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**O-11-621-1**

## Germination, growth and callus responses in EMS treated local cultivars of wheat (Triticum aestivum L.) under in vitro salt stress

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Wheat is an important cereal crop and main staple food in Pakistan. The use of new in vitro techniques has revolutionized the crop improvement scenario. In present study, in vitro germination, growth and callusing response of EMS treated M1 seeds of three wheat cultivars, Marvi 2000, Sindh 81 and TJ 83, was evaluated under NaCl stress. The sterilized seeds were inoculated on MS medium with various NaCl concentrations and for callusing, MS medium was supplemented with various concentrations of BAP, 2,4-D and Kn. It was found that TJ 83 showed higher germination (71%) at 100mM NaCl while Marvi 2000 showed higher shoots length (3.65±0.45cm), fresh weight (0.77g) and dry weight (0.263g) after twenty days and chlorophyll a contents (16.36mg/g of fresh weight) after seven days of inoculation on 100mM NaCl containing medium. Total soluble protein and carbohydrate contents were higher in Marvi 2000 plantlets with 19.48±0.54 and 11.21±0.12mg/ml respectively at 150mM NaCl. In callusing response under salt stress, MS medium containing 4.0mg/L 2, 4-D showed the maximum callusing response in all varieties. Gradual increase in fresh weight of calli Marvi 2000 was observed at 75mM NaCl concentration but these calli were failed to regenerate.

**Keywords**: wheat, in vitro salt stress, callus

**O-11-808-1**

## Characterization of crude xylanase produced by edible mushroom Pleurotus eryngii

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Xylanases has been increasingly forthcoming in recent years because of their possible involvement in numerous industrial processes including bioconversion of lignocellulose drived sugars in to fuels, processing food and the paper and fibre industries. Edible mushrooms are emerging as important source of xylanolytic enzymes and this study has concentrated to produce and characterize xylanases by pleurotus eryngii. The crude enzyme was characterized on the basis of various parameters such as incubation time, substrate specificity, substrate concentration, enzyme volume, buffer, pH, pH stability, temperature, temperature stability, and effect of various metal ions or compounds. The xylanase activity was noted maximum at 15 minutes of incubation time, 2.0% xylan and 0.5 ml enzyme volume. The highest enzyme activity was found at pH 4.5, whereas xylanase exhibited maximum stability in the range of pH 4.0 to 10.0. The maximum xylanase activity was noted at 60˚C, while enzyme was most active and retains more than 40% activity at 90˚C with in 10 minutes of incubation. ZnCl2 (10mM) stimulated the xylanase activity as compare to other metal ions or compounds. It is concluded that pleurotus eryngii is capable to produce pH stable and thermostable xylanase for industrial purposes.

**Keywords**: characterization, xylanase, edible mushroom Pleurotus eryngii

**O-11-831-1**

## Effect of water stress on some physiological parameters of wheat

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Water stress is the one of the major environmental constraints for wheat crop. Twenty-five wheat genotypes including 4 commercial check varieties were evaluated under four different water stresses conditions in the field. The physiological parameters viz., relative water content (%), leaf area, chlorophyll content (%) and osmotic potential were studied. Genotype NIA-8/7, BWM-3, NIA-9/5, NIA-37/6, NIA-10/8, NIA-28/4, NIA-30/5, NIA-25/5, ESW-9525, SI-91196, SI-9590, MSH-14, MSH-36, MSH-22, BWQ-4, BWS-78, BWM-47 indicated significantly below osmotic potential at severe stress (single irrigation applied at tillering stage); suggested that these genotypes possess more tolerance to drought conditions as compared to other genotypes including check varieties. Genotype NIA-10/8 possessed more relative water content than other genotypes under severe moisture stress. Five genotypes BWM-3, NIA-8/7, NIA-37/6, SI-91196 and BWS-78 had also significantly higher relative water content (RWC) percent under water stress, which indicated that these genotypes could be more tolerant to high water stress. Similarly genotypes NIA-8/7, Chakwal-86, NIA-25/1, MSH-22, BWM-84, BWS-77, Margalla-99, NIA-10/8 and Sarsabz showed more leaf area at various water stresses. At medium (two

irrigations) moisture stress NIA-8/7, NIA-10/8, NIA-25/1 and MSH-14 showed increase in chlorophyll content as compared to other genotypes.

**Keywords**: water stress, physiological parameters, wheat

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**P-11-837-1**

**Utilization of agricultural and industrial waste as carbon sources for α-amylase production by Bacillus megaterium**

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Amylases are one of the most important industrial enzymes and the sale is about 30% of the world's enzyme. Amylases have a wide range of applications in conversion of starch to sugar syrups, food, feed fermentation, textile, detergent and paper industries. In present work Bacillus megaterium was isolated from soil on starch agar medium by zone analysis method. Work was carried out for the optimization of α-amylase production by Bacillus megaterium using different agricultural (acid and alkaline hydrolyzed) and industrial waste carbon source such as molasses, date syrup, rice husk and sugarcane bagasse with different concentration (0.5 and 1.0 %). Maximum production of α-amylase was obtained on 0.5 % sugarcane bagasse hydrolyzed with 0.6N ammonium hydroxide (686Units/ml) at 7 hour of incubation. α-amylase production was also optimized by using different nitrogen sources such as peptone (control), tryptone, yeast extract, corn steep liquor, urea, sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride and ammonium sulphate and the maximum production of α-amylase were found in the presence of 1.5% peptone.

**Keywords**: amylase, industrial waste, Bacillus megaterium

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**P-11-851-1**

**In vitro callus proliferation in Melia azedarach L. and comparative antimicrobial potential of callus, leaf, bark and fruit extracts of bakain (Melia azedarach L.) & neem (Azadirachta indica A. Juss)**

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Melia azedarach L. is flashy fruited small to medium sized tree of "meliaceae" family. Different parts of this plant have repellant and antifeedant property. The improvement of the production of repellant and antifeedant compounds through plant tissue cultures could potentially provide a replacement for synthetic insecticides that are environmentally detrimental and often lose their effectiveness. The main theme of this study is to develop protocol for callus proliferation in M. azedarach in vitro and to investigate the comparative antimicrobial activity of callus, leaf, bark and fruit extracts of M. azedarach and A. indica. For callus induction, nodular stem sections were inoculated on MS medium containing different concentrations of 2-4-D, BAP and IBA. 20% extracts were prepared, form different explants of M. azedarach and A. indica, using water and methanol as solvent. The efficacy of extract of callus, leaf, bark and fruit of both species was determined by observing zones of inhibitions against four bacterial species Bacillus subtilis, Agrobacterium tumifacians, Staphylococuss aureus and Escherichia coli and four fungal species including Aspergillus candidas, Aspergillus niger, Fusarium solani and Pencillium lilicium. All the extracts from different explants showed variable growth inhibition responses.

**Keywords**: Melia azedarach L., callus, antimicrobial, bakain, neem

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**P-11-729-5**

**Microsattelite studies for screening of genetic variability among direct regenerants of sugarcane clone NIA-98**

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Direct regenerants in sugarcane was examined for genetic fidelity through SSR markers. The SSRs are comparatively a better tool for detecting polymorphism; it is so sensitive that it can reveal high allelic diversity. Fifteen regenerants were selected for this study. The total numbers of scorable bands were 37, out of which 30 were polymorphic and only 07 were monomorphic, indicating an intrinsic polymorphism rate of 81%. Fragments ranged in size from 117 bp to 2.19 kb. The number of fragments produced by various primers ranged from 2- 8, with an average of 3.7 fragments per primer. The level of polymorphism was varied with different primers. Maximum 08 bands were amplified with primer MsCir 55 and minimum 02 bands were amplified with primer MsCir-07, 12 and 27. Similarity coefficient reflects that all the selected directly regenerated plantlets irrespective of its treatments were genetically different with its parent and this difference is ranged from 5-70%. Direct regeneration method can be use for the genetic improvement instead of using it as tool for rapid clonal propagation.

**Keywords**: sugar cane, direct generation, SSRs

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**P-11-440-1**

**Optimization of the condition for tryptophan production by E.coli in the presence of Iranian cane molasses**

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Tryptophan is a fundamental precursor for a number of neurotransmitters in brain. In addition, tryptophan is an important material in the chemical synthesis of a range of pharmaceuticals. It has been used in treatment of depression, schizophrenia, and hypertension. Microbial fermentation strains allow the production of amino acids from cheap and renewable carbon source such as molasses. We have previously reported the importance of Iranian cane molasses to produce tryptophan by E.coli. In the present study, optimum condition has been introduced for production of tryptophan. The parameters that have been investigated are determination of the optimum concentration of salt and carbon source (molass), the optimum amount of inoculated bacterium, pH of culture medium and optimum shaking speed. Tryptophan production was measured using thin layer chromatography and HPLC followed by the chromatogram staining and scaning. The results showed that maximum growth is

obtained in the presence of 10 g/l cane molass. The optimum concentration of yeast extract was determined as the best nitrogen source. In addition, the 0.1% NaCl and 180 rpm shaking speed were assessed in the culture medium. After determining the optimum growth, the existence of tryptophan was followed in the culture medium. HPLC, TLC chromatograms and their scans showed that adding serine and PLP had not significant effect on tryptophan production.

**Keywords**: tryptophan, E.coli, Iranian cane molasses, optimization

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**P-11-729-6**

**An assessment of genetic diversity among wheat (Triticum aestivum L.) genotypes using random amplified polymorphic DNA (RAPD)**

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Twelve wheat varieties were assessed through RAPD for genetic diversity. Of forty primers, twelve were able to amplify the genomic DNA and it yielded 70 bands in which 48 were polymorphic. Maximum of 13 bands were amplified with primers A-10 while a minimum of 1 fragment was amplified with primer B-10. The size of amplified products was range between 220 bp to 4.48 kb. Some specific RAPD bands were also identified thus, reflecting the RAPDs application for the identification of wheat. Results revealed that variety C-591, Pasban, Barani, Punjab-81 and LU-26-S contain a specific segment of 1048 bp while Bakhar-2002, 2KCO-50 and Chakwal-87 contain a specific segment of 1112 bp amplified with primer B-10 and A-10 respectively. Genetically most similar genotypes were Punjab-81 and LU-26-S (71%) while most dissimilar genotypes were C-591 and 2KCO-50 (32%). On the basis of results achieved, the varieties could be divided into three groups, Punjab-81, LU-26-S, Pasban, and C-591 in a group, 2KCO-50, Chakwal 87, Rawal, and Bakhar-2002 clustered in C group. Another group B formed among QM-4531 and QM-4934. GA-2002 was falling in both clusters i.e. first and second showing 51% similarity with both the groups.

**Keywords**: wheat, genetic diversity, RAPD

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**P-10-813-1**

**OEC is the only biological factor oxidizing water**

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PhotosystemII from oxygenic organisms is unique among photosynthetic reaction centers in that it has the capability to oxidize H2O to O2. The oxygen produced by PSII is the source of oxygen in the atmosphere and has fundamentally changed the development of life on earth. PSII is the only biological system known that is capable of oxidizing water to molecular oxygen. The most fascinating and complicated aspect of PSII is accomplished by oxygen-evolving complex (OEC). The OEC is located on oxidizing side of PSII, and isolated within organelles known as chloroplasts, a plastid found in all plants and algae. The OEC is also found in one group of bacteria, the Cyanobacteria. The chemical reaction carried out by the OEC is oxidation of water to molecular oxygen, as shown here $2H2O \rightarrow O2 + 4H+ + 4e-$ The thermodynamics of this redox process are formidable. Water is an extremely poor electron donor, as expected, because oxygen is such a powerful electron acceptor. The OEC contains tetranuclear Mn cluster, which accumulates four oxidizing equivalents from four photochemical turnovers of the photoactive pigments and evolves one molecule of oxygen after four oxidations of the Mn ions. Checking structure and function of OEC we can achieve biological way of oxidizing water which will be a great achievement.

**Keywords**: photosystemII (PSII), oxygen-evolving complex (OEC), cyanobacteria

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**P-11-860-1**

**Optimization of culture condition for protease production by Aspergillus niger**

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Production of protease by Aspergillus niger was studied on the basis of various fermentation parameters such as incubation time (24-168), carbon sources (0.3%), nitrogen sources (0.02%), pH (3.0-10.0) and temperature (25-50°C). On the basis of present study, it was noted that Aspergillus niger secreted maximum protease (0.131Units/ml) when grown on mineral medium containing 0.3% glucose and 0.02% yeast extract as a carbon and nitrogen sources respectively in comparison to other carbon and nitrogen sources at 30±2°C for 96 hour. Where as, pH 7.0 and 35°C was found favorable for the higher yield of protease (0.199 Units/ml) by Aspergillus niger.

**Keywords**: Aspergillus niger, protease, yeast extract

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**P-11-873-1**

**Production of pectinase by Bacillus subtilis using date syrup as a carbon source in batch wise submerged condition**

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Nowadays, various research laboratories are trying to utilize inexpensive substrates like agricultural, industrial wastes and byproducts. In this study the waste of date industry was used as a carbon and energy source for the production of industrially important enzyme, which have wide range of applications in different areas. Pectinase production was investigated in batch wise submerged fermentation by Bacillus subtilis using date syrup as carbon source. The effect of different variables such as time of incubation, carbon sources, nitrogen sources and pH was determined for optimum production. The maximum level of pectinase production was noted when Bacillus subtilis was grown on mineral medium containing 1.5% date syrup and 0.75% yeast extract as carbon and nitrogen source respectively when incubated at 37°C for 8 hours with initial pH 8.0.

**Keywords**: Bacillus subtilis, date syrup, pectinase

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**P-11-733-1**

**Analysis of regulatory effects of FUR on expression of iron response gene in Escherichia coli**

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Escherichia coli counteract iron problems such as toxicity and poor bioavailability by employing at least two iron storage proteins. FtnA (ferritin), which acts as a major iron store and a haem-containing bacterioferritin (Bfr) whose role, is not clear. The global iron regulator protein, FUR (Ferric uptake regulator) regulates the expression of many iron response genes including the ftnA and bfr genes, which encode FtnA and Bfr respectively. Fe+2 –Fur complex represses transcription by binding to a 19 bp sequence (iron box) normally located near the pribnow box of cognate promoters. Fur can also act as a transcriptional activator switching on several genes including bfr and ftnA. A 71 bp intergenic region is located between the bfr and its upstream gene bfd. Herein, we studied the intergenic region to find a regulatory sequence effecting the bfr expression. To enable such analysis, different DNA fragments were made from bfr gene by cutting some nucleotids each time from bfr-bfd intergenic region gene. Plus some modification at them so, different constructs were made and have inserted in coding sequence of T4 vector containing Lac Z as reporter gene then the b-galactosidase activities were measured by ELISA photometric method. The results showed a sequence that affect regulation of bfr gene expression and have regulatory role for bfr expression.

**Keywords**: Escherichia coli, FtnA, bacterioferritin, iron box, b-galactosidase activities

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**P-11-840-1**

**Study the effect of different media on cell growth and recombinant protein expression in Pichia pastoris**

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Pichia pastoris is a species of methylotrophic yeast, which is frequently used as an expression system for the production of proteins. A number of properties make Pichia suited for this task, like a high growth rate and its ability to grow on a simple, inexpensive medium. Pichia can grow in either shake flasks or a fermenter, which makes it suitable for both small and large scale production. Different inducible and constitutive promoters have been used in P. pastoris for recombinant protein expression. In the present study, constitutive glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter was used for expression of a serine protease inhibitor in three different media. The rate of growth was studied at $OD_{600}$ and the level of protein expression was analyzed by ELISA method. In shake flask, the complex media had a better effect on growth rate than two other salty defined media, after 96h. The yield of recombinant protein reached to 450ng/ml in complex medium in compared with 300 ng/ml in defined media in 96 h. In conclusion; the complex medium is suitable for expression of our protein in shake flask.

**Keywords**: complex medium, defined medium, Pichia pastoris, recombinant protein

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**P-10-877-1**

**Optimization of cultural condition to improve production of recombinant bacteriorhodopsin mutant in E.coli**

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Bacteriorhodopsin (BR) is a light activated proton pump organized in the purple membrane patches of Halophilic archaeon. BR is involved in phototaxis of H. salinarium via generation of membrane potential change across the membrane. At present study, we evaluated the expression of a C-terminal fragment of bacteriorhodopsin by recombinant E. coli. The bacteriorhodopsin mutant gene was synthesized by consideration of E. coli codon usage. The synthesized gene was cloned in pET31a+ expression plasmid at Nde I and Hind III restriction sites and expressed under T7 promoter successfully. The expressed protein was analyzed by SDS-PAGE. The effect of temperature, induction time and ending time of induction were optimized.

**Keywords**: bacteriorhodopsin (BR), production, fermentation, expression

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**P-11-945-1**

**Properties of halophilic, acidotolerant maltogenic α-amylase from Nesterenkonia sp. F, moderately halophilic bacterium from Howz Soltan hypersaline lake**

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The use of amylases from moderate halophilic bacteria in industrial processes would have the advantage of the enzymes having optimal activities at high salt concentrations. We have selected the indigenous moderate halophile, *Nesterenkonia* sp. F, which produces an α-amylase that has shown remarkable properties. The pH and temperature profile for the cell-free filtrate was determined in various pH (4.0-10.0) using appropriate buffers and several temperatures (20° to 60° C), respectively. The NaCl in the culture medium was removed by extensive dialysis of the cell-free broth for an overnight. Dialysis overernight against buffers of 0.5-4.5 M NaCl was performed to assay the stability of enzyme in different NaCl concentrations. The activity of amylase in the culture filtrate was assayed in the presence of various metal ions, EDTA (1, 5, 10mM) and SDS. The reaction end products of amylase were analyzed by TLC. The enzyme showed maximum activity in 40° C and pH 5.0-7.0 and fairly stable in pH 5.0. Amylase activity was almost completely lost after dialysis against buffer without NaCl but optimally active at 2.5-3.0 M NaCl, although a remarkable activity was detected up to 4.5 M salts. Investigation of the end products after 24 h of reaction time, were formed in the order G2>G3>G4>G5>G6. The activity, except for Cu2+, Fe2+, and Fe3+ ions, extraordinarily

increased at 5 and 10 mM concentrations and decreased in 1mM of most of the ions even heavy metals, suggesting dependency to salts but it was sensitive to the presence of EDTA.

**Keywords**: halophiles, Nesterenkonia, alpha-amylase, activity

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**P-11-967-1**

**Knock down the expression of steroid receptor RNA activator (SRA) gene by microRNA adapted short hairpin RNA (shRNAmir)**

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SRA is a coregulator of steroid receptor transcriptional activities and enhances estrogen receptor(ER) transactivation by several folds. Since there is an increase in SRA expression in breast tumor in comparison to normal adjacent tissue, it has been suggested that SRA may play a role in cancer cells. In this study, we designed and developed a RNAi expression vector, microRNA adapted short hairpin RNA (shRNAmir) that mimics microRNA pathway inside the cell. shRNAmir-SRA was specific to SRA and constructed by SOE-PCR and cloning techniques then transfected into human breast cancer (MCF7) cells. SRA expression was estimated by Real Time PCR after two weeks selection with neomycin antibiotic. Our data showed about 60-70% expression reduction of SRA construct compared to control cells. From the obtained result we found that the shRNAmir-SRA could successfully knock down the expression of the target gene and may be suitable for a variety of applications, including tissue-specific knockdown and in vivo forward genetic screens. Further complementary studies are under progress in this regard.

**Keywords**: RNAi, steroid receptor activator (SRA), breast cancer, shRNAmir

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**O-11-729-8**

**Production of b-glucosidase by Penicillium lilacinum using agricultural waste as a substrate**

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Nowadays different laboratories of the world are trying to utilize renewable resources for the production of useful daily used goods. In this regard great attention has been focused on the utilization of agricultural and industrial waste and by products. Agricultural wastes treated with chemical and enzymatic method before using as a substrate for the growth of microorganism and production of various useful goods. In this study, sugarcane bagasse and leaves were treated with 0.6N H2SO4, HCl, HNO3 and HClO4 and the hydrolysate was used as a carbon source for the growth of Penicillium lilacinum and production of ß-glucosidase. The results reveals that high yield of ß-glucosidase 16.40 units/ml was achieved at 240 hrs, when 0.6N HClO4 treated sugarcane leaves hydrolysate was used as carbon source.

**Keywords**: beta-glucosidase, Penicillium lilacinum, sugarcae bagasse

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**P-11-934-1**

**Analyzing proteins and Isoenzymes of Listeria onocytogenesis**

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Listeria monocytogenes have 9 serotypes, in which serotypes 4a and 4b are most popular as human food borne diseases via dairy and meat type foods due to their psychrophilicity. Based on the lab reports the rate of Listerial infections has increased in recent years in Iran. In current study 2 isolated serotypes of Listeria monocytogenes from dairy foods including 4a and 4b were studied for protein profiles and isoenzyme analysis using SDS-PAGE and MLEE. In SDS-PAGE the protein profiles of 2 serotypes were mostly similar to each other but in compression to standard protein marker, there were many differences in 18 to 29 KD and zone of 68 KD. It was obvious that the most concentration of protein bands was distributed between zones of 14 to 97 KD. In PAGE for MLEE, 8 enzymatic systems included AP, GPI, ME, 6PGDH, and in 4b serotypes 13 isoenzyme were active; Based on the zymograms, it is concluded that the most differences between 2 serotypes were seen in GLDH, GaLDH, ME and 6PGDH systems, therefore, using these enzymatic systems can differentiate L.monocytogenes isolates.

**Keywords**: Listeria monocytogenesis, serotyps, proteins, isoenzymes, analysis

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**P-11-973-2**

**Comparing mannose binding lectin genetic diversity in intracellular and extracellular pathogens**

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One of the important immunological factors in diseases is mannose binding lectin (MBL). The aim of present study is to determine the distribution of the alleles of mannose-binding lectin gene codon 52, 54, 57 and promoter variants H/L, X/Y, P and Q in confirmed visceral leishmaniasis (VL) patients as an intracellular pathogen while compares with extracellular pathogens (in renal infection) and seek correlation between these variants and intracellular and extracellular infections. Fifty eight confirmed VL patients' blood samples were compared with fifty eight blood samples of patients received renal in results of renal infections. MBL genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Frequency of defective allele B in extracellular pathogens was more than intracellular pathogens (P=0.0001), and in contrary prevalence of wild type allele A in intracellular pathogens was more than extracellular pathogens (P=0.0001), and in other alleles and variants there was not any significant difference. In conclusion, there was more prevalence of alleles with low mannose binding lectin serum level in extrallelular pathogens which can be considered as a risk factor for these infections. On other hand, prevalence of high concentration alleles in intracellular pathogens indicates the role of mannose binding lectin level for susceptibility to intracellular pathogens.

**Keywords**: extracellular, genotype, intracellular, mannose binding lectin, pathogen

**P-11-989-1**

**The co-relation of genetics and phenology of Iranian onion genotypes to pink rot resistant**

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The cytology and phenology of some of the onion genotypes throughout Iran, include: Kashan-White, Azarshahr-Red, Isfahan-Dorcheh, Ghom-White, Zangan-Gholigheseh, Yasouj-Local, Herssin, Nishabour-Ishaghabad, Yazd-Abarkoh, Ramhormoz with various level of susceptibilities to pink root rot disease, against the two important commercial varieties, Texase-Erly-Grano, Yellow sweet Spanish were taken into consideration. The results after the Arc Sin and subjection to DMRT-analysis and, also to cluster analysis according to Ward's minimum variance method, using the cluster procedure of SAS computer software, the genotypes basal chromosome number was=4 for all the studied genotypes. Number of chromosomes, length of the longest chromosome, length of the shortest chromosome, longest/shortest length ratio, average of long arm/short arm ratio, average of chromosomes ratio were recorded. The types of chromosomes were metacantric, submetacentric and subtelocentric. Comparison of relative length of the shortest chromosome (s %) showed that, there are cultivars, with the higher symmetric karyotype and also in reverse directions, that is with the lesser symmetric karyotype respectively. The growth indices for these genotypes in Isfahan conditions in the two levels of green house and field in a randomized complete design with 4 replications in the field and 15 replications in the green house, including Crop Growth Rate=CGR, Relative Growth Rate=RGR, Net Assimilation Rate=NAR, and Leaf Area Index=LAI were assessed. The results revealed that cultivars were significantly different in growth indices. Ramhormoz had the smallest and Kashan white the greatest Leaf Area Index in the field and green house conditions. Ramhormoz and Gholi ghese had Smallest and Greatest respectively in Crop Growth Rate and Net Assimilation Rate of Kashan white and Yellow Sweet Spanish were higher than other genotypes. There was also a significant effect as far as the interactions between the cytology, morphology and resistance levels of genotypes are concerned.

**Keywords**: genetics, phenology, onion, resistance, pink rot

**P-11-624-1**

**Thermostable alkaline amylase production by a native bacterium isolated from soils around Tehran**

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Amylolytic enzymes are among the most important enzymes from a biotechnological view point. Amylases account for about 25% of enzyme market with applications in sugar, textile, paper, bakery, pharmaceutical industries. Thermostable amylases have advantages including decreased risk of contamination, cost of external cooling and increased diffusion rates. A thermostable alkaline α-amylase producing bacterium was isolated from soils around Tehran. The bacterium was isolated under selective growth temperature of 60ºC. This bacterium was able to produce biomaterials including amylase and high amounts of extracellular polymers giving interesting features to it. Molecular identification of this bacterium using PCR with universal primers (27F and 1492R) and sequencing 16s rDNA was indicating to highest similarity with Bacillus licheniformis. However the morphology was totally different from that of a control strain. Amylase production was studied in a medium containing (%) starch 1, trypton 0.2, K2HPO4 0.1, Na 2HPO4 0.25, NaCl 0.1, (NH4)2 SO4 0.2, CaCl2 0.005, MgSO4 7.H2O 0.005 under various culture conditions. The supernatant was used as amylase source for enzyme assays. Optimum temperature for enzyme production was 48ºC. The effects of pH ranging from 5-11 were studied on enzyme production and growth. Both growth and enzyme production were highest at pH 9. Assays showed significant thermostability keeping about 78% of its maximum activity at 80ºC.

**Keywords**: amylolytic enzymes, α-amylase, thermostable

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**Evaluation, isolation and identification of salmonella typhimurium in milk by PCR method**

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Salmonella is one of the important food poisoning agents. Identification of these bacteria is by using routine media cultures that is time consuming and does not have good precision. In the present study we compared PCR as an alternative, rapid and more precise method in identification of Salmonella typhimurium in milk with routine media cultures method. Six pasteurized milk sample bags were purchased and divided in 2 parts. Then half of them were contaminated with different numbers of Salmonella typhimurium (10-1 -103) manually. The second half was used as a control sample and was without any contamination. Then all were enriched in BPW (buffer Peptone Water) media and analyzed by PCR and routine methods. Comparing two methods, PCR method identified 5-10 Salmonella typhimurium in contaminated culture media during 8-12 hours of enrichment with minimum of one bacterium after 16 hours. In routine culture media Salmonella typhimurium were also identified, however, it required longer time (4-6 days) and was less precise. The control milk samples revealed that the purchased product were safe to be consumed. Our observations revealed that comparing two methods, time required for PCR is less than using routine methods (<24 hours vs. 5 days) for isolation, identification, and typing of Salmonella typhimurium in milk. Furthermore, its precision and sensitivity is more. Therefore, it can be considered as a good alternative for this purpose.

**Keywords**: Salmonella typhimurium, PCR, microbial culture, milk

**O-11-624-2**

**Microbial production of lycopene by using the metabolic engineering tools**

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Carotenoids are a diverse class of natural products that play an important role in all living organisms. Carotenoids are naturally synthesized by plants, fungi, algae and some bacteria. Animals can't produce carotenoids de-novo and they receive them from their diet. Lycopene, a proven antioxidant, is one of these carotenoids preventing the oxidation of low density lipoprotein (LDL) and cholesterol, thereby reducing the risk of developing atherosclerosis and coronary heart disease. Some research suggests that lycopene is associated with reduced risk of macular degenerative disease, serum lipid oxidation, and cancers of lung, bladder, cervix and skin. So lycopene is a very useful phytochemical nutraceutical and holds great commercial value. Despite the availability of synthetic carotenoids, in recent years, carotenoid production from microbial source is receiving great attention owning to public sensitivity regarding "synthetic food additives" and availability of microbial carotenoid genes. In this study, the genes that are required to convert lycopene precursor (IPP) to lycopene are expressed in E.coli. The lycopene pathway genes crt E, crt B, crt I from Erwinia herbicula were used for lycopene production by E. coli. The recombinant E.coli produced lycopene aerobically up to 167mg/L. The effect of idi gene encoding isopentenyl diphosphate (IPP) isomerase on the lycopene production was studied. This enzyme catalyzes a key step on carotenoids production. The obtained result highlights the significant effect of IPP isomerase on improved accumulation of lycopene in E. coli. The optimized expression construct of these four genes increased lycopene production up to ±335 mg/L.

**Keywords**: carotenoid, lycopene, metabolic engineering, recombinant E.coli

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**P-11-1017-1**

**Lentiviral-transduced human mesenchymal stem cells persistently express therapeutic levels of alpha-1 antitrypsin**

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Alpha-1 antitrypsin deficiency (AATD) is a common genetic disorder that primarily affects the lungs and liver and can lead to emphysema and progressive liver dysfunction and failure. AAT is a protease inhibitor the primary function of which is to inhibit neutrophil elastase, a protease that can degrade all of the constituents of the pulmonary connective tissue matrix. Gene therapy, whereby a normal AAT gene is delivered to the target cell by an efficient vector can result in the synthesis and secretion of the functional protein into plasma and offers the opportunity to correct the primary abnormality and restore synthesis of AAT. Genetically modified mesenchymal stem cells (MSCs) are potentially valuable tools for the novel treatment of human AATD. In the present study we investigate the utility of BMMSCs as a novel cellular vehicle for delivery of the correct AAT gene to patients suffering from AATD. To test this hypothesis, hMSCs were isolated using a combination of density gradient centrifugation and plastic adherence culture-expanded. The ex vivo expanded cells were applied to FACS analysis to confirm the exclusion of hematopoietic cell contamination and the expression of BMMSC markers by the use of surface antigens CD34, CD45, CD44, CD166, C105, CD106, and CD73. The transgene AAT-Jred chimer was transferred to hMSCs using a lentivirally vector and its expression was visualized by fluorescent microscopy. The expression of the AAT gene in hMSCs was further confirmed using RT-PCR. Secretion of AAT from hMSCs into the medium was determined using ELISA. Flow cytometric analysis revealed that isolated hMSCs were positive for CD44, CD73, CD105 and CD166, but showed no expression of CD34 and CD45. The integrity of hMSCs was further confirmed by their differentiation potential to osteogenic and adipogenic lineages. By employing a lentivirally vector efficient genetic loading of hMSCs with the AAT gene was achieved. ELISA assay confirmed the secretion of AAT from hMSCs to medium. These finding showed that hMSCs posses the key properties that ensure their employment as a cellular vehicles to deliver therapeutic gene to body and could provide a means for replacing AAT proteins in AAT deficient patients or other essential serum protein in other deficiencies.

**Keywords**: AAT deficiency, mesenchymal stem cells, gene therapy, bone marrow, 1-antitrypsin, lung

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**P-11-919-1**

**Application of Industrial biotechnology for the production of bio-based chemicals – a cradle-to-grave perspective**

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This review gives an indication of the current developments in the transition to bio-based production, with a focus on the production of chemicals, and points out some of the challenges that exist in the large-scale implementation of industrial biotechnology. Environmental pressures and a shift towards the use of agricultural-based raw materials, as well as rapid developments in the science supporting biotechnology, have stimulated the increasing focus on sustainable industrial processes for the chemical industry. The recent sequencing of the human genome and the associated sequencing of industrial bacterial and yeast genomes have also played their part. In addition, the fields of metabolic engineering, bioinformatics and computer-based modeling and process optimization are opening up opportunities for new products and cost reductions. Furthermore, the importance of evaluating the environmental impact of bio-based products with respect to their entire life cycle is highlighted, demonstrating that the choice of the raw material often turns out to be an important parameter influencing the life cycle performance.

**Keywords**: industrial biotechnology, human genome, metabolic engineering

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**O-10-1092-1**
**Exploitation of eco friendly bacteria for sustainable agriculture**

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Considerable attention has been focused recently on microbial involvement in degradation and synthesis processes in the environment, known as biodegradation and biosynthesis. Eco friendly bacteria are of tremendous importance in various aspects of biotechnology including agriculture biotechnology. Several indigenous bacterial strains were found capable of performing various beneficial processes for improving agricultural productivity, such as biopolymer production, phosphate solubilization, releases of free phosphate and essential micronutrient and production of plant growth hormones. Two properties biopolymer production and plant growth promotion have been studied in detail. The biopolymers were water absorbing compounds and involved in soil fertility. The biopolymer, alginate produced by CMG 1421 was extracellular, acidic in nature consists of mannouronic acid exclusively and has molecular weight ranging from 20,000-250,000 Da. The operon for alginate biosynthesis in CMG 1421 (alg D, alg 8, alg 44, alg E, alg x) has been isolated, and sequenced. Plant growth promoting bacteria (PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms. The ability of some microorganisms to convert insoluble phosphorus (P) to accessible form, like orthophosphate, is an important trait in increasing plant yields. In addition to release of free phosphate our isolates were also found to produce plant growth hormones, indole acetic acid and insole butyric acid. These were found to promote growth of maize and mung been plants. A pqq gene cluster producing PQQ was detected in some strains; the operon having the genes involved (pqq) in phosphate solubilization has been isolated and sequenced. A consortium of these has been prepared to enhance agricultural productivity. The details shall be described during presentation.

**Keywords**: indole acetic acid, indole butyric acid, plant growth promoting bacteria, phosphate solubilization, pqq operon