza sativa* L.) yields in saline condition through breeding require a good understanding of genetic factors that control component traits, such as grain yield, number of filled grain, flowering date and other agronomic traits. The objective of this study was to identify and characterize Quantitative Trait Loci (QTLs) salt tolerance at reproductive stage in saline condition using $F_{2:3}$ populations derived from the cross between Tarommahalli (*indica*) and Khazar

(*indica*). The linkage map constructed by 74 SSR molecular markers covered a total of about 1231.50 cM. Three QTLs were detected for number of primary branches under salt stress on chromosomes 1, 2 and 6. Also, Two QTLs (on chromosome 1) and two QTLs (on chromosome 3) were identified for biomass and plant height, respectively. Tarommahalli alleles (except qNB-6) increased salt tolerance in these loci. In this study, the three major QTLs with the very large effect, qUFG-1b for number of unfilled grain, qBI-1a and qBI-1b for biomass explained 22.58, 22.24 and 26.83% of the total phenotypic variance, respectively. In this study, new QTLs play essential roles in the growth of rice at reproductive stage in Iranian local population under salt stress and provide a rich source of information about the natural genetic variation underlying breeding rice in saline condition. *(Biotechnology 8 (1): 144-149, 2009; **doi:** 10.3923/biotech.2009.144.149)*

Optimization of Culture Conditions for Bacterial Cellulose Production by *Acetobacter* sp. 4B-2

G.Z. Pourramezan, A.M. Roayaei and Q.R. Qezelbash

One of the bacterial cellulose (BC) application problems in industry is its low productivity. So, the researchers have tried to increase the productivity of bacterial cellulose using various biochemical. In this study, production of BC using *Acetobacter* sp. 4B-2 by two categories of carbon sources (monosaccharide and disaccharides) were examined in the modified HS (Hestrin-Shramm) medium by simply replacing D-glucose with other carbon sources. Sucrose gave the highest yield, followed by glucose, xylose and lactose. The highest production of sucrose might be because of the rate of its consumption (80%), which is lower than that of glucose (93.5%). The best yield was achieved by using of 1.5% sucrose. The optimum pH of BC production was 7 and the optimum of temperature for producing BC was 30°C. After optimization of culture conditions, production was reached up to 11.98 g L^{-1} BC. *(Biotechnology 8 (1): 150-154, 2009; **doi:** 10.3923/biotech.2009.150.154)*

Investigations of *in vitro* Selection for Salt Tolerant Lines in Sour Orange (*Citrus aurantium* L.)

N.K. Koç, B. Baş, M. Koç and M. Küsek

The present study was conducted to create new stable somaclonal variants of sour orange in citrus. Embryogenic calli of *Citrus aurantium* that used widely as a

rootstock were successfully used *in vitro* selection for salt tolerance. Calli were cultured on basal MT medium containing three different concentrations of NaCl 100, 200 and 300 mmol. A great number of salt tolerant cell lines were isolated evaluating some morphological aspects of the callus material then, totally 67 plantlets were obtained from embryoids of these selected callus clusters from selective medium containing of 100 mmol NaCl. Further attempts should be made to support the level of salt tolerance through physiological and biochemical analysis. *(Biotechnology 8 (1): 155-159, 2009;* ***doi:*** *10.3923/biotech.2009.155.159)*

Bioadsorbtion of Arsenic by Prepared and Commercial Crab Shell Chitosan

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The present research investigated the arsenic removal performance of prepared and commercial crab shell chitosan by adsorption filtration method. The ability of chitosan to remove arsenic from prepared arsenic contaminated solution was examined by Atomic Absorption Spectrometry (AAS). It is found that the saturation volume of prepared chitosan for 10 mg L^{-1} As^{3+}/As^{5+}solution was 8.55 L and its arsenic removal capacity was 6244.2 mg kg^{-1}. For commercial chitosan, the saturation volume for low and high molecular weight chitosan was 1.3 and 0.45 L and their arsenic removal capacity were 461.8 and 149.5 mg kg^{-1}, respectively. The FT-IR study also confirmed that prepared chitosan's arsenic removal capacity was higher than that of low and high molecular weight commercial chitosan due to the free amino group. *(Biotechnology 8 (1): 160-165, 2009;* ***doi:*** *10.3923/biotech.2009.160.165)*

The Use of Locus Specific Microsatellite Markers for Detecting Genetic Variation in Hatchery bred *Probarbus jullienii*

N. Ghiasi, Z.A. Rashid, S. Hooshmand, K. Yusoff, S.G. Tan and S. Bhassu

This study is to demonstrated that microsatellites markers developed for *Tor tambroides* can be used to amplify microsatellite loci in other family. It is assumed that microsatellite loci are more conserved for aquatic species compared to terrestrial ones due to aquatic environments are less mutagenic than terrestrial ones. Development of microsatellites still requires investment of time and

resources. Thus using loci already developed in a related species may provide a cost-effective alternative to microsatellite isolation and development in a species of interest in present study, *Probarbus jullieni*. In this study we investigated the possibility of the conservation of microsatellite flanking regions among different species. Nine pairs of SSR primers, five gave very strong banding profile (SYK1, SYK 2, SYK 5 SYK 8 and SYK 9) which could be used for population studies by using the nested protocol. Results showed that SYK 2 and SYK 9 flanked the same $(CA)_n$ repeats and thus are highly conserved in a different species. The products of the SYK 5, 8 and 1 primer pairs showed differences in the microsatellite regions which they flanked in *Probarbus jullieni* when compared to those of the source species, *Tor tambroides*. The mean observed heterozygosity levels for all the primers ranged 0.23-0.81. The primers are all polymorphic with the mean number of alleles from 2-5. *(Biotechnology 8 (1): 166-170, 2009;* ***doi:*** *10.3923/biotech.2009.166.170)*

Micropropagation of Some Dwarf and Early Mature Walnut Genotypes

K. Vahdati, R. Razaee and M. Mirmasoomi

In current study, the proliferation and rooting ability of some low vigor and early mature seedlings of Persian walnut were compared with those of semi- and high vigor seedlings in *in vitro* condition. To do this, from each vigor group, nodal explants of newly grown shoots of 5- year-old seedlings were cultured on DKW medium. These explants were subcultured every mount and up to 13 times to increase the number of microshoots. Results showed that the number of axillary shoots arising from the microshoots was the highest in the dwarf and semi-dwarf genotypes compared to the high vigor ones (3.3 vs. 2.3). The low vigor genotypes also showed the highest number of nodes per a given size of shoot, smaller shoot size (2.6 vs. 4.5 cm), lower callus formation and higher rooting percentage (63.5 vs. 37.1%). Moreover, these genotypes showed *in vitro* flowering on micro-shoots, which are consistent with the field observations. These results proved the consistency of low vigor, precocity, basitonic growth tendency and easy rooting of dwarf and precocious genotypes under *in vitro* condition. In conclusion, a simultaneous recurrent selection program is recommended for both dwarfing and rooting ability (selection of dwarf/semi dwarf as well as easy to root clones) to utilize their advantages in high-density orchard systems. *(Biotechnology 8 (1): 171-175, 2009;* ***doi:*** *10.3923/biotech.2009.171.175)*

Study of Expression of Low-Temperature-Responsive Genes for Selected Barley Accessions

M. Keykhahsaber, A.A.V. Sedehi, A. Zakeri, H. Khaje and Z. Keykhah

In order to determine the level of low temperature response genes expression in ten selected barley accessions were obtained from Sistan Agricultural Research at spring of 2008. RNA from each of barley germination was extracted in two cold acclimation and one control condition. The result of real time PCR, using cDNA from three low temperature response gene (*bit2*, *bit14* and *bit101*), showed that there were significant difference in gene expression between three treatments and in each gene the highest percentage of gene expression belonged to accession 5 while accession 8, 9 and 10 composed one separate. In *bit14* gene, the increase in the amount of mRNA was carried out when the maximum level of freezing (4°C day/2°C night) apply. All of these genes are shown to be transcriptional regulated and root meristem had maximum level of RNA under cold treatment. *(Biotechnology 8 (1): 176-179, 2009;* ***doi:*** *10.3923/biotech.2009.176.179)*

Selective *in vitro* Activity of Marine Extract on Genes Encoding Membrane Synthesis of Methicillin Resistance *Staphylococcus aureus*

N.S. Mariana, M.A. Norfarrah, F.M. Yusoff and A. Arshad

Resistant strain issues of *Staphylococcus aureus* remain a global challenge and strategic drug discovery programs have been initiated to confront the issue through drug design based on infective target site. Methicillin resistant *Staphylococcus aureus* (MRSA) strains treated with a marine extract, exhibiting potential inhibitory activity through plate and tube assays were screened for activity on selected genes, namely genes encoding for important survival structure of bacteria. Bacterial cytoplasmic membrane is a vital structure and a critical barrier separating inside of cell from the environment. Disruption in membrane integrity will result in leakage of internal contents and followed by cell death. The necessity for bacteria to have membranes makes the membrane a practical target. With this premise, studies on MRSA membrane synthesis genes; *msr*R and *mpr*F genes were conducted via molecular biotechnological approaches. The effect of the resistant gene *mec*A was also investigated. Alteration of nucleotide sequence after treatment was observed only in the *mpr*F gene and was not evidence in nucleotide sequence of *msr*R gene. The selective targeting of *mpr*F gene by the marine extract is an invaluable finding which requires further investigations on the feasibility of the target gene to be

utilized in the development of anti-infective agent against MRSA. The research constitutes a scientific advancement in the field of medical treatment of drug resistant bacteria and a forefront study of drugs discovery program focusing drugs target genes. *(Biotechnology 8 (1): 180-183, 2009; **doi:** 10.3923/biotech.2009.180.183)*

Improvement of Excretory Overexpression for *Bacillus* sp. G1 Cyclodextrin Glucanotransferase (CGTase) in Recombinant *Escherichia coli* through Medium Optimization

P.K. Lo, C.Y. Tan, O. Hassan, A. Ahmad, N.M. Mahadi and R.M. Illias

In the study presented, Design of Experiments (DOE) was combined with statistical analysis such as fractional factorial design and small central composite design Response Surface Methodology (RSM) to significantly increase the extracellular recombinant CGTase yields in fermentation flasks. The new medium obtained by the statistical analysis for the significant medium components comprised of 12 g L^{-1} NZ Amine A, 24 g L^{-1} yeast extract, 9.44 g L^{-1} KH_2PO_4, 4.4 g L^{-1} K_2HPO_4, 4.58 mL L^{-1} glycerol, 7 mg L^{-1} sucrose and 3 mg L^{-1} $CuCl_2$. Yields were improved about 68% from 9.54 to 16.07 kU mL^{-1} in flasks when using the optimized cultivation medium. The results suggest that the overexpression level of recombinant CGTase excreted into the culture medium using the recombinant *Escherichia coli* could be improved through medium optimization. *(Biotechnology 8 (2): 184-193, 2009; **doi:** 10.3923/biotech.2009.184.193)*

Isolation and Characterization of Glyceraldehyde-3-phosphate Dehydrogenase Gene of *Trichoderma virens* UKM1

S.S.L. Oh, F.D.A. Bakar, A.M. Adnan, N.M. Mahadi, O. Hassan and A.M.A. Murad

In this study, the isolation and characterization of *Trichoderma virens* glyceraldehyde-3-phosphate dehydrogenase gene (GPD1) and its promoter is described. A cDNA clone of a partial GPD1 had been identified from an ongoing work on *T. virens* Expressed Sequence Tag (EST) analysis. This led to the isolation of a 2.9 kb *T. virens* GPD1 that encompasses the 5'-regulatory flanking region (1,364 bp), open reading frame (1,448 bp) and 3'-regulatory flanking region (31 bp) by DNA walking. Based on this sequence, a 1.017 kb cDNA fragment encompassing the Open Reading Frame (ORF) that encodes for GPD1

was subsequently isolated by reverse transcription-polymerase chain reaction. Comparison of the GPD1 and its cDNA sequences demonstrated that the complete gene sequence encodes a polypeptide chain of 338 amino acids interrupted by 2 introns. Sequence comparison analysis of the 5' non-coding region with the 5' flanking sequences of other fungal GPD genes show several regions of similar sequence. The segments from positions -68 bp relative to the start codon is potentially a transcription start site and is mapped within the pyrimidine rich region. The presumptive TATA and CAAT boxes are mapped at -363 to -358 and -109 to -105 from the initiation of translation sites, respectively. The deduced protein product is 71 to 96% identical to glyceraldehyde-3-phosphate dehydrogenases of other filamentous fungi. Phylogenetic analysis based on deduced amino acid sequences shows that GPD1 of *T. virens* forms a cluster with filamentous ascomycetes. The sequence of this gene and its promoter can be used for the development of genetic tools in molecular studies of *T. virens* and in the expression of heterologous genes. *(Biotechnology 8 (2): 194-203, 2009;* ***doi:*** *10.3923/biotech.2009.194.203)*

Functional Analysis of *Fusarium oxysporum* Nitric Oxide Reductase Expressed in Plant Suspension-Cultured Cells

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The biological function of Reactive Nitrogen Species (RNS) is not well understood, however, they actively contribute to the effect of green house gases. Development of plants that could efficiently denitrify intermediates of the RNS to the dinitrogen (N_2) is a rationale that could help amelioration the effect of these gases. *Fusarium oxysporum* cytochrome P-450 nor gene (Fnor) was constitutively expressed in tobacco BY-2 cells. The gene product functions as nitric oxide reductase (nor), which catalyzes the reduction of nitric oxide (NO) to nitrous oxide (N_2O) in the fungal denitrification pathway. Intact transgenic BY-2 cells cultured in ^{15}N-labeled nitrate ($^{15}NO_3^-$) actively produced $^{15}N_2O$ gas up to 59 folds higher than the wild-type cells. Activity of the enzyme was also confirmed by an *in vitro* nor activity assay. Tungstate (a nitrate reductase inhibitor) and cyanide (an inhibitor of the last protein complex of electron transport chain) strongly inhibited $^{15}N_2O$ production. These observations together suggest that Fnor enhanced the reduction of nitrate to N_2O in plant cells. This finding indicates that plant cells are capable to tackle the denitrification pathway. *(Biotechnology 8 (2): 204-211, 2009;* ***doi:*** *10.3923/biotech.2009.204.211)*

Improved Production of Endoglucanase Enzyme by *Aspergillus terreus*; Application of Plackett-Burman Design for Optimization of Process Parameters

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In this study, bagasse was used as substrate for endoglucanase (carboxymethyl-cellulase; CMCase) production using locally isolated *Aspergillus terreus* and the culture parameters were optimized for enhancing cellulase yield. The fungus showed 0.9 U mL^{-1} endoglucanase (CMCase) activity, during growth on basal salts medium at 35°C, initial pH value of 5.5 and in presence of 5% bagasse as a sole c-source. Preliminary experiments to address the most suitable nitrogen source as well as the optimal substrate (bagasse) treatment revealed that the optimal enzyme activity were 2.1 and 2.43 U mL^{-1} in presence of 3 g yeast extract and 1 N HCl or 1 NaOH, respectively. Statistically based experimental design was applied to optimize the production of endogluconase by *A. terreus*. To evaluate the effect of different culture conditions on the production of CMCase enzyme, Plackett-Burman factorial design was carried out. Twelve variables were examined for their significance on enzyme production. Treated bagasse (T_2), non treated bagasse (NT), K_2HPO_4, $NaNO_3$, trace elements, KCl, temperature and pH were the most significant factors encourage CMCase enzyme production, whereas treated bagasse (T_3), yeast extract and $MgSO_4$, were the most significant factors decreasing CMCase enzyme production. The pre-optimized medium showed approximately 4 folds increase in cellulase enzyme production. *(Biotechnology 8 (2): 212-219, 2009; **doi:** 10.3923/biotech.2009.212.219)*

Molecular Cloning of Glutamine Synthetase cDNA from *Lactuca sativa*: Sequence Analysis and Gene Expression during Storage

Dulal Chandra, Toshiyuki Matsui, Haruo Suzuki, Yusuke Kosugi and Koichi Fujimura

In order to understand the factors contributing to postharvest deterioration of lettuce, the changes in ammonia content as well as activity and gene expression of GS were investigated in the outer and inner leaves of lettuce head during storage at 20°C. About two times higher ammonia content out of its initial content was found in the outer leaf portion after the end of 96 h storage period. GS activity in this portion declined to about 27% of the initial level by 24 h of storage, while

activity in the inner leaf portion almost unchanged throughout the storage. To understand clearly these biochemical changes, a cDNA encoding GS was isolated, cloned and sequenced from lettuce leaves. The partial cDNA clone referred to as *LsGS* (*Lactuca sativa* Glutamine Synthetase; AB440673) consisted of 799 nucleotides which showed more than 80% similarity for both nucleotide and amino acid level with the GS genes of other dicotyledonous plant. Northern blot analysis showed that the level of transcript of GS decreased in the outer-leaf portion after 24 h of storage which well correlates with the enzyme activity of this portion. Although, we found no specific trend in GS activity in the inner-leaf portion, the transcript level gradually increased until the end of storage period. The inconsistency between enzyme activity and gene expression may suggest that GS expression in lettuce is controlled by multiple levels of regulations in a tissue-specific manner. *(Biotechnology 8 (2): 220-227, 2009;* ***doi:*** *10.3923/biotech.2009.220.227)*

Augmented Survival of Bacteria Within Biofilms to Exposure to an Atmospheric Pressure Non-Thermal Plasma Source

S. Salamitou, M.J. Kirkpatrick, H.M. Ly, G. Leblon, E. Odic and M.S. DuBow

Bacteria embedded within biofilms present a challenge to surface decontamination by conventional means. Atmospheric pressure non-thermal plasma processes have emerged as a promising approach to overcome this problem. We used a non-thermal atmospheric pressure plasma, operated in a humid atmosphere, to assess planctonic versus biofilm-resident bacterial (*Escherichia coli*) susceptibility to treatment. The concentrations of stable chemical species at the treatment reactor gas outlet were monitored by FTIR. The decontamination efficiency of the process was evaluated against bacteria embedded within a biofilm, as well as planctonic cells placed on a glass surface. Bacterial survival was assessed using a combination of Colony Forming Unit (CFU) ability and vital staining with a combination of DAPI plus Propidium Iodide. Both methods revealed an increased resistance of biofilm-resident bacteria, when compared to planctonic cells, after a 40 min exposure to the post discharge gas. Present results show that biofilm-resident bacteria demonstrate augmented survival when exposed to atmospheric pressure non-thermal plasma treatment and thus that decontamination procedures should take into account this survival when evaluating surface decontamination measures. *(Biotechnology 8 (2): 228-234, 2009;* ***doi:*** *10.3923/biotech.2009.228.234)*

Cloning and Expression of a Biosurfactant Gene from Endosulfan Degrading *Bacillus* sp.: Correlation Between Esterase Activity and Biosurfactant Production

S. Khanna, K.K. Sekhon and N.T. Prakash

The *srfA* gene (1976 bp) was cloned from *Bacillus* sp. SK320 into *E. coli* DH5α using plasmid vector pGEM-T (3 kb). Higher esterase activity was observed in the clone *E. coli* pSKA with olive oil as sole carbon source, as compared to that from *Bacillus* sp. SK320. Purification of esterase from *E. coli* (pSKA) on Q-Sepharose resolved the extracellular esterase into three components designated as A1, A2 and A3. All the three esterases were heterogeneous in nature. Sephadex G-75 further resolved the esterase into sub components which were purified to homogeneity as seen by activity as well as silver staining. Clone *E. coli* pSKA showed esterase enzyme with mol. wt. ranging from 12 to 53 Da indicating the multiplicity of the enzyme. An extracellular esterase from clone *E. coli* pSKA grown on olive oil was purified and shown to possess biosurfactant activity. Clone *E. coli* pSKA showed an enhancement in the biosurfactant production (2.45 g L^{-1}) as compared to 1.2 g L^{-1} from *Bacillus* sp. SK320. Clone *E. coli* pSKA reduced the surface tension to 32 dynes cm^{-1} as compared to 40 dynes cm^{-1} by *Bacillus* sp. SK320. *(Biotechnology 8 (2): 235-241, 2009;* ***doi:*** *10.3923/biotech.2009.235.241)*

Molecular Cloning and Expression of a *Caenorhabditis elegans* Cathepsin B-Like Protease

E. Miranda-Miranda, A. Zamora-Ruíz and R. Cossío-Bayúgar

The present study reports the cloning and expression of a *Caenorhabditis elegans* Cathepsin B-like Protease (CBP), with the objective of obtaining a recombinant enzyme bearing biochemical properties similar to natural CBP reported in the literature for *C. elegans* and parasitic nematodes. The gene was isolated by PCR from *C. elegans* cDNA, resulting amplicon was cloned into a baculovirus expression plasmid, insect cells were used for assembly of a recombinant baculovirus containing the *C. elegans* CBP gene. Thirty five and 45 kDa recombinant proteins were identified from baculovirus infected crude cells containing a His-tag antigenic marker identified by a specific polyclonal antibody in a Western blot assay, both of these recombinant proteins were capable of digesting gelatin in a SDS-PAGE-gelatin assay. Affinity chromatography purified fractions of this recombinant protease, were assayed for peptidase activity against

synthetic fluorogenic peptides, including specific cathepsin B substrates and a caspase tetra peptide substrate, maximum cathepsin activity was detected at pH 6.0 for all synthetic substrates and total inhibition was achieved by cystein protease inhibitor E-64 but not by EDTA, pepstatin or PMSF protease inhibitors. Recombinant *C. elegans* cathepsin B-like protease can be obtained in large amounts from the infected insect cell culture. *(Biotechnology 8 (2): 242-247, 2009; **doi:** 10.3923/biotech.2009.242.247)*

Antimicrobial Peptides in Aqueous and Ethanolic Extracts from Microbial, Plant and Fermented Sources

Koshy Philip, Saravana Kumar Sinniah and Sekaran Muniandy

The objective of this research was to isolate novel peptides from extracts prepared from native microbial, plant and fermented sources. The antimicrobial properties of these extracts were initially tested using *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus*. The exact species and strains of these test microorganisms were confirmed by identifying its 16S RNA sequence. The most pronounced inhibition zone for ethanolic extracts was obtained with *Andrographis paniculata*. For peptide/protein extracts only *Allium sativum* showed promising results. The particular compound responsible for the inhibition in each case is undergoing characterization by using High Performance Liquid Chromatography (HPLC) and mass spectrometry. *(Biotechnology 8 (2): 248-253, 2009; **doi:** 10.3923/biotech.2009.248.253)*

Characterization of Collagen from Eggshell Membrane

Yu-Hong Zhao and Yu-Jie Chi

Collagen was extracted by acid-pepsin digestion and isolated by salt precipitation from eggshell membrane. The characteristics of eggshell membrane collagen were investigated with amino acid analysis, sodium dodecyl sulphate-polyacrylamide gel electrophoresis, Fourier transforms infrared spectroscopy and differential scanning calorimetry. The amino acid composition of the eggshell membrane collagen is rich in glycine, proline and hydroxyproline. Electrophoresis revealed two different α (α_1 and α_2) chains. FTIR showed regions of amides A, B, I, II and III were 3325, 2926, 1653, 1550 and 1240 cm^{-1}, respectively. Analysis of differential scanning calorimetry revealed that thermal denaturation temperature of eggshell membrane collagen was 55.10°C and collagen of eggshell membrane retains intermolecular crosslinks after extraction process. Collagen of eggshell membrane

was typical type I collagen and may be applicable to variety of usage including functional food, cosmetic, biomedical and pharmaceutical industries. *(Biotechnology 8 (2): 254-258, 2009;* ***doi:*** *10.3923/biotech.2009.254.258)*

In vitro Shoot Cut: A High Frequency Multiplication and Rooting Method in the Bamboo *Dendrocalamus hamiltonii*

Rajneesh K. Agnihotri and Shyamal K. Nandi

A rapid and high frequency reproducible *in vitro* regeneration protocol of a multipurpose bamboo species *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro has been developed through single node cutting taken from the lateral branches of a 20 year old field grown elite bush. Axillary buds on the nodal explants sprouted within a fortnight of culture on Murashige and Skoog (MS) medium without any Plant Growth Substance (PGS). After 3-4 weeks of incubation, the sprouted buds were excised from the mother stumps and placed on MS medium supplemented with different concentrations of 6-benzylaminopurine (BAP; 2.0-12.0 μM) and 1.0 μM α-naphthaleneacetic acid (NAA). Enhanced proliferation was induced on the propagules (small clusters with 3-5 multiple shoots and rhizomatous part) on medium supplemented with 8.0 μM BAP and 1.0 μM NAA; subsequent removal of the shoots (about 1.5 cm) from the rhizomatous portion (shoot cut) and placing them on the same media combination influenced multiplication capacity. A multiplication of 20 folds was achieved on MS medium supplemented with 8.0 μM BAP and 1.0 μM NAA at the end of the 2nd subculture. Enhanced root formation (>90%) occurred when the propagules following shoot cut were placed on to MS medium supplemented with 100 μM indole-3-butyric acid (IBA) for 10 days and then transferred to IBA-free medium. This is the first report from this species where 20-fold multiplication was obtained and subsequent enhanced rooting (>90%). The hardened plants, established in the field, exhibited normal growth even after 2 years. *(Biotechnology 8 (2): 259-263, 2009;* ***doi:*** *10.3923/biotech.2009.259.263)*

Degradation of D,L-2-chloropropionic Acid by Bacterial Dehalogenases that Shows Stereospecificity and its Partial Enzymatic Characteristics

S. Thasif, S. Hamdan and F. Huyop

A *Pseudomonas* sp. strain S3, which can utilise a halogenated compound of D,L-2CP as sole carbon and energy source, catalyses the hydrolytic

dehalogenation of both D- and L-isomers of 2-chloropropionic acid. Two kinds of dehalogenase enzymes were isolated from cells of *Pseudomonas* sp. strain S3. A thermostable L-specific dehalogenase (DehL) and non-thermostable D-specific dehalogenase (DehD) can be obtained when cells grown only in the presence of D,L-2CP. These inducible enzymes were then further characterised. The maximum activity of D-specific dehalogenase (DehD) enzyme on D-2CP was found at pH 9.5 at 35°C, whereas the L-specific dehalogenase is thermostable and retained its full activity upon heating at 55°C for 15 min. The pH and temperature optima for dehalogenation of L-2CP were 7.5 and 50°C, respectively. *(Biotechnology 8 (2): 264-269, 2009; **doi:** 10.3923/biotech.2009.264.269)*

Rhizobial Lipo-Chitooligosaccharides and Gibberellins Enhance Barley (*Hordeum vulgare* L.) Seed Germination

M. Miransari and D. Smith

Gibberellins are plant hormones, enhancing seed germination. The bacterium-to-plant signal, lipo-chitooligosaccharides (LCOs) or Nod factors, are of great importance for roots organogenesis and hence, nodule formation and N fixation. Hence, we hypothesized that LCOs like gibberellins may also enhance barley (*Hordeum vulgare* L.) germination. The objectives were to test the effects of gibberellins on barley germination and to test the hypothesis that LCOs may increase seed germination in barley. The concentrations, tested were 10^{-5} M for gibberellins and 10^{-6} M and 10^{-7} and 10^{-8} M LCOs. Although, gibberellins were able to numerically increase barley germination (up to 18%), the LCOs seemed to be more effective on barley germination as they significantly increased seed germination (up to 44%). Hence, the novel finding indicates that for LCOs may also be very effective on barley seed germination, through inducing morphogenesis and physiological changes in seeds. This finding can have very important agricultural implications. *(Biotechnology 8 (2): 270-275, 2009; **doi:** 10.3923/biotech.2009.270.275)*

Application of Protease Isolated from *Bacillus* sp. 158 in Enzymatic Cleansing of Contact Lenses

Rasika Pawar, Vasudeo Zambare, Siddhivinayak Barve and Govind Paratkar

A neutral protease, isolated from *Bacillus* sp. 158 was used for removing protein deposits from contact lenses. Partial purification of the protease was carried out using ammonium sulphate and factors affecting the enzyme activity, such as assay

temperature and assay pH were characterized. The optimum pH and temperature for protease were found to be pH 7.0 and 30°C, respectively. The partially purified protease was stable at temperature range of 30-40°C and pH 6-7. However, protease was maximum stable at 30°C and pH 7.0. The enzyme could be effectively used to remove protein deposit from contact lenses indicating its potential to increase in transmittance of lenses. *(Biotechnology 8 (2): 276-280, 2009; **doi:** 10.3923/biotech.2009.276.280)*

Evaluation of TaGSK1 Gene Expression in Selected Wheat Genotypes as Salinity Marker Assisted Selection

S. Bahrami, M. Solouki, B. Siasar and A. Ghanbari

In order to determine the level of TaGSK1 gene expression in 9 selected wheat genotypes an experiment was carried out in Agricultural Biotechnology Institute, University of Zabol. Seeds of wheat cultured on solid MS medium plate under positive and control condition and RNA was extracted for each genotype and treatment. Relative gene expression using cDNA as template was done in Real Time PCR. The results of real time PCR showed that the Bam genotype has maximum level expression of TaGSK1 gene between 9 genotypes. Minimum expression was found in ER-Salt-85-17 line originated from Tehran Province. TaGSK1 gene expression between genotypes is varied from 39% in ER-Salt-85-17 until 71% in Bam genotypes. In order to determine the level of TaGSK1 gene expression in 9 selected wheat genotypes an experiment was carried out in Agricultural Biotechnology Institute, University of Zabol. Seeds of wheat cultured on solid MS medium plate under positive and control condition and RNA was extracted for each genotype and treatment. Relative gene expression using cDNA as template was done in Real Time PCR. The results of real time PCR showed that the Bam genotype has maximum level expression of TaGSK1 gene between 9 genotypes. Minimum expression was found in ER-Salt-85-17 line originated from Tehran Province. TaGSK1 gene expression between genotypes is varied from 39% in ER-Salt-85-17 until 71% in Bam genotypes. *(Biotechnology 8 (2): 281-284, 2009; **doi:** 10.3923/biotech.2009.281.284)*

Transfer of the Arcelin-Phytohaemagglutinin-α Amylase Inhibitor Seed Protein Locus from Tepary bean (*Phaseolus acutifolius* A. Gray) to Common Bean (*P. vulgaris* L.)

K.P. Mbogo, J. Davis and J.R. Myers

Phaseolus vulgaris cultivars ICA Pijao, Rojo and 5-593 were crossed to the *P. acutifolius* wild accessions G40199 and an F_2 selection from a cross between

G40199 and cultivated brown seeded unnamed tepary accession (designated Brown Tepary). G40199 is highly resistant to the two major bruchid pests of common bean: *Acanthoscelides obtectus* and *Zabrotes subfasciatus*, but the mechanism for resistance is unknown. Interspecific F_1 hybrids with the three common bean parents were generated via., embryo rescue. Recovered hybrids were from ICA-Pijao and 5-593 and were highly sterile and were backcrossed as females to ICA Pijao. Seeds from the BC_1F_2 plants were screened for protein phenotype and the inheritance of seed storage protein profiles contributed by the tepary bean parents. Most of the F_1 hybrids demonstrated introgression of a lectin-like protein of 33 kDa that was found in G40199, but not in the Brown Tepary or common bean lines. This lectin related protein complex was similar to the arcelin (ARL), phytohaemagglutinin (PHA) and α-amylase inhibitor (α-AI) seed storage protein family of *P. acutifolius*. Genomic DNA sequences from wild accession G40199 and the interspecific hybrids revealed a high sequence similarity to ARL2 and α-AI genes of *P. acutifolius*. Because lectin-related proteins of *P. acutifolius* have been associated with strong resistance to bruchids, we hypothesize that these proteins alone or in conjunction with other factors, may contribute to the disputed bruchid resistance mechanism in G40199. *(Biotechnology 8 (3): 285-295, 2009; **doi:** 10.3923/biotech.2009.285.295)*

Optimisation of Lignin Peroxidase Production Using Locally Isolated *Pycnoporus* sp. Through Factorial Design

Z. Zanirun, S. Abd-Aziz, F.H. Ling and M.A. Hassan

Lignin peroxidase has been extensively studied and has been reported to produce by white rot fungus. The highest lignin peroxidase producer from local isolates, identified as *Pycnoporus* sp. was selected for the optimisation study. Factorial design approach was significant to determine the optimum conditions that significantly influenced the production of lignin peroxidase by *Pycnoporus* sp. Several factors were selected in a range indicated by -1 and +1 for lower and upper level, respectively. The results of ANOVA were analysed to check for the significant factors. Optimum condition for the highest lignin peroxidase activity of 51.1 U L^{-1} was obtained at 24 mM of nitrogen concentration, agitation speed at 110 rpm, pH 3.5, inoculum concentration of 6×10^6 spores mL^{-1} and with the addition of inducer (veratryl alcohol). Considering the results obtained, this statistical design was effective in improving the lignin peroxidase production from *Pycnoporus* sp. *(Biotechnology 8 (3): 296-305, 2009; **doi:** 10.3923/biotech.2009.296.305)*

A Consensus Approach for Intrinsic Disorder Analysis for Heat Shock Protein Family

B.K. Bhowmick, W. Dawson, P. Majumder and K. Shimzu

Intrinsic Disorder (ID) regions are implicated with various regulations, but, structure of few ID proteins is experimentally determined and huge amounts of such proteins are still unknown. Computational methods are novel ways to determine such ID regions rapidly and efficiently. There are some popular methods remain to predict that, although, their output varies. Present study attempted to take aggregated efforts to solve the problem. With that regard, we identified and analyzed the ID parts by a consensus approach for Heat Shock Protein (HSP) family and to interpret their significant functional relations. Heat shock proteins are also called tenseness proteins, are a group of protein, are present in all cells in all life lines. This family is very important for cellular function and regulations. Our approach is relevant with several others computational and experimental results. Results are considered only when ≥ 60% accuracy maintained with a common state parameters. From HSP analysis, we headed to conclude that ID prediction for HSP are more univocal and thus their functional implications what we interpreted deserve for good sense. ID possesses more Serine/Thiamine sites and functional domains which are related with disorders and phosphorylations. *(Biotechnology 8 (3): 306-315, 2009; **doi:** 10.3923/biotech.2009.306.315)*

Molecular Characterization of Pomegranate (*Punica granatum* L.) Landraces Grown in Jordan using Amplified Fragment Length Polymorphism Markers

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Amplified Fragment Length Polymorphism (AFLP) technique was used to asses the genetic diversity among twelve pomegranate landraces collected from three locations in Jordan. Eight AFLP primer combinations detected a total of 1433 bands with an average of 14.9 bands per landrace. *Mse*I+CTG and *Eco*RI+ATG primer combinations have the highest ability to discrimination the landraces; they revealed 265 bands with an average of 22.1 band/landrace. The polymorphism that detected by individual primer combinations ranged from 56.7 to 100%. The average genetic similarity ranged from 0.46 to 0.87 among the twelve tested landraces. The highest similarity was recorded between the landraces Qrati and Khdari1, Táeafi and Helow Khashabi. Landrace Zeglabi

showed broad diversity comparing to the other landraces. However, all landraces were discriminated with the tested primer combinations. This study has emphasized the ability of AFLP in determining the genetic diversity among pomegranate landraces. *(Biotechnology 8 (3): 316-322, 2009; **doi:** 10.3923/biotech.2009.316.322)*

Semiquantitative RT-PCR Analysis to Assess the Expression Levels of *Wcor14* Transcripts in Winter-Type Wheat

E. Valiellahi, A. Niazi and M. Farsi

The objective of this study was to determine the quantitative expression of COR gene (*Wcor14*) in different cold treatment in Iranian winter-type wheat, using semiquantitative RT-PCR analysis. Semiquantitative RT-PCR analysis showed that *Wcor14* specifically induced by low temperature. The transcripts of *Wcor14* were up-regulated within 3-6 h of cold acclimation at 4°C. *(Biotechnology 8 (3): 323-328, 2009; **doi:** 10.3923/biotech.2009.323.328)*

Functional Prediction of *Calamus manan* Inflorescence ESTs Through Motif Detection

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Calamus manan floral cDNA libraries were constructed for four stages of flowering in male and female plants, respectively. The *Calamus manan* inflorescence ESTs were generated to provided a better understanding of the flowering process through the identification of genes that are expressed in the floral tissues of this plant. The BLASTX homology search showed that 119 ESTs that were generated from this study had significant matches to unknown proteins and an additional 127 ESTs did not match any protein sequences in the NCBI database. Therefore, a motif search was carried out for the unknown ESTs to predict their putative functions. A total of 136 EST clusters were used in the motif analysis and the InterProScan software was chosen as the motif search tool. There were 66 types of motifs detected from this search. Based on the motifs detected within the query sequences, putative function predictions were successfully performed on 49 EST clusters. *(Biotechnology 8 (3): 329-342, 2009; **doi:** 10.3923/biotech.2009.329.342)*

The Effects of Naphthaleneacetic Acid and Gibberellic Acid in Prolonging Bract Longevity and Delaying Discoloration of *Bougainvillea spectabilis*

Mohammed Saifuddin, A.B.M.S. Hossain, O. Normaniza, A. Nasrulhaq Boyce and K.M. Moneruzzaman

In this study, experiments were conducted to investigate the effects of NAA and GA_3 on bract longevity under exposed sunlight conditions and six months of observation. *Bougainvillea* bracts at four different stages of bract development were sprayed with gibberellic acid (100 ppm GA_3), naphthaleneacetic acid (50, 100 and 150 ppm NAA) and mixed GA_3 (100 ppm) and NAA concentrations (50,100 and 150 ppm). Bract longevity was found to be almost 10 days longer in NAA (50, 100 and 150 ppm) than in the water control and in GA_3 (100 ppm) treatment. In the case of GA_3 and NAA (50, 100 and 150 ppm) treatment on alternative days, bract longevity was 30 days longer when compared with the water control. It was also observed that a delay in discoloration and stomata conductance were increased in the presence of GA_3 with low a concentration of NAA. The results indicated that the prolonging effect of low concentrations of NAA at the initial budding stages was more effective compared with its application at other stages of development and at higher concentrations. Maximum bract weight and shoot length were observed in the GA_3 and GA_3 plus NAA treated flowers. *(Biotechnology 8 (3): 343-350, 2009; **doi:** 10.3923/biotech.2009.343.350)*

Isolation of a Cold-Responsive Gene (*Wcor14*) Encoding a Chloroplast-Targeted Protein from *Aegilops tauschii*

E. Valiellahi, A. Niazi and M. Farsi

To determine the genetic nature of these mechanisms, several cold-responsive genes were identified. *Wcor14*, a member of the wheat cold-responsive (*Cor*) gene family, in this study has been isolated and characterized from ancient wheat ancestor *Aegilops tauschii*. The deduced polypeptide *Aegilops tauschii WCOR14* a hydrophobic polypeptide with 140 amino acids (MW = 13.5 kDa) showed high homology to the previously identified wheat and barley *COR* proteins. Analyses of the cDNA and genomic DNA sequences in this study suggested that, *Aegilops tauschii Wcor14* and its related sequences constitute a small multigene family with different intron sizes. The transcripts Analyses of *Wcor14* suggested that *Wcor14* transcripts were up-regulated within 3-6 h of cold acclimation at 4°C. We used SCRATCH server for protein structure prediction. *(Biotechnology 8 (3): 351-357, 2009; **doi:** 10.3923/biotech.2009.351.357)*

Effective Improvement of Genetic Variation in Maize Lines Derived from R08×Donor Backcrosses by SSRs

Q. Shanbao, W. Yuhua, R. Tingzhao, Y. Kecheng, G. Shibin and P. Guangtang

Introduction of exotic germplasm into local adapted maize (*Zea mays* L.) inbred lines might enhance genetic variability and led to greater progress from selection. Eighteen outstanding maize inbred lines with high resistance to Northern Corn Leaf Blight (NCLB) extensively utilized in Northern China were used as donor parents to improve elite line 08-641 (R08), which has being played a central role in breeding hybrids in Southwest of China. As a result, 36 backcross-derived lines (BC-derived lines) including 18 BC_1F_3 and 18 BC_2F_2, were obtained. To evaluate improvement efficiency and genetic variation for BC-derived lines, microsatellites were used to genotype these lines together with recurrent parent, R08 and 18 donor parents. Each genotype had a unique banding profile, the genetic similarity coefficient ranged from 0.398 to 0.981. Average genetic diversity (He) and Shannon's information index were 0.344 (Range: 0.036-0.774) and 0.629 (Range: 0.092-1.542), respectively. The hierarchical analysis of molecular variance (AMOVA) and the coefficient of gene differentiation (G_{ST}) values revealed that most of the variations (average: 65.8%) were presented among lines from different combinations given generation, nearly half of variations (Average: 47.6%) were found between BC_1F_3 and BC_2F_2 given combination. Four SSR markers associated with *Ht2* and *Ht3* genes were also screened in this study and the practicability of these markers assisted selection was discussed. The present study demonstrated that one generation of backcross based on the selection of right donor parents, identification of target traits and general combining ability in self-cross process would be best effective in maize backcross improvement. *(Biotechnology 8 (3): 358-364, 2009;* ***doi:*** *10.3923/biotech.2009.358.364)*

Response of Onobrychis Genotypes to PEG 10000 Induced Osmotic Stress

L. Imanparast and D. Hassanpanah

In order to evaluation of drought stress tolerance of seven onobrychis genotype *in vitro* condition, was done in Ardabil, Iran in 2009. This experiment was performed by use of factorial design on the basis of completely randomized in three replications. A factor includes five osmotic potential levels (0, -3, -6, -9 and -12 bar) and B factor includes seven onobrychis genotypes (Syntetic, Mako Shoot, Osko Asfanjan, Khosro Shahr Tazekand, Osko Askandaran, Ardabil

Garjan and Ardabil Hasanbarogh). For making the different osmotic potentials were used the PEG 10000 and distilled water as control. The variance analysis results showed that there is significant difference between drought levels, genotypes and their interaction as attributes such as coleoptile length, germination uniformity and percent, between drought different levels as attribute germination speed. The Synthetic genotype had the most coleoptile length, germination uniformity and germination percentage in -3 bar and control in compare of the other genotypes. Synthetic genotype showed the most tolerance to drought stress in comparison of the other genotypes and selected the tolerant genotype. *(Biotechnology 8 (3): 365-369, 2009; **doi:** 10.3923/biotech.2009.365.369)*

14α-Hydroxylation of Androst-4-en-3, 17-dione by the Whole Cells of Cyanobacterium *Nostoc piscinale*

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The potential of microalga *Nostoc piscinale* was evaluated in bioconversion of androst-4-en-3, 17-dione (I). Bio-reaction was performed in BG-11 liquid medium supplemented with 0.05% steroid substrate under continuous light photo-regime of 3000 lux at 25°C for 5 days. Three major metabolites were purified chromatographically and identified on the bases of their spectral data and physical constants as 17β-hydroxyandrost-4-en-3-one (II), 14α-hydroxyandrost-4-en-3,17-dione (III) and 14α,17β-dihydroxyandrost-4-en-3-one (IV). Bioconversion characteristics observed were 14α-hydroxylation and 17-keto reduction. Present results showed that the green biocatalyst is suitable for some specific alterations on androst-4-en-3,17-dione including 14α-hydroxylation. *(Biotechnology 8 (3): 370-374, 2009; **doi:** 10.3923/biotech.2009.370.374)*

Determination of Sperms' ATP Content of Golden Grey Mullet (*Mugil auratus*) at Different Conditions

S. Sadeghi, M. Hedayati and S. Jamili

Aim of this study was determination of sperms' ATP content of golden grey mullet in different time, temperature and extenders. Caspian Sea Mugilidea is one of the most important fish of the sea fishery which nowadays is the predominant catches fish of the mentioned sea. The ATP content of mentioned fish sperm is an important index of fish fertility determination of the sample's ATP concentration was done by ultra sensitive bioluminescence method. ATP content of sperms were determined at two different sampling temperature (10-12 and 18-20°C) and two different keeping temperature (4°C and room temperature) for 6 h and also ATP

content assayed until 10 days storage in the three extender types (glycerol, 0.7 and 0.65% salt solution). Results of the present study showed that, ATP content of sperms, collected at 10-12°C was 74.04±7.22%, in comparison to 18-20°C. The ATP content of sperms during 6 h keeping at 4°C and room temperature were 90.26±0.91% and 17.17±1.49%, respectively. Determination of sperms' ATP content after 5 days keeping in glycerol, 0.7 and 0.65% salt solution revealed that glycerol or 0.65% salt solution is better extender than 0.7% salt solution. But sperms which were kept in mentioned extenders for 10 days showed that glycerol was better than salt solutions based on sperm ATP content saving. Results revealed that, in order to save sperms' ATP content of golden grey mullet, sampling at 18-20°C and keeping in glycerol as extender is recommended. *(Biotechnology 8 (3): 375-379, 2009; **doi:** 10.3923/biotech.2009.375.379)*

Effect of Nitrogen and Phosphorus Fertilizers on Growth and Oil Yield of Indigenous Mint (*Mentha longifolia* L.)

Mahmoud S. Alsafar and Younis M. Al-Hassan

A field study was conducted to determine the effect of different rates of application of N and P fertilizers at different time intervals on the growth and essential oil yield of indigenous mint (*Mentha longifolia*) during 2000-2001 and 2001-2002 cropping seasons. The response of growth and essential oil yield of crop to different fertilizer treatments was consistent in both the years. The Leaf Area Index (LAI) increased significantly with the increasing the rate of fertilizer application from 75/50 kg N/P_2O_5/ha (F_4) to 100/75 kg N/P_2O_5/ha (F_5) than the control and the lower rates of fertilizer application. Application of 75/50 kg N/P_2O_5/ha significantly increased the total dry matter and essential oil yield. Essential oil yield increased with the corresponding increase in the total number of leaves/plant and leaf area. The time of fertilizer application did not affect significantly the essential oil yield in both the cropping seasons. Overall, the essential oil yield of indigenous (wild) mint was maximum in F_4 treatment (75/50 kg N/P_2O_5/ha) under the agro-ecological conditions of Al-Hassa, Saudi Arabia. *(Biotechnology 8 (3): 380-384, 2009; **doi:** 10.3923/biotech.2009.380.384)*

Degradation of 3-Chloropropionic Acid by *Escherichia coli* JM109 Expressing Dehalogenase (*deh*) Gene used as Selection Marker

Tan Yea Yusn and Fahrul Huyop

3-Chloropropionic acid (3CP) in its carboxylate ionic form is a synthetic compound found in herbicide. The biodegradability of 3CP is not well documented

but a microbe that has the ability to utilise 3CP as sole carbon and energy source has been isolated. The dehalogenase gene (*deh*) cloned from *Rhodococcus* sp. HJ1 could be used as a selection marker gene for vector in *E. coli*. Halogenated compound, especially 3CP inhibit the growth of some microorganisms. In current investigation, a 4 kb *Eco*R1 fragment of genomic DNA from *Rhodococcus* sp. HJ1 was cloned into pUC18 plasmid and transformed into an *E. coli* JM109 conferred 3CP resistance on them. Therefore, *E. coli* transformed with vector marked with *deh* could be easily selected on plates containing 3CP. The *E. coli* JM109 transformed with pTY096 (deh^+) weakly expressed the *deh* gene as shown from its slow growth with cells doubling time of 22 h with minimal amount of chloride ion released in the growth medium. *(Biotechnology 8 (3): 385-388, 2009; **doi:** 10.3923/biotech.2009.385.388)*

Comparative Evaluation of the Sensory Properties of Doughs Fermented with Yeasts Isolated from Orange

B. Boboye and I. Dayo-Owoyemi

Five different yeasts were isolated from the juice of orange. The yeasts were identified as *Brettanomyces bruxellensis, Hanseniaspora uvarum, Saccharomyces rosei, Pichia fermentans* and *Hypopichia burtoni*. Yeast population (1.41×10^9 cfu mL^{-1}) of each of the isolates was used to ferment wheat flour dough in order to determine their individual fermentative abilities. Sensory evaluation of the baked fermented doughs using parameters namely: leavening, texture, aroma, taste and appearance revealed that the yeasts, *Saccharomyces rosei and Pichia fermentans* produced loaves having sensory properties ($p \leq 0.05$) comparable with two baker's yeasts (Fermipan and Sat-instant) commonly used in many of the bakeries in Ondo State, Nigeria. *(Biotechnology 8 (3): 389-392, 2009; **doi:** 10.3923/biotech.2009.389.392)*

In vitro Shoot Regeneration of *Citrullus vulgaris* Schrad (Watermelon)

F. Suratman, F. Huyop and G.K.A. Parveez

In this study, shoot regeneration using cotyledons derived from seedlings of diploid and triploid yellow watermelon (cultivars Hwang Fong Yellow Queen, Round Dragon and Chin San Seedless) was investigated. Multiple shoots from auxiliary meristems were obtained without adventitious shoot. Shoot regeneration system for watermelon was successfully established from cotyledon sections of 4 to 5

day-old *in vitro* seedling. The explants were collected from the proximal cotyledon with hypocotyls segment. Highest mean number of multiple shoots were obtained (9.83±0.54) for cultivar Hwang Fong Yellow Queen on MS medium supplemented with 20 μM BAP as compared to (8.87±0.81) for Chin San Seedless on 5 μM of BAP and (6.00±0.32) for Round Dragon on 10 μM of BAP. From these results, cultivar Hwang Fong Yellow Queen was used for further experiment due to its highest percentage of germination and mean number of shoots. Subsequently, the cultivar successfully rooted (80%) on half strength MS medium supplemented with 0.5 μM IAA. Finally, 50% of these rooting plantlets were acclimatized on soil. *(Biotechnology 8 (4): 393-404, 2009; **doi:** 10.3923/biotech.2009.393.404)*

Evaluation for the Production of Antialgal Substances from *Streptomyces neyagawaensis*

S.A. El-Sherbiny, Y.M. El-Ayoty, M.F. Ghaly and N.S. Fleafil

Optimization of *S. neyagawaensis* N_{60} (Egyptian isolate) for the production of natural antialgal substance was carried out. The Clear Inhibition Zone (CIZ) in the different algal species indicated the maximum biological activity of metabolite was attained at 6 g L^{-1} maltose 1.05 g L^{-1} NH_4Cl and 1 g L^{-1} K_2HPO_4 under pH 6.5, temperature 28°C and incubation period 7 days. For microanalysis, xylene was the most efficient solvent for extraction of the lytic substance which has one spot under UV lamps at RF 0.65 using TLC. Identification of the antialgal substance produced by *S. neyagawaensis* was carried out on the basis of elementary analysis, IR, mass and NMR spectra. The earlier analysis emphasized that the molecular weight equal 369.45 kDa with chemical formula $C_{19}H_{21}NO_6$ (Anthracidin A). Different concentrations of Anthracidin A were tested against *Anacystis nidulans* revealed that chlorophyll a, nucleic acids were reduced with increasing the concentration of Anthracidin to 40 μg mL^{-1}. *(Biotechnology 8 (4): 405-415, 2009; **doi:** 10.3923/biotech.2009.405.415)*

Molecular Cloning of Cellulose Synthase Gene, *SpCesA1* from Developing Xylem of *Shorea parvifolia* spp. *parvifolia*

E.T. Lau, W.S. Ho and A. Julaihi

This study reported the isolation and *in silico* characterization of full-length cellulose synthase (*CesA*) cDNA from *Shorea parvifolia* spp. *parvifolia*, an important tropical hardwood tree species. Cellulose synthase (CesA) is a member

of processive glycosyltransferases that involved in cellulose biosynthesis of plants. The full-length of *SpCesA1* cDNA with size 3308 and 3120 bp open reading frames encoding a 1040 amino acid was isolated using RT-PCR and RACE-PCR approaches. The predicted *SpCesA1* protein contained N-terminal cysteine rich zinc binding domain, 7 putative transmembrane helices (TMH), 4 U-motifs that contain a signature D, D, D, QxxRW motif, an alternating conserved region (CR-P) and 2 hypervariable regions (HVR). These entire shared domain structures suggest the functional role of *SpCesA1* is involved in cellulose biosynthesis in secondary vascular tissues of *S. parvifolia* spp. *parvifolia*. Sequence comparison also revealed the high similarity (87%) among *SpCesA1* and PtrCesA2 of *Populus tremuloides*. This further implies the involvement of *SpCesA1* in catalyzes the cellulose biosynthesis of secondary cell wall rather than primary cell wall. Thus, identification of new *CesA* genes from tropical tree genomes is essential for enhancing knowledge of cellulose biosynthesis in trees that has many fundamental and commercial implications. *(Biotechnology 8 (4): 416-424, 2009; **doi:** 10.3923/biotech.2009.416.424)*

Optimization of Fermentation Conditions for the Biosynthesis of Inulinase by the New Source; *Aspergillus tamarii* and Hydrolysis of Some Inulin Containing Agro-Wastes

W.I.A. Saber and Noura E. El-Naggar

From the rotted Jerusalem artichoke tubers, 11 fungi were isolated on synthetic medium containing inulin as a sole carbon source. On the base of inulinase activity on inulin (I), one of them was selected and identified as *Aspergillus tamarii* AR-IN9. Incubation of *A. tamarii* AR-IN9 for 72 h, pretreatment of inulin-containing agro-wastes in autoclave at 20 Ib/in^2, 3% corn steep liquor in the growth medium, pH 5.5 and 35°C were the best conditions for inulinase production. The overall production reached up to 71.97 U mL^{-1}. *Aspergillus tamarii* AR-IN9 showed invertase activity on sucrose (S), with high values of I/S ratio which indicating that the fungus is active in inulinase production. Inulinase activity reached its maximum at pH 5.2 and 45°C. The enzyme was still stable by 80% or more at the pH range from 4.4 to 7.2 for 24 h and by 75% at 50°C for 90 min. The metal ions; $MgCl_2$, $CoCl_2$ and $MnCl_2$ positively modulated inulinase activity. The resultant inulinase showed high hydrolysis activity on Jerusalem artichoke (71.64%), dahlia tubers (67.55%) and chicory roots (55.11%). Therefore, various agro-wastes and inulin-containing materials could be economically hydrolyzed with *A. tamarii* AR-IN9 inulinase into fructose, which has many therapeutic and industrial aspects. Besides the beneficial environmental

impact by the bioremediation of such agro-wastes. *(Biotechnology 8 (4): 425-433, 2009; **doi:** 10.3923/biotech.2009.425.433)*

In Silico Analysis of Evolution in Swine Flu Viral Genomes Through Re-assortment by Promulgation and Mutation

S. Sur, G. Sen, S. Thakur, A.K. Bothra and A. Sen

Availability of the sequences of latest strains of H1N1 virus and their comparison with other viral strains may provide significant clues to the nature of H1N1. The objective of the study was to look into the characteristics of genes and proteins of the swine flu and related viruses to understand their lifestyle and evolutionary relationship. Sequences of genome segments were analysed using ACUA, Codon W and DAMBE. Evolutionary relationships were determined via condensed matrix method. CAI values were quite high in the studied viruses and pI values of proteins showed a bi-modal distribution. All HIN1 strains as well as Influenza C, H3N2 and H2N2 had pI in the range greater than 8.1 with H1N1 CAL07/2009 having pI value of 8.87. Positive correlations of GC3 and GC content with CAI values were noticed. Hydropathy and aromaticity levels increased with the decrease of GC3. Phylogram revealed a rooted tree, which shows two major clades, Clade A and Clade B with subclades. Majority of H1N1 lie together in the same clade with the exception of H1N1 CAL04/2009 that lies in a different clade altogether along with H1N1 Puerto-Rico. Mutational bias is the main factor driving codon usage variation. High expression of pathogenicity related genes confirm its role as pathogen. Most of the H1N1 basic proteomes are influenced by mutational pressure. Genes associated with the hydrophilic proteins are favoured by translationally optimal codons. Phylogenetic analysis portrays the role played by reassortment in controlling the evolution of the studied strains. *(Biotechnology 8 (4): 434-441, 2009; **doi:** 10.3923/biotech.2009.434.441)*

Decolourization of Azo Dyes by a Strain of *Micrococcus* Isolated from a Refuse Dump Soil

O.D. Olukanni, A.A. Osuntoki and G.O. Gbenle

Bacterial degradation is a viable treatment option for azo dyes containing wastewater. However, a great drawback is the generation of potentially toxic and mutagenic end products (aromatic amines) by anaerobic bacteria. This study is part of efforts to develop textile effluent bio-treatment processes to produce reusable water by decolourization and degradation of azo dyes to non toxic

metabolites. The ability of fourteen bacterial strains isolated from various environmental sources to decolourize textile wastewaters aerobically using a simulated effluent made with three textile reactive azo dyes; Reactive Yellow 42 (RY 42), Reactive Blue 13 (RB 13) and Reactive Red 58 (RR 58) were investigated. Three strains showed >95% decolourization of the synthetic effluent within 24 h. The effect of culture condition (pH, temperature and media) on the degradation of methyl red, a standard azo dye, by the isolate with the highest decolourization level; identified as *Micrococcus* sp., was also studied. The strain showed optimum decolourization at pH and temperature around 7 and 37°C, respectively. It preferred nutrient broth to minimal media and 0.02 g dry mass decolourized 50 mL, 56 mg L^{-1} solution of methyl red within 6 h under adequate oxygen supply. UV-visible spectra analyses of aniline sulphate (an aromatic amine) and those of the metabolic products of methyl red suggest that methyl red was first converted to aromatic amine(s) which was subsequently mineralized by the bacterium. The high azo dyes decolorization ability of the *Micrococcus* strain suggested that aerobic decolourization of azo dyes could be as effective as the anaerobic counterpart if suitable organisms are employed. *(Biotechnology 8 (4): 442-448, 2009; **doi:** 10.3923/biotech.2009.442.448)*

Regeneration of *Stevia rebaudiana* and Analysis of Somaclonal Variation by RAPD

Md. Moktaduzzaman and S.M. Mahbubur Rahman

The objectives of this experiment were to develop the optimal concentration of auxin and cytokinin for regeneration of *Stevia rebaudiana* and finally analysis somaclonal variation by RAPD. Various degrees of callus induced from the leaf segments cultured on MS medium supplemented with the different concentrations and combinations of NAA+BA and 2,4-D +BA. Among them, 1.5 mg L^{-1} NAA with 1.0 mg L^{-1} BA was the best for callus induction (91.67%) which also produced highest fresh weight (621.7 mg) and dry weight (79.00 mg) of callus. For shoot formation, calli were transferred on to MS medium supplemented with different concentrations and combinations of BA and NAA with control. The highest number of shoots (2.17) and the highest average length of the shoot (3.22 cm) per culture was observed at 1.8 mg L^{-1} of BA with 0.12 mg L^{-1} of NAA. The regenerated shoots were then transferred to MS liquid medium supplemented with same concentration of IBA and NAA. All the treatments produced roots and 1.50 mg L^{-1} IBA produced highest percentage of root (93.33%), but 1.00 mg L^{-1} NAA produced highest number (no. 7.66) of roots and highest length of roots (13.33 cm) per culture. The regenerated plantlets were

successfully transferred into pots containing 75% soil and 25% sand and finally transferred into the field. Apparently somaclonal variations were examined among regenerated plants along with mother plant by RAPD. DNA samples from mother plant and 9 randomly selected regenerated plants were subjected to RAPD analysis. Bands generated through RAPD-PCR were scored according to whether they were present (1) or absent (0) to determine the extent of somaclonal variation. The estimation of genetic similarity coefficient based on RAPD band-sharing data analyzed indicated that some regenerated plants were 100% similar to the mother plants and some were 71, 57 or 14% similar may be due to variation *in vitro* condition. *(Biotechnology 8 (4): 449-455, 2009;* ***doi:*** *10.3923/biotech.2009.449.455)*

In vitro Antibiotic Bustle of Coral Reef Associated Gastropod, *Drupa margariticola* (Broderip, 1832) of Tuticorin Coastal Waters, Southeastern India

C. Chellaram, R.S. Sreenivasan, S. Jonesh, T.P. Anand and J.K.P. Edward

To test the antibacterial effect of the extracts of *Drupa margariticola* obtained using low to high polar solvents, like ethyl acetate, dichloromethane, acetone and methanol. Partial purification of the active crude extract was carried out using column chromatography employing a step gradient solvent system. A maximum inhibition of 7 mm against *E. coli* was shown by the 100% acetone column purified fractions of *D. margariticola* at a concentration of 0.125 mg. Minimum Inhibitory Concentration values were found to be lower for the 100% acetone fraction for pathogens, *E. coli* (0.05 mg), *Klebsiella pnuemoniae* (0.05 mg), *Pseudomonas aerogenosa* (0.07 mg) and *Streptococcus pnuemoniae* (0.07 mg). Thus 100% acetonated fraction of the extract of *D. margariticola* was considered as potent antibacterial compounds against some human pathogens. The antibacterial potential of the mollusc, *Drupa maragariticola* becomes a corner stone for the future development of novel biologically active compounds. *(Biotechnology 8 (4): 456-461, 2009;* ***doi:*** *10.3923/biotech.2009.456.461)*

Analysis of Genetic Diversity in Bangladeshi Chicken using RAPD Markers

M.B.R. Mollah, F.B. Islam, M.S. Islam, M.A. Ali and M.S. Alam

Understanding the genetic diversity at molecular level is a prerequisite in developing strategies for effective conservation and utilization of chicken genetic

resources. We studied the genetic variation within and between Bangladeshi native (Naked Neck, Frizzle and Non-descriptive indigenous) and exotic (White Leghorn, Rhode Island Red, Commercial layer and broiler) chicken populations by Random Amplified Polymorphic DNA (RAPD). Four out of the 20 random primers exhibited sufficient variability for studied populations. The four primers yielded a total of 39 distinct bands, 25 of which were polymorphic. Estimation of polymorphic loci, intra-population similarity indices and Nei's gene diversity suggested that genetic diversities within a population were high in non-descriptive, Frizzle, Naked Neck, Rhode Island Red and White Leghorn chicken populations compared to the commercial layer and broiler populations. The coefficient of gene differentiation (G_{ST} = 0.34) and gene flow (N_m = 0.98) values reflected a high level of population differences. UPGMA dendrogram segregated the chicken populations in various degree based on their genetic distance. The overall genetic distance among native chicken was relatively low comparison to the exotic populations. The results of present study might have significant impact on the breeding and conservation of native chicken genetic resources in Bangladesh. *(Biotechnology 8 (4): 462-467, 2009; **doi:** 10.3923/biotech.2009.462.467)*

In vitro Degradation Behavior of *Bombyx mori* Silk Fibroin Films Exposure to Protease XXIII

K. Nuanchai, S. Prasong and S. Wilaiwan

Proteolytic activity of protease XXIII on Silk Fibroin (SF) films was studied. The films were prepared from the SF solution by casting on the polystyrene plates and used as substrate for enzymatic degradation. The SF films were incubated with 1.0 mg mL^{-1} protease XXIII at 37°C up to 21 days. After incubation, those of secondary structure and thermal behavior of the SF films were investigated. FT-IR spectra indicated that the SF films predominantly β-structure. There was found that secondary structure of the films did not change even at 21 days of incubation times. However, slightly decreased of FTIR spectra were also observed by shoulder absorption peaks. The result suggested that some crystalline regions might be digested by the enzyme. This related to the thermal stability from thermogravimetric analysis since the SF films gradually decreased their thermal stability followed the increasing of time exposure to protease XXIII. It is a promising that protease XXIII could be digested SF and will be used this enzyme as a model system for enzymatic study on SF. *(Biotechnology 8 (4): 468-472, 2009; **doi:** 10.3923/biotech.2009.468.472)*

COD and BOD Reduction of Domestic Wastewater using Activated Sludge, Sand Filters and Activated Carbon in Saudi Arabia

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The objective of this study was to determine COD and BOD reduction from domestic wastewater using sedimentation, aeration, activated sludge, sand filter and activated carbon. Mean maximum COD and BOD reduction was 92.17 and 97.66%, respectively. Other water quality parameters such as TSS, TDS, NO_2, TKN and PO_4 showed significant reduction except NO_3 which increased significantly using different materials in the Wastewater Treatment Plant (WTP). The sewage treatment system using different materials showed excellent potential for COD and BOD removal from domestic wastewater. Also, the concentration level of COD and BOD in the treated water was within the permissible limits for industrial cooling and agriculture use especially for landscape development. *(Biotechnology 8 (4): 473-477, 2009; **doi:** 10.3923/biotech.2009.473.477)*

Territorial Investigation Based on the Chemical Composition of Chemlali Virgin Olive Oils

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The purpose of this study was to evaluate differences in the chemical composition of virgin olive oils from the Chemlali variety cultivated in different olive growing areas of the Centre of Tunisia. All samples were harvested using the same controlled procedures and were submitted to a controlled processing in the same laboratory mill. Several analytical parameters and indices were determined. Results showed that the oils quality was attributed not only to the olive variety but also to the plantation site, therefore to climatic and pedologic factors. All these parameters showed an important effect on the fatty acid, phenol, α-tocopherol, sterol and volatile contents of the oils. *(Asian Journal of Biochemistry 4 (1): 1-12, 2009; **doi:** 10.3923/ajb.2009.1.12)*

Nephrotoxicity Reduction by Fixed Dose Combination of Cephalosporins and Aminoglycosides in *Mus musculus* Mice

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Free radicals are causative factors for aminoglycoside induced renal toxicity. The aim of present study was to evaluate effect of fixed dose combination of

cefepime+amikacin (Potentox) as well as ceftazidime+tobramycin (Tobracef) antibiotics on antioxidant enzymes (Super oxide dismutase, Catalase and Glutathione reductase) along with (free radical mediated damage) malonaldialdehyde levels and extracellular antioxidant enzymes (creatinine, total bilirubin and uric acid enzymes) in kidney tissue of *Mus musculus* mice. Present findings showed that the activities of the antioxidant enzymes were significantly lowered along with increase in MDA (malonaldialdehyde) levels and extracellular antioxidants after single treatment of aminoglycosides (amikacin and tobramycin) as compared to control group. A significant improvement in antioxidant enzymes along with significant decrease in creatinine, total bilirubin, uric acid and malonaldialdehyde (MDA) levels were observed in fixed dose combination of cefepime plus amikacin as well as ceftazidime+tobramycin treated groups compared to amikacin and tobramycin alone treated group. These results indicate that a fixed dose combination of cephalosporins with aminoglycosides using chemical vector mediated technology acts as an antioxidant and prevents nephrotoxicity induced by aminoglycosides. *(Asian Journal of Biochemistry 4 (1): 13-21, 2009; **doi**: 10.3923/ajb.2009.13.21)*

Selenium and α-Difluromethylornthine in Combination have Strong Activity Against Elevated Polyamines and Glucose Levels in Serum

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In the present study mice were supplemented with 2% of both L-arginine and L-ornithine in drinking water for four weeks. L-arginine and L-ornithine intake elevate polyamine levels in serum of female Swiss albino mice. The effect of selenium (Se) administration (as sodium selenite: 0.5 or 1 mg kg^{-1} body weight) or/and α- difluromethylornthine (DFMO: 2 mg kg^{-1} body weight) on the elevated polyamine levels was studied. The elevated polyamine levels were decreased significantly by administration of low and high doses of Se with DFMO. Glucose concentration in the serum increased significantly with high polyamine level of groups and reduced back around the normal values by Se and DFMO treatment. The concentrations of triglycerides and cholesterol are not effected by the elevated levels of polyamines in the serum. These results suggest that administration of Se in combination with DFMO protect cells from the harmful effect of high levels of polyamines. *(Asian Journal of Biochemistry 4 (1): 22-29, 2009; **doi**: 10.3923/ajb.2009.22.29)*

Impact of *Plasmodium berghei* and Chloroquine on Haematological and Antioxidants Indices in Mice

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The effect of malaria parasites and chloroquine in mice was examined. The importance of this study derives from the prevalence of malaria in the tropical and subtropical regions, as well as the declining therapeutic efficacy of chloroquine as a first line treatment against malaria infection in these endemic areas. This study aimed to determine the pattern of possible alterations in some haematological and antioxidant molecules in mice treated with either Plasmodium or chloroquine. Three groups of ten mice each categorized as control, non parasitized chloroquine treated (NPcqT) and Parasitized non treated (PnT) were used in this study. Observations from the work show that parasites in mice significantly ($p<0.05$) increased plasma total protein, globulin, erythrocyte fragility, total bilirubin, oxidative stress, glucose-6-phosphate dehydrogenase (G6PD), liver superoxide dismutase (SOD) and catalase (CAT) enzyme activities. Also the study showed that there is a significant ($p>0.05$) decrease in plasma SOD, CAT, reduced glutathione (GSH), liver G6PD and GSH. Parasitemia also reduced significantly ($p<0.05$) mice packed cell volume (PCV). Chloroquine treatment of Non Parasitized (NP) mice increased significantly ($p<0.05$) erythrocyte fragility, plasma total bilirubin, oxidative stress, but reduced ($p<0.05$) mice PCV, plasma SOD, CAT, G6PD, GSH but increased ($p<0.05$) liver SOD, CAT and reduced GSH significantly ($p<0.05$). The results obtained from the statistical analysis of data suggest that both malaria parasites increase oxidative stress in mice and chloroquine increases SOD and CAT activity in hepatic tissue of mice. *(Asian Journal of Biochemistry 4 (1): 30-35, 2009; **doi:** 10.3923/ajb.2009.30.35)*

Molecular Existence of Mature Odontoblast and Osteoblast Cells in Adult Human Pulp Tissues

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The dental pulp tissue is essential in dentine development. The existence of Dental Pulp Stem Cells (DPSCs), i.e., osteoblast and odontoblast are to assist in dentine repair and tooth regeneration. The existence of osteoblast that secreted bone matrix directly from pulp tissue has not been reported. The purpose of this study is to determine the existence of odontoblast and osteoblast cells excavated directly from pulp tissues by using molecular markers. The isolated RNA expressing two

gene markers, i.e., dentin sialophosphoprotein and osteocalcin which were secreted by odontoblast and osteoblast cells, respectively. The expression of dentin sialophosphoprotein and osteocalcin demonstrated that both odontoblast and osteoblast cells exist in adult human pulp tissues. *(Asian Journal of Biochemistry 4 (2): 36-44, 2009;* ***doi:*** *10.3923/ajb.2009.36.44)*

Study on Apparent Amylose Content in Context of Polymorphism Information Content along with Indices of Genetic Relationship Derived through SSR Markers in *Birain*, *Bora* and *Chokuwa* Groups of Traditional Glutinous Rice (*Oryza sativa* L.) of Assam

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Amylose content was determined in 41 traditional glutinous rice varieties of Assam classed as *Birain*, *Bora* and *Chokuwa* group during 2004-06. Average apparent amylose content in 6 accessions of *Chokuwa* (9.368%) was higher than 20 accessions of *Bora* group (0.502%) and 15 accessions of *Birain* (0.191%) genotypes. *Mahsuri,* a non-glutinous rice variety contained intermediate amylose content (21.2%). Eight SSR markers were used to assess genetic variability. The size of amplified fragments ranged from 100 to 500 bp. Among all genotypes, average Polymorphism Information Content (PIC) was 0.923. The average genetic similarity within the *Birain* accessions ranged from 0.119 to 0.571. Within *Bora-Chokuwa* accessions, similarity value ranged from 0.047 to 0.667. The average similarity was 0.228, which reflected that the *Bora* group could be more diverse than the *Birain* group. Amylose content is said to be highly influenced by environmental conditions. Since, *Birain* accessions were from the same Barak valley agro-climatic condition and *Bora* as well as *Chokuwa* were from the Brahmaputra valley, an analysis was made with corresponding pair-wise relative rate of increase (%) in apparent amylose contents as well as corresponding values of pair-wise Jaccard's co-efficient of similarity among the accessions of *Birain*, *Bora* and *Chokuwa* groups of glutinous rice. It showed the existence of a matching relation between the increased values of respective apparent amylose content and the genetic similarity. It seems that apparent amylose content though cannot play a solid indicator for genetic variability in glutinous rice germplasm. However it may help to gauge biochemical bases towards genetic variability under same environmental condition. *(Asian Journal of Biochemistry 4 (2): 45-54, 2009;* ***doi:*** *10.3923/ajb.2009.45.54)*

Effect of Artemisinin with Folic Acid on the Activities of Aspartate Amino Transferase, Alanine Amino Transferase and Alkaline Phosphatase in Rat

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Studies on the effect of artemisinin alone and artemisinin with folic acid on the activities of aspartate amino transferase (ASAT), alanine amino transferase (ALAT) and alkaline phosphatase (ALP) in the serum of male Wistar rats were carried out. Different groups of rats (8 group^{-1}) were orally given 0.75, 1.50, 3.00 and 6.00 mg kg^{-1} b.wt. of artemisinin. Each of these doses was also administered concurrently with 1.50 mg kg^{-1} of folic acid, respectively. Artemisinin only elevated the activities of serum ASAT, ALAT and ALP significantly at the four dose levels. When 1.50 mg kg^{-1} of folic acid was concurrently administered with artemisinin, the elevated serum level of ASAT, ALAT and ALP was significantly reversed almost completely at low dose of 0.75 and 1.50 mg kg^{-1} artemisinin. Folic acid only reversed the elevated activity of ASAT, ALAT and ALP by artemisinin partially when the dose of artemisinin was high (i.e., 3.00 and 6.00 mg kg^{-1} of artemisinin). These results suggest that folic acid offers complete relief to metabolic disorders at low artemisinin concentration while the relief is partial at high concentrations. *(Asian Journal of Biochemistry 4 (2): 55-59, 2009;* ***doi:*** *10.3923/ajb.2009.55.59)*

Nigella sativa Modulates Cytokines Expression in Mature Bovine Adipocytes

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In this study, we examined the effect of either lipopolysaccharide (LPS, 1 μg mL^{-1}), *Nigella.sativa* extract (NS, 5 μg mL^{-1}), or co-treatment of both for 24 h on cytokines expression in mature bovine adipocytes using RT-PCR analysis. The results showed that separate treatment by LPS and NS stimulated the expression of interleukin-1 (IL-1β), IL-6, IL-8 and IL-10. Co-treatment of cells by *N. sativa* with LPS inhibited LPS induced IL-6 and TNF-α expression and induced additive stimulatory effect on LPS induced IL-8 and IL-10 expression. The results indicate that *N. sativa* extract has immuno-modulatory effect on bovine adipocytes by stimulating different cytokines expression that potentiate different inflammatory and anti-inflammatory functions in bovine adipocytes. *(Asian Journal of Biochemistry 4 (2): 60-67, 2009;* ***doi:*** *10.3923/ajb.2009.60.67)*

Fermented Soybean Products: Some Methods, Antioxidants Compound Extraction and their Scavenging Activity

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Antioxidant compounds in food such as phenolic compounds played various roles as health promoting factors (e.g., cancer and cardiovascular disease), antimicrobial agents; flavor active compounds, colorants precursors and colloidal stability affecting factors as well as chelating agents. Fermented foods such as soy products have been in existence for thousands of years and received attention as sources of many effective antioxidants. This review discusses about the most commonly used fermented soybean food antioxidants, methods of their preparation and description of their scavenging activity/antiradical property. *(Asian Journal of Biochemistry 4 (3): 68-76, 2009;* ***doi****: 10.3923/ajb.2009.68.76)*

Characterization of a *Capsicum chinense* Seed Peptide Fraction with Broad Antibacterial Activity

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Habanero chili pepper (*Capsicum chinense*) is widely consumed as a fresh vegetable, although its extremely high capsaicin content has led to other uses (e.g., medicine and self-defense). Recently described antimicrobial peptides from *C. annuum* were very efficient in inhibiting growth in human and plant pathogenic bacteria and fungi. In order to explore the potential use of *Capsicum chinense* seeds as a source of antimicrobial peptides, in the present study a peptide fraction from *C. chinense* pepper seeds, denominated G10P1, was enriched, partially purified and its antimicrobial activity tested against the plant and human pathogens *Xanthomonas campestris*, *Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Erwinia carotovora*, *Agrobacterium* sp., *Shigella flexnerii*, *Escherichia coli*, *Staphyllococcus aureus* and *Bacillus subtilis*. The minimum inhibitory concentration of the G10P1 peptide fraction against *X. campestris* was 12.5 μg mL^{-1}. Electrophoresis of the G10P1 in a denaturant 15% polyacrylamide gel showed it to be composed of ~7.57 and ~5.6 kDa polypeptides, both associated with an area of strong antibacterial activity. The sequencing of 18 amino acids from the N-te rminal of the ~7.57 peptides and 12 from the ~5.6 kDa peptides showed no clear association with previously described antimicrobial peptides. However, the ~5.6 kDa peptides were related to the NAC and WRKY transcription factors, both involved in direct regulation of the plant defense

response against pathogen attack and the ~7.57 kDa peptides had low homology with a 3-oxo-[acyl-carrier-protein] synthase from *Capsicum chinense*. *(Asian Journal of Biochemistry 4 (3): 77-87, 2009; **doi:** 10.3923/ajb.2009.77.87)*

Extraction, Characterization and Nutritional Properties of Two Varieties of Defatted Foxtail Millet Flour (*Setaria italica* L.) grown in China

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In this study, we examined the various protein fractions and protein concentrates of two selected varieties (white and yellow) of foxtail millet grown in China. Characterized by amino acid analysis, differential scanning calorimetry (DSC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein content for the white increased after defatting though not significantly different ($p<0.05$) from 11.50 to 11.59 and the yellow decreased from 11.41 to 11.39. Fat content shows a significant decreased ($p<0.05$) after defatting from 2.38 to 0.41(white) and 2.90 to 0.66 (yellow). Prolamin yellow and glutelin white were the major fractions (38.8 and 47.2%, respectively), followed by albumin yellow and white as 2.6 and 1.5%, respectively and globulin yellow and white as 2.5 and 1.4%, respectively and the difference was significant ($p<0.05$) among the various protein fractions. Results showed a significant amount of amino acids with essential amino acids above the recommended amount by FAO/WHO for humans. Albumin white possessed the highest DSC result (Tp = 79.583°C, ΔH = 5.115 J g^{-1}), glutelin white the lowest (Tp = 66.682°C, ΔH = 0.313 J g^{-1}). Fractions and concentrates had molecular sizes below 14.0 and above 97. 0 kDa. Protein fractions and concentrates are potential as functional food ingredient. *(Asian Journal of Biochemistry 4 (3): 88-98, 2009; **doi:** 10.3923/ajb.2009.88.98)*

Influence of Cultural Conditions on Glutathione Peroxidase Synthesis in *Candida albicans*

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The influence of cultural conditions that affect GPX production in *Candida albicans* grown in Lee's medium was investigated. Optimum temperature and pH for GPX activity were 25°C and 7.2, respectively. Substrate specificity for *C. albicans*. Glutathion peroxidase was in the order of cumene hydroperoxide>t-butyl hydroperoxide> hydrogen peroxide>benzoyl peroxide. Aeration as well as

large head space volume enhanced the growth of *C. albicans* and GPX production. Arabinose and ammonium sulphate significantly increased the GPX synthesis. Among nitrogen sources, polypeptone enhanced both the growth and GPX synthesis. Various cellular activities are regulated by the level of GSH. Therefore, the level of GPX might be used as one of the criteria in developing new drugs against *Candida albicans*. *(Asian Journal of Biochemistry 4 (3): 99-105, 2009;* ***doi:*** *10.3923/ajb.2009.99.105)*

Entropy Driven Binding of *O*-Glycan and Glycoproteins to *Artocarpus hirsuta* Lectin: An SPR Study

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In this study, thermodynamics of binding of *O*-glycan (Galβ1→3GalNAcα1→*O*Ser) and the glycoproteins possessing it, viz., fetuin and mucin to *A. hirsuta* lectin was studied using Surface Plasmon Resonance (SPR). The binding affinities were in the order of asialomucin >mucin>asialofetuin>fetuin>*O*-glycan and found to increase with increase in valency of the ligand. Unusual for a lectin-ligand interaction, the binding was endothermic and entropically driven and the higher affinity was associated with a large favorable entropy term. The native fetuin and mucin showed lower affinity than their desialylated counterpart. Kinetic analysis of the binding revealed that the difference in the affinity of different ligands was due to different rates of their association, whereas the dissociation rates were similar and showed decrease with temperature. The activation energy of the association process was lower with desialylated glycoproteins than that of sialylated one resulting in their faster association and higher affinity. *(Asian Journal of Biochemistry 4 (4): 106-116, 2009;* ***doi:*** *10.3923/ajb.2009.106.116)*

Mutagenesis of Gln-142 and Phe-143 of *O*-Acetylserine Sulfhydrylase

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In order to examine the substrate-binding site of *O*-acetylserine sulfhydrylase (OASS) from *Escherichia coli* (*E. coli*), we mutated Gln-142 and Phe-143, which exist at a β-turn region in the active site. The mutants retained one molecule of pyridoxal 5'-phosphate (PLP) per subunit and PLP was covalently bound in Schiff base linkage, similar to what was observed for the wild type enzyme. Q142A and F143Y OASS inhibited the reaction with *O*-acetylserine and the subsequent formation of the amino acrylate intermediate. The F143A, S and D

mutants were able to form the amino acrylate intermediate, but the rate was significantly slower than that of the wild type enzyme. These results suggest that mutagenesis of Gln-142 and Phe-143 residues in OASS influence catalytic properties, possibly due to modulation of the substrate-binding site. *(Asian Journal of Biochemistry 4 (4): 117-124, 2009;* ***doi:*** *10.3923/ajb.2009.117.124)*

Biochemical and Histological Changes Associated with Long Term Consumption of *Gnetum africanum* Welw. Leaves in Rats

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Changes in some biochemical and haematological indices including serum protein, haemoglobin, cholesterol, lipid peroxidation, white blood cells, Glutathione-s-transferase, Superoxide dismutase, Alanine transaminase, Aspartate transaminase and Alkaline phosphatase were investigated in male rats fed with a diet supplemented with leaves of *Gnetum africanum.* The histological changes on the liver, intestines and testes were also examined. The long term feeding of the *Gnetum africanum*-supplemented diet caused significant increases ($p<0.05$) in weight, haemoglobin and white blood cells. There were also significant increases ($p<0.05$) in Glutathione-s-transferase and Superoxide dismutase enzymes. However, *Gnetum africanum*-supplemented diet caused a significant reduction ($p<0.05$) in serum protein and lipid peroxidation. The liver enzymes namely Alanine transaminase, Aspartate transaminase and Alkaline phosphatase were unaffected while the reduction in cholesterol was not significant. Histologically, the liver hepatocytes and hepatic plates were respectively elongated and enlarged while the intestinal mucosa showed elongated villi and enlarged submucosa. There were however no histological changes on the testes. *(Asian Journal of Biochemistry 4 (4): 125-132, 2009;* ***doi:*** *10.3923/ajb.2009.125.132)*

Protective Effect of Squalene on Endogenous Antioxidant Vitamins in Experimentally Induced Myocardial Infarction in Rats

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In the present study an attempt has been made to assess the cardioprotective effect of squalene on isoprenaline-induced myocardial infarction in male albino rats with respect to changes in the levels of endogenous antioxidant vitamins in heart tissue. Levels of endogenous antioxidants such as ascorbic acid, α-tocopherol and

endogenous squalene content in heart tissue were determined. Significant ($p<0.001$) reduction was observed in the levels of ascorbic acid, α-tocopherol and endogenous squalene content in the heart tissue of isoprenaline administered rats compared to normal control rats. It is worth noting that, the prior administration of squalene at 2% level along with feed for 45 days significantly ($p<0.001$) reduced the isoprenaline-induced decline in the levels of these vitamins and restored the membrane bound squalene content at near normal. The results of the present study indicates that the cardioprotective effect of squalene might be ascribable to its antioxidant property thereby sharing the responsibility of these antioxidant vitamins in counteraction of free radicals generated during isoprenaline-induced oxidative stress. *(Asian Journal of Biochemistry 4 (4): 133-139, 2009;* ***doi****: 10.3923/ajb.2009.133.139)*