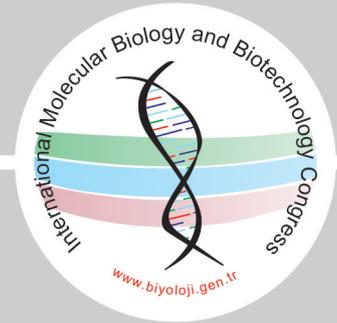


3rd International Molecular Biology and Biotechnology Congress

June 02 - 06, 2014

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ABSTRACT BOOK

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**ORAL PRESENTATION
ABSTRACTS**

Investigation of Replication of A Novel Plasmid (pHIG22) from *Thermus scotoductus* K6

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Abstract

A small, novel and multicopy cryptic plasmid, named pHIG22, was isolated from *Thermus scotoductus* K6. The nucleotide sequence of pHIG22 revealed that the plasmid was 2222 bp long, with a total G + C content of 63%. According to BLAST result, the sequence of pHIG22 didn't show any similarities to any other plasmids. The sequence of novel plasmid were analysed by various bioinformatic tools such as motif analyser, ORF finder, possible rep protein determinator, promoter predictor, direct repeats-palindroms finder, etc.

To determine the replication origion and replicase of pHIG22, it was amplified by PCR from five different locations and cloned into pUC18-HTK (including highly thermostable kanamycin cassette). pUC18-HTK can not replicate in *Thermus*. All cloning experiments were done in *E.coli* JM101 strain. All pUC18-HTK-pHIG22 clones were transformed into *Thermus thermophilus* HB27, *Thermus thermophilus* TH104, *Thermus sp.* M5, *Thermus sp.* 6 and *Thermus* 7. Only one of the construct (pUC18-HTK-pHIG22/5) was able to replicate in *Thermus thermophilus* HB27 at 70°C in modified TM medium meaning that pHIG22 is amplified out of rep ori and replicase regions.

Deletions, RT PCR and TEM searches with pHIG22 is continuing to find out the exact limits of rep ori and its replicase strain.

Keywords: Hybrid plasmid, pHIG22 plasmid, thermophilic transformation, highly thermostable kanamycin cassette.

Acknowledgement: This study was supported by TUBITAK (Project No: 112T277).

Determination of Self-Compatibility Status of *Thermopsis turcica* Through Histological Analysis

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Abstract

Thermopsis turcica Kit Tan, Vural & Kucukoduk (*Fabaceae*) is the critically endangered and endemic Turkish species located between the south-western part of Aksehir Lake and the southern part of Eber Lake. The uniform occurrence of at least two free carpels of *T. turcica* is the first record in the subfamily *Papilioideae* (= *Faboideae*) of *Leguminosae*. Although this genotype are valuable as females, to date there is no consideration has been given on fertilization biology of *T. turcica*. In the present study, 2 populations of species *T. turcica* (Eber and Aksehir populations) were used. Selfing with plants from two populations and reciprocal crosses between two populations of *T. turcica* were performed at Nezahat Gökyiğit Botanical Garden during pollination period of May and June 2012. Pistil samples were collected from 1th to 10th day of the pollination without damaging the population. All pistils collected were fixed in FPA-70 solution and stored at +4 °C until microscopic observations. Pistil samples were stained with aniline blue. After staining, pistils were cut into two parts (stigma with style and ovary) and were further cut longitudinally, split into two parts. All samples were observed under a fluorescence microscope. In all third day-old samples of *T. turcica* (both of self- and interpopulation pollinated samples), pollen grain germination was observed. As a consequence of histological analysis's results, all samples of *T. turcica* have been clearly identified self-compatible.

Keywords: *Thermopsis turcica*, hybridization, self-compatible, pollen grain germination

Acknowledgement: We would like to thank the Nezahat Gokyigit Botanical Garden, of Istanbul, for providing the research materials presented in this study. We are also grateful to Prof. Yesim Yalcin-Mendi and Prof. Yildiz Aka-Kacar from Cukurova University, of Adana, to provide laboratory facilities for histological analysis.

Genotoxicity Induced by Amorphous Silica Nanoparticles

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Abstract

Silica nanoparticles (SiO₂, NPs) are used in various industries such as food, cosmetics, optical imaging, cancer therapy, targeted and controlled drug release. Although human exposure to SiO₂ NPs is highly frequent, the impact on human health is remain unclear. In this study, genotoxicity induced by SiO₂ NPs in human lymphocytes was investigated using chromosome aberration (CA), sister chromatid exchange (SCE), micronucleus (MN) and comet assays. Firstly, SiO₂ NPs (Sigma-Aldrich, amorphous, 12 nm) examined by transmission electron microscopy (TEM) and dynamic light scattering (DLS). They were generally spherical and polydispersed in deionized water. However, multilateral, shapeless, agglomerates and branched structures were also observed. Size distributions were between 12.07-115.50 nm by EM. Average hydrodynamic diameter was 709 nm, zeta potential was -31±1, showing that the surface of SiO₂ NPs is negatively charged. Lymphocytes were treated with 10, 50, 250 and 1000 µg/ml concentrations of SiO₂ NPs. The frequency of CAs increased at all treatments, but this increase was significant at only 250 and 1000 µg/ml at 24h and, at only 1000 µg/ml at 48h treatment. Statistically significant increase was also observed in SCE/cell at all treatments. All concentrations of SiO₂ NPs (except the lowest, 10 µg/ml) significantly and dose dependently (r=0.94) increased the frequency of MN. The primary DNA damage detected by comet assay significantly increased at all concentrations and both treatment times (2h and 3h) compared to control. It can be concluded that SiO₂ NPs have clastogenic, mutagenic, and DNA damaging effect on human lymphocytes.

Keywords: SiO₂ nanoparticles, chromosome aberration, sister chromatid exchange, micronucleus, comet assay.

Antiproliferative Property and Apoptotic Effect Of A Novel Coordination Compound Containing Au^I(CN)₂ On Some Cancer Cell Lines

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Abstract

Introduction; Cancer is the second leading cause of death after diseases of cardiovascular system in the world. Coordination compounds have been used in medicine for treatment of various diseases including cancer. The present study was designed to determine antiproliferative and apoptotic effect for newly synthesized cyano-bridged {Au^I(CN)₂} coordination compound, coded as AK11b (ZnC₁₄H₃₂N₆O₄Au), against on HeLa, C6 and HT29 cancer cell lines.

Material&method; The new coordination compound containing Au^I(CN)₂ was synthesized using "brick-mortar" method [1]. The antiproliferative and cytotoxic activities of AK11b on tumor cell lines were determined using BrdU Cell Proliferation Assay (BCPA) and lactate dehydrogenase assay (LDH assay) respectively. The mechanism of action of the AK11b was clarified using DNA laddering assay and TUNEL assay.

Result; According to BCPA and LDH test results, AK11b was significantly antiproliferative and cytotoxic on tumor cell lines compared to control anticancer drug, 5-fluorouracil (5-FU). The LDH test results revealed that AK11b was significantly cytotoxic than 5-FU, suggesting that AK11b may be detrimental to the cell membrane. The compound AK11b caused laddering of genomic DNA, indicating that it may act through inducing apoptosis on the cells. The results of the study revealed that the AK11b is a promising potent antiproliferative agent for cancer cell lines by inducing apoptosis.

Keywords: Coordination Complexes, Anticancer Activity, AK11b

Anti-cancer and Apoptotic Activity of A Novel Coordination Compound Containing $\text{Ag}^{\text{I}}(\text{CN})_2$ In Some Cancer Cell Lines

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Abstract

Background: Coordination compounds have been providing exciting development of metal-based therapeutics. We have been exploring the antiproliferative and apoptotic effect of newly synthesized cyano-bridged $\{\text{Ag}^{\text{I}}(\text{CN})_2\}$ coordination compound, AN11 ($\text{CuC}_{11}\text{H}_{16}\text{N}_7\text{O}_2\text{Ag}_3$), against on HeLa, C6 and HT29 cancer cell lines.

Materials and Methods: The coordination compound containing $\text{Ag}^{\text{I}}(\text{CN})_2$ was synthesized using "brick-mortar" method [1]. *In vivo* cytotoxicity of AN11 was evaluated by lactate dehydrogenase assay (LDH assay) against on cancer cell lines. The antiproliferative activity of AN11 was assessed against cancer cell lines using BrdU Cell Proliferation Assay (BCPA). DNA laddering and TUNEL assays were used to determine whether this compound induce DNA degradation and apoptosis in tumor cells.

Results: According to BCPA and LDH test results, this coordination compound was more significantly inhibited the viability of cancer cells than 5-fluorouracil (5-FU), an anticancer drug. Remarkably, the LDH test results disclosed that AN11 was significantly cytotoxic than 5-FU, suggesting that this compound may affect membrane integrity of tumor cells. Furthermore, AN11 caused the laddering of genomic DNA, indicating apoptosis.

Conclusion: Our preliminary data strongly indicate that this compound may be potential therapeutic agent for cancer therapy.

Keywords: Coordination Complexes, Anticancer Activity, AN11

Pharmacokinetics and Dosage Regimen of Ciprofloxacin Following Single Intramuscular Administration in *Nili/Ravi* Buffalos

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Abstract

The present study was undertaken with the objective to determine the pharmacokinetics and optimal dosage regimen of ciprofloxacin in *Nili/Ravi* buffalos. For this purpose, the drug was administered intramuscularly at 5 mg/kg body weight in each of eight animals. Following ciprofloxacin administration, blood samples were collected at different time intervals and analyzed for ciprofloxacin using HPLC. Pharmacokinetic parameters were calculated using two compartment open model. Peak plasma concentration (C_{max}) of ciprofloxacin, 4.89 ± 0.28 µg/mL was achieved at 0.87 ± 0.03 hours (T_{max}). Values for half life of absorption (t_{1/2} abs), distribution (t_{1/2} α) and elimination (t_{1/2} β) were 0.45 ± 0.03 , 0.45 ± 0.03 and 3.05 ± 0.20 hours, respectively. The value for apparent volume of distribution (V_d) was 1.09 ± 0.06 L/kg, area under the curve (AUC) was 20.28 ± 1.13 µg.hr/mL and total body clearance (CL) was 0.25 ± 0.02 L/hr/kg. Based on these parameters, an optimal intramuscular dosage of ciprofloxacin in adult *Nili/Ravi* buffalos was calculated as 17.86 mg/kg, to be repeated after 24 hours interval. These results show that ciprofloxacin in these buffalos has the general pharmacokinetic characteristics of a typical fluoroquinolone antimicrobial agent. That is, it has distribution, clearance and half life that are similar to other studies. Based on these results, it was concluded that calculated dose was higher than the dose recommended by the manufacturer and to avoid drug residues in the meat and antimicrobial resistance, this locally investigated dosage regimen should be strictly followed in local buffalos.

The Effects of Nonylphenol on Gamete Physiology in Bovine

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Abstract

Alkylphenol ethoxylates (APEOs) are used as non-ionic surfactants in variety of industrial, agricultural and domestic products such as pesticides, detergents, paints and cosmetics. Therefore, these compounds can reach to human being through foodchains. These endocrine disrupters also called Xenoestrogen have estrogenic, carcinogenic and toxic effects. Among the degradation products of APEOs, the main groups such as alkylphenol (AP) and nonylphenol (NP), which generate the molecule are revealed. NP exert its estrogenic effects by binding to estrogen receptors. NP causes morphological and functional alterations in male and female genital tract and mammary glands. These alterations may reduce fertility, mammary and prostate cancer. Therefore, the main purpose of this study is to determine the adverse effects of NP on sperm and oocytes. The effects environmentally relevant NP concentrations such as 0.01, 0.1, 1, 10 and 100 µg NP/ml were chosen. NP mediated abnormalities in sperm DNA and oocyte maturation were investigated. Tunel assay was employed to determine the adverse effects of NP on sperm DNA, whereas the effects of NP on oocyte maturation in tissue culture medium-199 (TCM-199) were tested. The present study demonstrated that 100 µg NP/ml concentration induced apoptosis by causing DNA breaks in bovine sperm cells. This study also showed that 100 µg NP/ml concentration inhibits oocyte maturation. It is concluded that NP have adverse effects on the integrity of sperm DNA and oocyte maturation.

Keywords: Nonylphenol, sperm, oocyte, Tunel Assay, endocrine disruptors

Can Toxicity of Imidacloprid With Water Extracts of Various Plants Be Removed?

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Abstract

Pesticides are chemicals that have been produced against pests that threaten public health and agricultural places. Compounds which affect on insects of these chemicals are called insecticide. The newest classes of insecticides are neonicotinoids which have been specifically produced for invertebrates.

This study is intended to show the toxic effects of imidacloprid (IMI) which are one of neonicotinoids insecticides on life span of *Drosophila melanogaster* removed with the water extracts of *Salvia lavandifolia*, *Hypericum scabrum*, *Capsella bursa-pastoris* and *Teucrium orientale* plants.

Sets of experiments containing application groups at different concentrations (0.5, 1.0, 1.5 and 2.0 ppm) and IMI + plant extract (1:1 v / v) were prepared. All applications were separately made at the female and the male populations of Oregon R wild type strain of *D. melanogaster*. The counting was continued until the last individual died.

According to the data obtained, the IMI shortened the lifespan of *Drosophila melanogaster* depending on increase of the dose. All plant extracts significantly reduced toxic effects of IMI in both female and male populations and have increased the longevity of *Drosophila melanogaster*

Keywords: *Drosophila melanogaster*, Plant extract, Imidacloprid, Life span.

Drinking water denitrification using a novel sulfur-based autotrophic membrane bioreactor

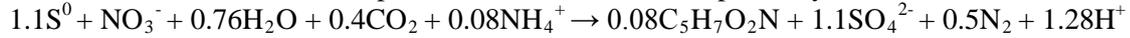
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Abstract

In the present study, a novel sulfur-based autotrophic denitrifying membrane bioreactor was tested in detail for nitrate and nitrite removal from drinking water. In recent years, sulfur-based autotrophic process has drawn significant attention due to its high efficiency, elimination of carbon requirement, and the possibility of contaminating treated effluent by organic compounds. In the process, nitrate and sulfur are used as electron acceptor, and electron source, respectively, as illustrated below.



In the literature, generally column based bioreactor processes have been used for sulfur-based autotrophic denitrification. In this kind of applications, sulfur granules should be big enough not to cause clogging and to eliminate the washout of sulfur granules. Also, treated effluent may be contaminated with the bacteria sloughing from sulfur-bed. Considering these kind of disadvantages of the column based applications, in our study, a novel sulfur based autotrophic denitrification process has been used for the first time. A bench-scale 4 L membrane bioreactor equipped with hydrophilic flat sheet polyethersulfone (PES) membrane (0.45 µm) was used. Trans membrane pressure (TMP), pH and oxidation reduction potential (ORP) were measured on-line. The reactor and the membrane performances were evaluated at varying hydraulic and nitrate loading conditions. Sulfur was externally added to the reactor two times in a week considering the theoretical requirement according to the equation given above. Almost complete denitrification efficiency was achieved when the influent nitrate concentrations were 25-50 mg/L NO₃⁻-N at HRT as low as 5 h. The generated sulfate was close to the theoretical value. Reactor was operated successfully at fluxes of 20 and 40 L/m²/h.

Keywords: Autotrophic denitrification, drinking water, membrane bioreactor, sulfur-based autotrophic denitrification

Characterization of symbiotic capsules containing lactulose and *Pediococcus pentosaceus* OZF

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Abstract

The incorporation of probiotics and prebiotics into foods and diet supplements is considered to be very important due to their benefits on the host. However, one of the major problems of probiotic treatment is the loss in the viability of the strains during gastrointestinal (GI) transit. The aim of this study was to develop a safe and efficient delivery system targeted into the host intestine for a probiotic *Pediococcus pentosaceus* OZF, isolated from human breast milk, with a complementary prebiotic. The effect of different prebiotics (fructo-oligosaccharide, lactulose, Hi-maize starch and inuline) supporting the growth of OZF strain, was investigated by *in vitro* fermentation. Compared to other tested prebiotics, lactulose was significantly improved the growth of OZF strain ($p<0.05$). Encapsulation parameters such as alginate, CaCl₂ and lactulose concentration, and gelling time were optimized and second coating was performed using the different bio-polymers (alginate, chitosan, poly-L-lysine, whey proteins) for the purpose of improving the stability of the symbiotic capsules. The morphological and surface properties of the capsules were analyzed by Scanning Electron Microscope and the viability of the OZF strain in simulated gastric and bile conditions was evaluated. In conclusion, double coated co-encapsulation was significantly improved the viability of OZF strain compared to its non-encapsulated form under GI system conditions ($p<0.05$) and provided a controlled release of the cells into the host intestine.

Keywords: *Pediococcus pentosaceus* OZF, encapsulation, probiotic, prebiotic, symbiotic

Two New Fungal Isolates with a Potential for Ethanol Production from Lignocellulosic Materials and Their Molecular Identification

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Abstract

Recently there has been a growing interest in identification of new ethanol producing microorganism strains with lignocellulolytic activities due to the non-edible property of lignocellulose as a feedstock and the high potential of lignocellulosic bioethanol as a major fuel type for the replacement with fossil fuels in the near future.

The aim of the present study was determined as isolation of fungal strains with lignocellulosic activity and determination of their ethanol production capabilities. Decaying woody materials were collected from Erzurum and near locations, and aseptically transferred to the laboratory. Purification of isolates was done according to general procedures. After lignocellulolytic activity determination tests, the ethanol production determination for each active isolate was done by cultivation in modified BMC media and ethanol levels were determined by gas chromatography method. Molecular identification of the isolates was done by using PCR with universal ITS primers, sequencing of amplicons and the BLAST analysis of NCBI database.

According to the lignocellulolytic activity results, two active strains (MG46 and MG59) were determined. MG59 produced bioethanol at 3.38 g/L a concentration in modified BMC media for 5 days fermentation process, but MG46 did not at the same conditions. Further, MG46 was identified as *Botryotinia fuckeliana* and MG59 as *Trichoderma citrinoviride*.

In a conclusion, the experimental data and results offer that *Trichoderma citrinoviride* MG59 strain show valuable properties for development of technologies in the bioethanol production from lignocellulosic biomass. The amount of ethanol production can be increased by optimization studies in the future.

Keywords: Bioethanol, Biomass, Lignocellulose, Renewable Energy.

Acknowledgment: The authors would like to express appreciation for the support of the sponsors [Republic of Turkey – Ministry of Food, Agriculture and Livestock: TAGEM-13/ARGE/17].

Investigation of the transcriptomics alterations in human colon cancer development and progression

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Abstract

Aim: The aim of this study was to determine the differences of gene expression in normal colon (CRN) and colorectal cancer (CRC) by using microarray system.

Introduction: Cancer is the most important diseases characterised by unregulated cell growth and the invasion and spread of cells from the site of origin, or primary site, to other sites in the body. Colon cancers have become the most common malignancy in both developed and developing countries including Turkey.

Methods: Total RNA was extracted from tissue samples of 12 patients with colorectal cancer using the Qiagen miRNeasy Kit. miRNA was polyadenylated by using PolyA Tailing master mix. After Flash Tag Biotin HSR Ligation samples were hybridized, stained and washed. The arrays were finally scanned using AGCC Scan control programme according to the manufacturer's protocol of Affymetrix GeneChip software.

Results: It was found that some of miRNAs were found up or down regulated in CRC cells compared to non tumor cells. miRNA-21, miRNA-34, miRNA-181a and miRNA-let7g were overexpressed and miRNA-139, miRNA-486, miRNA-378 and miRNA133 were downregulated in patients with CRC. We have also found that three dysregulated miRNA's, which to our knowledge have not previously been associated with colorectal carcinogenesis.

Conclusion: Consequently, the results of this study will increase our understanding of development, progression and earlier detection and personnel treatment of colon cancer.

Keywords: Colon cancer, Microarray, miRNAs, Transcriptomics.

Ethic number : 74059997.050.01.04/020

Molecular Epidemiological Investigation of Multistate Outbreaks of *Salmonella* Enteritidis from Clinical and Environmental Samples in Turkey, 2000-2010.

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Abstract

In this study a total of 55 interrelated *Salmonella* serotype Enteritidis stock strains selected from the culture collection of Turkey National Enteric Pathogen Reference Laboratory were investigated by plasmid profile analysis with the method defined by Kado and Liu and pulsed field gel electrophoresis (PFGE) according to World Health Organization protocols using *Xba*I macrorestriction enzyme for better understanding of the molecular epidemiology of *S. Enteritidis*. Also, all strains were analysed antimicrobial susceptibilities with disc diffusion method. The study strains were selected from clinical and environmental samples from different regions in Turkey between 2000 and 2010. Strains were scanned against 20 antibiotics and 3 out of them (amikacin, ciprofloxacin, gentamicin) were found sensitive to all strains. 5 isolates had no plasmid. Most of test strains carry 57 kb plasmid in common and 15 genotypes were identified among the 55 isolates. By PFGE, 6 genotypes were related closely, 3 genotypes were undistinguished. Also, 6 genotypes were unrelated. To our knowledge, it is the first report on the phenotypic and molecular characterization of *S. Enteritidis* isolates from both environmental samples and clinical isolates in Turkey.

Keywords: *Salmonella enteritidis*, PFGE, Plasmid Profil analysis, Turkey

Antimicrobial, antioxidant and cytotoxic activities of *Satureja khuzestanica*

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Abstract

Satureja khuzestanica is an endemic annual plant of Lorestan province, Iran. This plant has been employed as analgesic and antiseptic in the southern parts of Iran. The main components of the wild *S. khuzestanica* were carvacrol (93.9%) eugenol (1.0%) p-cymene (0.8%) and thymol (0.6%). Carvacrol has been found to have significant antioxidant properties. Because of these medical features, several members of this genus have been widely used in alternative medicine. Methanol and ethanol extracts of *S. khuzestanica* were screened for their antimicrobial activity against eight bacteria. Both plant extract showed good inhibitory activity against both gram negative and positive bacteria. The best inhibitory activity was observed with methanol extract. In addition, the plant extracts were tested for their antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The methanol extract of *S. khuzestanica* had strong antioxidant activity. In addition, the cytotoxic activity of the methanol and ethanol extract was evaluated on HeLa cell line using MTT method, The methanol extract of *S. khuzestanica* showed good cytotoxicity which was concentration dependent. This result indicated the potential anticancer activity of this plant

Keywords: *S. khuzestanica*, antimicrobial activity, antioxidant activity

First Report On C-Banding and Nor Location of Endangered Leuciscine Fish, *Squalius anatolicus* (Bogutskaya, 1997) (Teleostei, Cyprinidae) From The Central Anatolia

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Abstract

In this study, the description of the karyotype of the endangered chub *Squalius anatolicus* (Bogutskaya, 1997) is presented by means of conventional staining (Giemsa-staining). As well C-banding and Silver staining (Ag-NORs) techniques were applied in the study. This endemic species have strict distribution where only central part of the Anatolia. *S. anatolicus* was found to have diploid chromosome number $2n=50$ (10 m+22sm+10st+8a, NF=82). Sex chromosomes were not detected. C-banding technique revealed that many chromosomes show clear pericentromeric blocks of constitutive heterochromatin. Ag-NORs treatments revealed consistent positive signals located at the end of the short arms of a submetacentric chromosome pair. Silver nitrate (AgNO₃) staining also showed that NOR (nucleolus organizer region) length heteromorphism in the homologous chromosomes. NOR locations are useful cytological characters for taxonomic and evolutionary studies. Therefore our data will be provide contribution for cytogenetic comparative against other members of the genus *Squalius*.

Keywords: *Squalius anatolicus*, karyotype, C-banding, NOR.

The Anti-cancer Effect of the Copolymer-drug Conjugate Containing Methotrexate (Amethopterin) on MCF-7 Cell Lines and Its Toxic Effect on L929 Cell Lines

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Abstract

Objective: The drug product Methotrexate, which is used for treatment of breast cancer, has a limited use due to the fact that it has too many side effects depending on its toxic effect on tissues. The objectives of this study are to bind the drug product Methotrexate to maleic anhydride vinyl acetate copolymer (MAVA) to increase its solubility in body fluids; to decrease its toxic effects; and to increase its anticancer activity compared to crude drug. Furthermore, antimicrobial activity of the synthesized copolymer was investigated.

Material and Methods: Synthesized MAVA copolymer's antibacterial and antifungal effects were determined through disc diffusion method. Structural characterization of the MAVA-Methotrexate copolymer-drug conjugate was performed by Fourier Transform Infrared Spectroscopy (FTIR) and Proton Nuclear Magnetic Resonance Spectroscopy (¹HNMR). Its solubility in water and the behavior of copolymer-drug conjugate in PBS (Phosphate Buffer Saline) at hour 1, 24 and 48 were investigated. Anticancer activity of copolymer-drug conjugate on MCF-7 cells was determined by XTT assay in comparison with anticancer activity of the crude drug, while its toxic effects on L929 cells were determined by XTT Assay again in comparison with the crude drug. The results were analyzed statistically with the Mann-Whitney U Test.

Results: It was found that MAVA copolymer doesn't have any antimicrobial activity according to our study. The synthesized copolymer-drug conjugate which is structurally characterized and whose solubility in water is studied was determined to be bound to each other with a quite successful reaction by means of amidation mechanism; to be water-soluble; and to have a long time effect in PBS. While the highest concentration of Methotrexate has a killing effect at a rate of 58,43% on MCF-7 cells, MAVA-Methotrexate conjugate has a rate of 65,19% (p<0,05). When the toxic effect of copolymer-drug conjugate on L929 cell lines was investigated, it was observed that its vitality rate (77,10%) was greater than the drug product (75,45%); that is to say, its toxic effect on cells was lesser.

Conclusion: Since the conjugation reaction of the synthesized MAVA-Methotrexate copolymer-drug conjugate was successful, the conjugate was determined to be biocompatible; to have good water-soluble characteristics; and to show compatible behaviors in PBS buffer solution as well, unlike the copolymer it contained. It was also observed that current anticancer activity of the active ingredient of the drug product was increased as the result of the formation of copolymer-drug conjugate, and that also its toxic effect was decreased, compared to the crude drug it contained. For the further stage, it is planned to test MAVA- Methotrexate conjugate in animal experiments and to develop it into chemotherapy drug, directing it to clinical research.

Keywords: Methotrexate, anticancer, antimicrobial, MAVA

DNA Interaction and Antimicrobial Activities of Ferrocenyl Cyclotetraphosphazenes

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Abstract

Phosphazenes (N=PX₂)_n are the compounds containing double bonds between phosphorus and nitrogen atoms which they may have the open chain (linear), cyclic or polymeric structures. Cyclophosphazenes are an important class of inorganic ring systems, and exhibit very different physical and chemical properties depending on the types and properties of the bonded substituents to the P-atoms. In this study, the tetrameric phosphazene derivatives which contain three different substituents are screened for antimicrobial activity against various microorganisms and interactions with pBR322 plasmid DNA. The pyrrolidino-substituted tetrameric phosphazenes (1a-3a) are found to be effective against all tested microorganisms. The morpholino-substituted tetrameric phosphazene (3b) is active against yeast strains. The results show that antimicrobial activity and DNA interactions considerably vary according to substituents bonded to P-atoms on the tetrameric phosphazene ring.

Keywords: Ferrocenyl cyclotetraphosphazene, antimicrobial activity, DNA interaction

Investigations into Structural and Biochemical Properties of Nuclear Lamins

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Abstract

The nuclear lamins are type V intermediate filament proteins which are important structural elements for the nuclear envelope. The lamins bind the chromatin domains to the nuclear periphery and localize some of the nuclear envelope proteins. In addition, they are related with the regulation of nuclear processes including chromatin organization, DNA replication, transcription and cellular differentiation. It is suggested that lamins may regulate nuclear functions by directly interacting with the chromatins and controlling the position of chromosomes within the nuclear space.

Interest in the nuclear lamina has rapidly increased. This is due to many devastating diseases caused by more than 400 distinct mutations in the LMNA gene; diseases such as Emery–Dreifuss muscular dystrophy (EDMD), dilated cardiomyopathy type 1A, the segmental premature ageing diseases Hutchinson–Gilford progeria syndrome (HGPS) and atypical Werner’s progeria. Different lamin expressions have also been reported in various cancers. The increase in mechanical stress resulting from high lamin levels and the alterations in lamin expressions could modulate cell proliferation, differentiation and migration, each of which is an important step in cancer progression. Different hypotheses have been proposed to explain the molecular mechanism underlying these diverse diseases. Although our knowledge on the functions of nuclear lamins has increased, additional studies are needed to understand its molecular mechanisms. These studies could shed a light on the diagnosis and treatment of the diseases.

Keywords: Nuclear Lamins, LMNA gene, progeria syndrome

Antifibrotic and Protective Effects of Vitamin D on Experimentally Induced Liver Fibrosis in Rats

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Abstract

Chronic or acute liver damage causes fibrosis characterized by the aggregation of ECM. In recent years, antifibrotic and antiproliferative properties of 1,25(OH)₂D₃, the active form of Vitamin D on renal and lung fibrosis has been investigated. Little is known about the role of Vitamin D in hepatic fibrosis. The aim of this study was to investigate the protective and antifibrotic effects of Vitamin D against hepatic fibrosis. 18 male rats were organized into 3 groups: Control (G1), CCl₄ group (G2) and Vitamin D administered CCl₄ group (G3). Hepatic fibrosis was induced by CCl₄ dissolved in corn oil (1:1) (1,5 µl/g) twice a week for 12 weeks. Corn oil (1,5 µl/g) was administered to G1 using identical methods and time intervals. At the beginning of CCl₄ injection, 1,25(OH)₂D₃ dissolved in corn oil (0,5 µg/kg) was administered daily. Liver tissues were fixed in 10% formalin, dehydrated and embedded in paraffin. Sections were stained with H&E and Masson's trichrome. G1 displayed normal structure in both stainings. In the Masson-trichrome stained sections, hepatic fibrosis was observed especially around the portal area, central vein and between portal veins in G2. Contrary to these findings, fibrosis disappeared in G3. In some of the H&E stained sections, there was sinusoidal dilatation around the central vein, mononuclear cell filtration and hemorrhage in the portal area of G2. These pathologic findings decreased in G3 and liver tissue showed similarity to G1. Histological evaluations suggest that Vitamin D may play a protective and antifibrotic role against CCl₄- induced liver injury.

Keywords: Hepatic fibrosis, CCl₄, Vitamin D, Light microscope

The Genotoxic and Oxidative Effects of Some Edible Insects *In vitro*

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Abstract

Edible insects offer an important nutritional resource for humans, because these insects are rich in protein, fat, carbohydrates, various vitamins, and minerals. While eating of insects has become widespread in parts of the world, very limited information is available concerning with effect on human health of their consuming as food. In this study, the genotoxic and oxidative effects potentials of extracts of *Caelifera* sp, *Gryllotalpa* sp., *Onitis* sp., *Omphisa fuscidentalis*, and *Oecophylla smaragdina* have been assessed on cultured human blood cells. The extracts were added to the cultures at 12 different concentrations (0-2000 mg/L). Micronucleus (MN) tests were used to monitor the DNA and chromosomal damage produced by aqueous extracts *in vitro*. In addition, to assess the oxidative effects, total antioxidant capacity (TAC) and total oxidant status (TOS) levels were also measured. The results of the study revealed that these extracts haven't genotoxic effects at the tested concentrations. However, dose-related alterations in both TAC and TOS levels were observed as depending on the type of extract. In the light of present findings, it was concluded that the studied insects can be consumed safely, but it is necessary to consider the cellular damage which is likely to appear depending on the extent of oxidative stress. *In vitro* approach used here which includes the collaborative use of genetic endpoints and oxidative stress markers is a valuable technique for comparing the possible health risks of edible insects in relation to mutagenesis and carcinogenesis.

Keywords: Genotoxicity, micronucleus test, oxidative status, edible insects.

Effects on Oxidative Stress of Hazelnut and Oleic Acid in Rats Fed A Rich-Cholesterol Diet

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Abstract

Objective: Hyperlipidemia; plasma triglycerides and/or cholesterol level is elevated. Are transported in the form of triglycerides and cholesterol in plasma lipoproteins. Hyperlipidemia therefore also depends on the increase in plasma lipoprotein levels. About this study, consumption of antioxidant compounds containing hazelnut and oleic acid serum lipid profile, Thiobarbituric acid Reactive Substances (TBARS) and blood Glutathione (GSH) levels were examined for their effect on.

Material and Methods: In our study, 32 Wistar Albino rats were used. Experimental animals were divided into 4 groups of 8 rats each: 1. Control 2. Hyperlipidemic 3. Hyperlipidemic-Hazelnut 4. Hyperlipidemic + Oleic acid. To create an effective model of hyperlipidemia were identified as 12 weeks experimental period. Blood samples were used to evaluate lipid profile (by using commercial assay kits), GSH (by using Ellman method), TBARS (by using Ledwozyw method).

Conclusion: When the hyperlipidemic group compared with the control group, in the hyperlipidemic group serum triglycerides, total cholesterol, LDL- C, Total lipid, LPO, AI and AIP levels significantly increased, HDL-C levels significantly decreased. In the hyperlipidemic+hazelnut group, serum HDL-C, blood GSH levels significantly increased while levels of serum LPO and AIP are significantly decreased. In the hyperlipidemic + oleic acid group, serum HDL-C and blood GSH levels significantly increased while levels of serum LPO and AIP are significantly decreased.

Results: Consequently fed a diet rich in cholesterol in persons hazelnut and/or oleic acid consumption the the protective nature of cardiovascular disease has been identified.

Keywords: hyperlipidemia, hazelnut, oleic acid, oxidant-antioxidant balance

Are there another laboratory parameters, which can be used in the interpretation of blood culture results?

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Abstract

Blood culture is gold standart for detection of bloodstream infections. Blood culture contamination leads to inappropriate or unnecessary antibiotic therapy, and likely to increase antibiotic resistance, prolonged hospitalization, additional hospital costs. Studies over the past decades investigated new tests or markers that allow more rapid and useful/cheap detection of infective agents. Several investigations have identified procalcitonin(PRC), the precursor of calcitonin, C-reactive protein(CRP), mean platelet volume(MPV), for prediction of blood culture contamination. In this study, we aimed to examine the effectiveness of NLR, MPV, PRC and CRP for decision-making process of blood stream infection and contamination. This retrospective study involved a total of 4216 patients; clinical and laboratory data from 615 positive, 132 negative and 84 contaminated samples were examined. Patients were randomly selected; no bacterial growth in blood culture(NBG), growth accepted pathogenic microorganisms(GAP), coagulase-negative staphylococci(CNS) and contaminated culture(CC). 10 ml blood sample inoculated into per BacT/ALERT culture bottle and incubations were performed using BacT/ALERT® 3D(bioMérieux, Inc. France) automated system. Blood sample were gathered in a hematologic sample tube with anticoagulant and following haematology parameters were investigated: white blood cell(WBC), neutrophils(NEU), lymphocytes(LYM), mean platelet volume(MPV), using with Cell-Dyn 3700SL haematology analyzer(Abbott, USA). WBC and MPV values were not statistically significant differences between groups. But NEU, LYM and NLR were shown statistically significant differences between groups($p < 0,001$). There were statistically significant differences PRC positivity and PRC serum concentration level with $p = 0,002$ and $p = 0,009$ respectively between groups. In conclusion Ultimately, our findings in the appropriate patient profile indicated that, NLR and PRC results might help to define bacteriemia.

Keywords: Blood culture, contamination, bacteremia

Efficient regeneration system from rye leaf base segments

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Abstract

Rye is extremely recalcitrant to tissue culture. Efficient plant regeneration system from leaf base segments of rye (*Secale cereale* L.) was developed. The factors affecting the callus formation and regeneration capacity of leaf segments of diploid and tetraploid *Secale cereale* plants were investigated. The highest callus formation rate (10.4%) and shoot formation (4.5%) were achieved in the first segments. Two different media type, N6 and MS medium were also investigated. The highest callus (15.78%) and shoot (6.7%) formation were observed in MS medium. Different carbohydrate sources which were 20 gr/L sucrose and 20 gr /L maltose were assessed to determine the effect on callus and shoot formation. The highest callus formation rate (11.72 %) was observed in medium containing 20 gr/L Maltose. Based on shoot formation, sucrose (5.9%) performed better than maltose (3.13%).

Keywords: auxins, cytokinins, regeneration, carbohydrates.

Proliferative and Apoptotic Effects of Lichen Secondary Metabolite Lobaric Acid on A549 Tumor-Cell Line

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Abstract

Aim: Cancer is currently known as the second most deadly disease today after heart attack, and many communities have begun to use alternative treatment methods as well as the normal treatment methods. Known as symbiotic associations of algae and fungi, lichens have been commonly used in antioxidant studies recently. Our study was directed to cancer studies as the antioxidative and antigenotoxic properties of lichens have frequently been determined recently. Therefore, in this study, the aim is to observe the effects of lobaric acid on A549 tumor-cell line, initiated from a human alveolar cell carcinoma.

Material and Methods: A549 tumor-cell lines that were obtained by cell culture assay treated with lobaric acid at 24 and 48th hours, proliferation and apoptosis situations were observed by cytotoxicity assays LDH and WST-1.

Results: In conclusion it was observed that lobaric acid was stopped the proliferation and induced the apoptosis at 48th hour. It was also observed that 100 and 200 micromolar doses were the best concentrations.

Conclusion: It was known that lobaric acid has antioxidative and antigenotoxic effects and this study is more important than the others as using lichen secondary metabolites instead of lichen extracts. In the future, we will confirm the reason of cell death by several late apoptosis tests.

Keywords: Lobaric acid, anticancer, Apoptotic effect

Resistance Mechanisms in Chronic Myeloid Leukemia Treatment: Why TKIs Don't Cure Chronic Myeloid Leukemia?

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Abstract

Chronic Myeloid Leukemia (CML) is a clonal multi-step myeloproliferative disorder of pluripotent hematopoietic stem/ progenitor cells. The molecular hallmark is the presence of a reciprocal chromosomal translocation (9;22) and fusion gene that is called BCR-ABL oncogene in myeloid progenitors. CML is one of the best understood cancer type probably because it has simple etiology than the other cancer and comparatively easy to monitor in the clinic. Two decades ago first tyrosine kinase inhibitor (TKI) Imatinib has been introduced in the market for the treatment of the disease. It was the first successful example of a TKI used for the treatment of CML patients with chronic phase and showed important improvements in response and overall survival rate. However, significant portions of CML patients failed to respond to therapy and acquired resistance against to drug have been reported. The recognition of the problem of imatinib resistance caused to the design second generation TKIs, nilotinib and dasatinib and they have higher efficiency in Imatinib-resistant/intolerant patients in clinical trials. Although impressive clinical responses are obtained in second generation TKIs treatment, some CML patients are also develop resistance to them. Research activities showed that the mechanism of TKI resistance are mutations in BCR-ABL gene, amplification of BCR-ABL gene, clonal evolution, low intracellular drug uptakes by disordered expression of influx and efflux proteins and activation of alternative signaling pathways by oncogenic proteins. The mechanisms of resistance against TKIs in CML should be discussed together with strategies to overcome and to prevent resistance with available drug.

Keywords: CML, BCR-ABL, resistance, TKIs

Enhancing of Cold Tolerance in Beans (*Phaseolus vulgaris*) by Using Bacteria Isolated from Apoplast of Cold-Resistant Wild Plants

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Abstract

It was investigated potential of that bacteria isolated from apoplast of cold-resistant wild plants can used in enhancing of cold tolerance of cold-sensitive plants. Bacteria were isolated from leaf apoplast of cold-resistant wild plants and inoculated at leaves of bean (*Phaseolus vulgaris*). Bean plants with/without inoculation were exposed to cold stress and some physiological parameters were evaluated.

Cold-resistant plants (*Verbascum cheirenthifolium*, *Capsella bursa-pastoris*, *Artemisia austriaca*) were collected at Palandöken Mountain (Erzurum/Turkey) in February-March months. Plants were transported to laboratory as soon as possible and their separated leaves were sterilized. Then apoplastic fluid (extracellular fluid) of leaves was obtained by vacuum-infiltrating. Apoplastic fluid including apoplastic bacteria was inoculated in Petri dishes including Nutrient-agar and they were transferred in an incubator (4 °C). After 10 days growing bacteria were purified and carried out identification of their species. *Serratia plymuthica*, *Staphylococcus aureus*, *Pseudomonas syringae* were isolated from apoplast of leaves of the wild plants. Bean plants were grown at 24/20 °C, and when seedlings were 10 day-old, the bacteria was inoculated at their leaves and they were transferred in a growing chamber (8/5 °C) for 3 days. %Freezing injury and ice nucleation activity were determined at fresh leaves of the seedlings.

%Freezing injury increased at cold conditions while decreased at leaves inoculated with the bacteria. %Freezing injury was 83% at cold while was 45% by inoculation of *Serratia plymuthica* at same conditions. Application of bacteria increased ice nucleation activity (decreasing of freezing point). Inoculation of *Serratia plymuthica*, for instance, decreased by 1.9 °C freezing point of apoplastic fluid by increasing by 27% the activity. Results from these two experiments were evaluated together with findings from SDS-PAGE of extracellular proteins secreted by the bacteria. In conclusion, it is suggested that our bacteria inoculated at bean can improve its cold tolerance by decreasing freezing injury and freezing point of apoplastic fluid.

Keywords: Apoplast, Ice nucleation activity, freezing Injury, *Phaseolus vulgaris*, PGPB bacteria

Callus Induction, In vitro Shoot Development and Somaclonal Variations in Cotton (*Gossypium hirsutum* L.)

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Abstract

Callus induction and regeneration were studied in the two Turkish cotton varieties (Nazilli 84S and Sahin 2000) possessing different sensitivity levels to salt. Also, the rate of polymorphisms was investigated using random amplified polymorphic DNA (RAPD) in the plant samples obtained from the shoot tip and node cultures.

The cotyledon and hypocotyl explants gave better response in terms of callus induction when compared with the root explants. Hypocotyl explants were cultured in the Murashige-Skoog (1962) medium supplemented with 0.1 mg L⁻¹ kinetin, 0.1 mg L⁻¹ 2,4-D and 0.1 mg L⁻¹ IAA and 77% and 84% callus induction was observed in Nazilli 84S and Sahin 2000 cultivars, respectively. When MS medium was supplemented with various plant growth regulators, callus induction from the cotyledon and hypocotyl explants were observed. However, plant regeneration was not evident.

When, 0.1 mg L⁻¹ TDZ and 0.1 mg L⁻¹ kinetin were added to the MS medium, 100% explant response was seen in both cultivars in the shoot tip cultures. Moreover, in the cotyledonary nodes grown in the MS medium with the addition of 0.25 mg L⁻¹ kinetin, the explant response was 86% and 68% for Nazilli 84S and Sahin 2000, respectively.

Also, the genetic similarity of the plants grown from the shoot tip culture was detected to be around 94–99%, whereas the polymorphism rate for these plants was calculated to be 10%. Interestingly, when the plants obtained from the node culture were used, the polymorphism rate was 16.6% for Nazilli 84S and 9.8% for Sahin 2000 cultivars.

Keywords: Cotton (*Gossypium hirsutum* L.), callus induction, shoot tip culture, node culture, RAPD.

A Comparison of the Parallel Sparse Index, and Wheeler's Data Compression Algorithm

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Abstract

Alignment of genomic reads involves mapping of short reads from a particular individual onto a pre-sequenced reference genome of the same or similar species. Individuals of the same or similar species share the majority of their genomes. Therefore, short reads alignment provides a much more efficient way to sequence the genome of a particular individual than does direct sequencing. Many strategies are proposed for this alignment process. Indexing the reference genome and performing short read search over the index is a dominant, and more preferable technique for both time and memory concerns. M. O. Kulekci et al recently developed a space-efficient indexing via sparse suffix arrays with fast searching capability to catch the lengthy short reads produced by the next generation high-throughput DNA sequencing technology. Our aim is to make a comparison between this technique, and the historical block sorting lossless data compression algorithm proposed by M. Burrows, and D.J. Wheeler. We have seen that parallel sparse index read aligner by defining the rightmost mismatch criteria that prioritize the errors towards the end of the reads, where errors are more probable, supplies approximate matching capability which was missing. We show that indexing a genome with sparse suffix arrays is a good alternative to the Burrows-Wheeler transform.

Keywords: Genome Indexing, Parallele Sparse Index, Data compression, Burrows Wheller Transform

Investigation of SIRT1-7 Gene Expression Levels with High Capacity RT-PCR in Multiple Sclerosis

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Abstract

Introduction and Aim:

Multiple sclerosis (MS) is a chronic disease characterized by de-myelination and axon damage causing hardened tissues in the brain and / or spine by causing dead tissues, which develop as a result of autoimmune mechanisms triggered by environmental factors in genetically sensitive individuals, that may persist for a lifetime, where involvement of multiple regions may be seen in the central nervous system. The exact cause of MS is not known, in spite of extensive investigations. Mammals have 7 sirtuin proteins (SIRT1-7). Although these sirtuins are relatively protected, N and C terminals are different. They can accomplish various biological functions by means of these differences. Sirtuin (SIRT) genes play a role in the regulation of many functions, including human metabolism, ageing, cancer, urea cycle, stress, and cell cycle. Activation of the SIRT1 gene was shown to prevent Alzheimer's disease, and suppression of this gene was shown to do the opposite in a study on the genetics of ageing. Investigation of expression of SIRT family of genes and those genes that they are in relation in biological processes and delineating a possible association with MS development was aimed in the present study.

Material and Methods

mRNA was measured in peripheral blood samples of 95 patients with MS and 95 healthy individuals without a known neurodegenerative disease and compared with expression of SIRT genes in both groups by Fluidigm quantitative RT-PCR Array. The data obtained were analyzed with Mann – Whitney U test, after GAPDH and Beta – Actin normalization and calculation of the ratios of the results of patients and healthy controls.

Findings and Discussion

Expressions of SIRT1*1 (p : 0.0000732), SIRT5*3 (p : 0.0000800) and SIRT5*4 (p : 0.0005274) genes were found to be significantly (p < 0.05) decreased after the statistical evaluations. Further analyses were planned for future, after this study. SIRT family and the genes that they are in interaction with in their pathways are considered promising for the diagnosis and treatment of MS disease.

Keywords: sirtuin, Fluidigm Dynamic Array, gene expression, multiple sclerosis

A Molecular Phylogeny of the genera of Apiaceae (Umbelliferae) inferred from nuclear ribosomal internal transcribed spacer (ITS)

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Abstract

The family Apiaceae (Umbelliferae) is one of the largest family in terms of genera in Turkey. Some members of Apiaceae are economically important because of their usages as vegetables (e.g., parsley, dill, parsnip, celery) and spices (e.g., coriander, anise and cumin). They also have wide application in traditional medicine. In this study, it is aimed to determine the genetic distances of nine genera (*Chaerophyllum* L., *Anthriscus* Pers., *Scandix* L., *Pimpinella* L., *Ferulago* W. Koch, *Malabaila* Hoffm., *Turgenia* Hoffm., *Daucus* L., *Artemisia* L.) from Apiaceae family naturally distributed in different places in Bitlis and Elazığ. For this purpose, 12 taxa were sampled and ITS region of nrDNA was sequenced to assess the genetic variation among the genera. Two major clusters were obtained with respect to the maximum likelihood phylogenetic tree. According to the result, *Artemisia* was found the most diverse genera among these taxa. Detail results will present in the meeting. It is expected to provide a new clear aspect of view in phylogenetic variation among the genera of Apiaceae with respect to our findings.

Keywords: Apiaceae, phylogeny, nrDNA

Fermentation Assisted Production Of PHA From Alternative Carbon Sources

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Abstract

Polyhydroxyalkanoates (PHA) are recognized as completely biosynthetic and biodegradable with zero toxic waste, and completely recyclable into organic waste. However, petroleum-based plastic products are not biodegradable, resulting in a significant burden on solid waste management. Polyhydroxyalkanoates are one of the possible solutions to replace some petroleum-based polymers. The major bottleneck with large production of PHA production is the cost associated with scale-up. The goal of this research will be to investigate the engineering parameters to improve on the production of PHA from *E.coli* harboring the pBHR68 plasmid. For this purpose, conventional air-sparged fermentations were conducted for the production of PHB at agitation rates of 350, 500, 750rpm and initial-oxygen sparge rates of 0.4 and 0.8vvm. Analysis of OD, CFU, DCW, Glucose assay and PHB production were carried out at every 4 hour. The highest PHB production was at 750rpm 0.8vvm as 57.15% mass of PHB in dry mass after 48h.

One of the major cost of associated with bacterial PHB scale-up is the carbon substrate. Selecting a carbon source that is inexpensive, readily available and renewable with little compromise to PHB yield and quality is desired. The carbon sources that were used in this study were: glucose, microalgae. Microalgae were collected from The Logan City Wastewater treatment plant and dried. Dry microalgae were hydrolyzed with H₂SO₄. After hydrolysis, centrifuge harvested algae was used as a carbon. Analysis of OD, CFU, DCW, Glucose assay and PHB production were carried out at every 4 hour. PHB production was 13.25% mass of PHB in dry mass after 48h in medium containing 7.5% algae

Keywords: Polyhydroxyalkanoates, Fermentation, Microalgae

Preparation of an Aminoacid Sensitive Biosensor and Determination of Its Working Conditions

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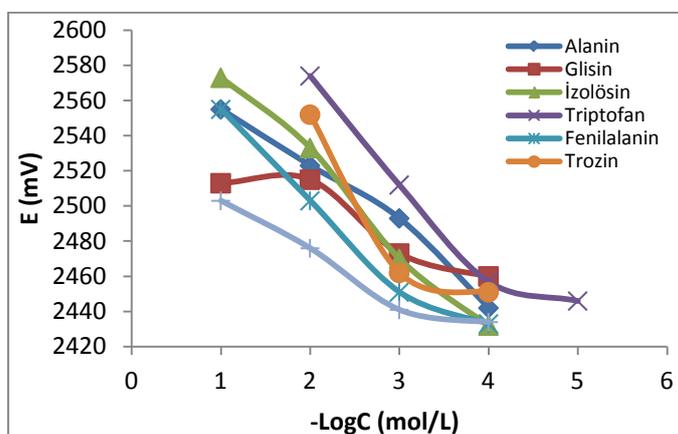
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Abstract

Simultaneous determination of free aminoacids are performed using liquid chromatographic methods. Most of the developed liquid chromatographic methods are indirect (post-column and pre-column) spectrophotometric methods. Due to that these methods are expensive, time consuming, non selective, and nonsensitive, some new methods have been investigated. This work deals with preparation of micro-size composite aminoacid sensitive biocensors and their usability as detector in liquid injection analysis systems for aminoacids. An aminoacid sensitive biosensor was prepared by coating biolayer on the surface of composite structured pH sensor surface.

Biosensor prepared in this way not constituted of internal reference electrode or internal reference solution.

Potentiometric performance of aminoacid sensitive biosensor (selectivity constant, linear working interval, detection limit, reaction time, pH working interval, repeatability, life time, and time dependent potential decay) was investigated by computer-controlled measurement system in static and dynamic conditions. Potentiometric attitude of biosensor towards aminoacids in 1.0×10^{-1} - 1.0×10^{-5} mol L⁻¹ concentration interval was shown in Scheme 1. Flow-through cell of developed micro-sized biosensor with 1 microliter dead volume was prepared. The prepared biocensor flow cell was exploited as a detector successfully in flow injection analysis system.



Scheme 1. Potentiometric behavior of aminoacid biosensor against free aminoacids.

*This work has been supported by the Turkish scientific and technological council with the Project (TBAG 110T793)

Asymmetric Matrices to Improve the Sensitivity of Sequence Alignment

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Abstract

In the literature efforts have been made to improve the sensitivity of sequence alignment by constructing specialized substitution matrices specific for particular protein classes. PHAT (21) and SLIM (22) matrices, are derived from curated collections of transmembrane proteins. Matrix-construction strategies starting from curated alignments, as for the PAM and BLOSUM series, cannot in principle yield valid asymmetric target frequencies and substitution scores. This is because the initial aligned sequences are treated symmetrically, with no justifiable distinction between query and subject. In this article we work on an asymmetric matrix that maintains consistency between the background and target frequencies. It requires as input only the pair of sequences being compared and a valid general-purpose substitution matrix.

Effects of Different Forms of Humic Substances on Physiological Properties and Shoot Regeneration of *Brassica napus* L. c.v. PR46W31

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Abstract

In this study, effects of various concentrations of potassium humate (KH), boron humate (BH) and iron humate (FeH) on physiological characteristics and shoot regeneration which is an important oil plant of the use of rapeseed were investigated. Sterilized seeds were cultured on MS, 3% (w/v) sucrose and 0.7% (w/v) agar containing 1 ppm, 3 ppm, 5 ppm and 7 ppm KH, BH and FeH. Stem segments 3-4 mm in length were excised from 3-4 week old seedlings obtained from the seeds. Stem explants were cultured on MS with 1mg^l⁻¹ 6-benzylaminopurine (BAP), 0,2 mg^l⁻¹ 1-naphthaleneacetic acid (NAA). According to the results, increasing doses of humate reduce the width of the lamina. 1 and 5 ppm FeH was measured the maximum length in root (8,16 cm) and the maximum length of the stem length (9,94cm) 3 ppm in BH. Fresh (0,215 gr) and dry weight (0,0125 gr) increased at 1 ppm KH. It was observed that fresh and dry weight decreased at 7 ppm of all humate forms. Humate doses increased with increasing amounts of photosynthetic pigments and the highest results of photosynthetic pigments were measured at FeH doses. It was observed that callus induction was 100%. The highest callus weight (0,134g) was obtained at 3ppm KH. The highest total shoot number per petri dish (15.33) was seen at 1 ppm FeH. The highest shoot regeneration (30%) were found at control medium and 7 ppm KH. As a results, it was shown that all the humate forms had positive effects on physiological parameters, but there were no effect on shoot regeneration.

Keywords: *Brassica napus* L., Humate, Shoot regeneration

Molecular Cloning and Expression of Bile Salt Hydrolase (BSH) Gene from *Lactobacillus plantarum* GD2 Strain

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Abstract

Bile Salts Hydrolase (BSH) is an enzyme produced by the intestinal microflora that catalyzes the deconjugation of glycine- or taurine-linked bile salts. BSHs from different lactobacilli strains exhibit higher variation in sequence, pH optimum, kinetic properties and substrate specificity. In this study, *bsh1* gene from *L. plantarum* GD2 was cloned into pET22b expression vector and expressed in BLR (DE3) strain of *E. coli* by induction of 0.01 mM of isopropylthiogalactopyranoside. The *bsh1* gene was amplified by PCR using species specific oligonucleotide primers and then cloned into pBluescript cloning vector and then pET22b expression vector. Clones were transformed to XL1-blue and BLR (DE3) strains of *E. coli* respectively. The overexpressed recombinant protein was purified by B-PER 6xHis Fusion Protein Purification Kit and deconjugation ability of the enzyme was tested with six different conjugated bile salts by Ninhydrin protein assay. Biochemical characterization of the purified bile salt hydrolase from *L. plantarum* GD2 revealed some distinct characteristics, not observed in other species of *lactobacillus*. Comparison of the deduced amino acid sequences of these four genes with previously known sequences revealed high homology with BSH enzymes of several microorganisms. The purified *bsh1* enzyme showed highest activity against taurodeoxycholic and glycodeoxycholic acids, moderate activity against taurocholic and glycocholic acids, and minimum activity against taurochenodeoxycholic and taurochenodeoxycholic acids. To investigate key residues of the active site, substrate binding pocket and substrate selectivity of BSH enzyme, site-directed mutagenesis on conserved amino acids of BSH enzyme and structural analysis will be required in the future.

Keywords: Bile Salt Hydrolase (BSH), Probiotics, *Lactobacillus plantarum* and gene cloning

This work was supported by Scientific Research Projects of Abant İzzet Baysal University (2011.03.01.406, to M.Ö.).

The effect of Baicalein on miR-25 and PDCD6IP expression in osteosarcoma cell line Saos-2

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Abstract

Baicalein has started to attract attention in recent years due to its ability to induce apoptosis and inhibit cell growth in various cancer types. It is derived from the *Scutellaria baicalensis* plant. The anti-cancer effects of baicalein is demonstrated in this study*, as well as its effects on miR-25 and *PDCD6* gene expression. miR-25 is expressed in different levels in number of cancer types and it has been suggested to have both oncogenic and tumor suppressor function. One of the target genes of miR-25 is *PDCD6IP* which blocks apoptosis in some cells. PDCD6IP binds to the product of the *PDCD6* gene, a protein required for apoptosis, in a calcium-dependent manner and may be responsible for the protection against cell death. The relationship between miR-25, which is changing the expression in tumors, and expression of PDCD6IP on the apoptosis connection was studied. The gene of *PDCD6IP* indirectly interacts with PDCD6 for apoptosis and also changes in *PDCD6* gene expression are determined.

2µM baicalein was applied to the Saos-2 cells. After treatment, at 2th, 4th, 8th, 24th and 48th hours, cells were collected and total RNA was isolated. cDNA was synthesized for microRNA and related gene expression by using reverse transcription. In order to determine the changes in expression of miR-25 and other genes, quantitative PCR was performed.

According to results, it was observed that miR-25 and *PDCD6* gene expression increased, also *PDCD6IP* gene expression decreased at 24th hour. These results demonstrate that there is a correlation between increased expression of miR-25 and *PDCD6IP* and *PDCD6* gene expression during occurrence of apoptosis induced by baicalein.

* In this study, data from FEF-13026 numbered project which was supported by Adnan Menderes University Scientific Research Projects Department were used.

Keywords: Baicalein, miR-25, Saos-2, *PDCD6*, *PDCD6IP*

Comparative Analysis of Phytochemical Constituents, Antioxidant and Antimicrobial Activity, Phenolic and Protein Content of Karaman-Grown Apples

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Abstract

A healthy diet, consisted of fruits and vegetables, has been known to be effective in the prevention of chronic diseases. Apples are a very significant part of the human diet since they are rich in phenolic compounds and antioxidant activity. Currently, Turkey is the fifth country for apple production in the world and Karaman province is the second biggest apple producer of Turkey.

The aim of this study was the comparative investigation of phytochemical composition, antioxidant and antimicrobial activities, total phenolic and protein content of apples. To mimic general public apple consumption, Golden Delicious, Red Delicious (Starking) and Granny Smith apple types, which are produced and consumed at the highest amount, were obtained from local market farmers in Karaman and water extracts of apples were investigated. Peel and fleshy parts of apples were separately peeled, dried, grinded and extracted. Compositions of the water extracts were determined by gas chromatography-mass spectroscopy (GC-MS) technique and twelve different components were identified in investigated samples. The highest chemical composition ratio was predicted in Golden Delicious type apples. The highest antioxidant activity was determined in peels of the Starking type apples (5.59 mg/ml for 50% DPPH inhibition) with corresponding total phenolic content (>40 µg/ml). Lowest antioxidant activity (16.53 µg/ml) was detected in peels of Granny Smith type apples. Antimicrobial activity was observed only in the peels of Golden Delicious type apples. Protein analysis revealed that protein concentrations in the peels are averagely 36% higher than the fleshy parts of the tested apple types.

Keywords: Karaman, apple, antioxidant activity, phenolic content, protein content

Molecular Characterization of Crown Gall of Grape (*Rhizobium vitis*) by PFGE in Turkey

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Abstract

Surveys were carried out to determine the occurrence of *Rhizobium vitis* in İzmir, Manisa, Denizli, Tekirdağ, Edirne, Kırklareli, Çanakkale, Diyarbakır, Elazığ, Malatya, Mardin, Nevşehir and Ankara, the important vineyard provinces of Turkey, during July and September of 2009-2010. 103 strains were obtained after the surveys and isolates were identified as *R. vitis* by classical bacteriological techniques, pathogenicity tests and PCR. Similarity rate of the isolates were made according to the PFGE (Pulsed Field Gel Electrophoresis) profiles of *PmeI* restriction endonuclease. The most genetical diversity was found in the isolates from Denizli, Ankara and Nevşehir provinces. Depends of our knowledge this is the first and pioneer research for the genotypic similarity rates of *R. vitis* isolates by PFGE in Turkey.

Keywords: *Rhizobium vitis*, vineyard, Pulsed Field Gel Electrophoresis

Genomics and Transcriptomics Analysis of Cu Accumulator Plant *Brassica nigra* L.

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Abstract

Brassica nigra has the second smallest genome size (~ 632 Mbp) among the six cultivated species of *Brassica*. *Brassica* species are well known as metal accumulators and some of them are being used for phytoremediation in contaminated soils. Approximately 25% of the documented metal hyper accumulating species are, like *A. thaliana*, members of the *Brassicaceae*. The super metal accumulating capacity of *Arabidopsis halleri* and *Thlaspi caerulescens* have been well documented. Because of their slow growth and low biomass, other fast-growing and high biomass brassica crop plants, for example *Brassica juncea* and *Brassica nigra* have been evaluated for their ability to hyper accumulate metals from contaminated soils. The Diyabeker ecotype of *B. nigra* collected from southeastern part of Turkey was found to be hyperaccumulator of Cu. We carried out the comparative transcriptome analysis in order to find out the expression level of metal induced genes and transcriptome changes both in low and high Cu treated plants. Microarray analysis showed that some of the genes were highly expressed (several hundred fold) with Cu treated plants compared to control. Our microarray data using Affymetrix GeneChip Arabidopsis Genome Array (ATH1-121501 Genechip) indicate that possibly several genes including the genes in glutathione pathway, metal ATPase and ABC transporters are involved in metal tolerances in this ecotype. In this communication the use of molecular tools and the exploitation of Arabidopsis knowledge will be presented in detail.

The role of the C2 domain of Inn1 during cytokinesis in the budding yeast *Saccharomyces cerevisiae*

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Abstract

Cytokinesis is the final stage of the cell cycle in which the cytoplasm is divided to form two independent daughter cells. In animal cells and yeasts a contractile ring of actin and type II myosin forms at the cleavage site and contracts at the end of mitosis. Constriction of the actomyosin ring is coupled to ingression of the plasma membrane, but the molecular mechanism is still poorly understood.

The Inn1 protein plays an essential role during cytokinesis in budding yeast and is important for actomyosin ring contraction to be coupled to ingression of the plasma membrane and septum formation, although its mode of action remains unclear. The amino terminal portion of Inn1 comprises a C2 domain that is essential for cytokinesis.

To understand the role of the C2 domain of Inn1, we have screened for suppressors of the lethal defect in cytokinesis produced by a mutated version of Inn1 with a defective C2 domain and showed that the defect is suppressed by specific mutations in chitin synthase Chs2. We also showed that Inn1 associates with Chs2 in yeast cell extracts. In parallel, we found evidence for the possible involvement of another cytokinesis protein Cyk3 in activation of Chs2.

These findings suggest a model for activation of Chs2 proteins by orthologues of Inn1 and Cyk3, and hopefully provide the basis for future studies of cytokinesis in fungal species including a variety of human pathogens. This is especially important for developing future drugs against important fungal pathogens.

Keywords: cytokinesis, Inn1, Chs2, budding yeast

Investigation of polymorphic variants of LOX-1 genes in patients with Gestational Diabetes Mellitus in Turkish Population

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Abstract

Gestational diabetes (GDM) is a chronic metabolic disease with a high prevalence worldwide. The etiology of diabetic complications is multifactorial, and is closely associated with genetic background. The lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), encoded by the ORL -1 gene, plays critical role in multiple signal transduction pathways and is involved in the process of pro-atherosclerotic conditions, such as dyslipidaemia and diabetes.

The aim of the present study was to investigate the polymorphisms of LOX-1 3'UTR188T>C and K167N (G501C) gene which can may cause susceptibility to GDM. We analyzed the distribution of LOX-1 K167N (G501C) and LOX-1 32UTR 188T>C polymorphisms in 84 GDM pregnant women and 110 pregnant healthy women in Turkish population, using PCR-RFLP method.

The frequencies of LOX-1 3'UTR188T>C, TT, CT and CC, were 63%, 26% and 11% in women with GDM and 58%, 32% and 10% in pregnant healthy women. There was no significant difference in the distribution of genotypes and alleles of LOX-1 3'UTR188T>C between the two groups. In addition, the frequencies of LOX-1 K167N (G501C) exon 4 G>C, KK, KN and NN, were 23%, 10% and 66% in women with GDM and 13%, 33% and 54% in pregnant healthy women. There was no significant difference in the distribution of alleles of LOX-1 K167N(G501C) between the two groups. Patients having LOX-1 K167N (G501C) KN (OR: 5.067; 95% CI:1.872-13.716, P=0.001) genotype had a significantly higher risk of GDM than those with LOX-1 K167N (G501C) KK genotype.

This is the first report showing an association between LOX-1 gene polymorphisms and GDM development, and suggests that LOX-1 K167N polymorphism may confer increased risk for the development of maternal and fetal complications in diabetes. Further larger studies should be performed to confirm these results.

Keywords: LOX-1, polymorphism, gestational diabetes

GMO issue in B&H – five years after the enactment of Law on GMO B&H

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Abstract

In February 2009, Parliamentary Assembly of Bosnia and Herzegovina adopted Law on GMO, which came in force in March 2009. The Law is harmonized with EU Legal Framework on GMO so the same provisions related to authorization, trade, transportation and labelling apply to GMO food and feed in B&H. In addition, Regulations related to the preparation of dossier for requesting the authorization for a GMO event, as well as the procedure for applying for a GMO event authorization were adopted in 2012. As of 2014 no requests for authorization of any GMO event were made. Therefore, no GMO events are currently authorized in B&H for use in food in feed, which in practical terms means “zero tolerance”. Consequently, no requirement for labelling currently exists. This situation causes several issues related to GMO. Producers take advantage of generally poor understanding of what constitutes a GMO by the consumers and include unregulated “GMO free” label in their marketing strategies. In return, consumers exert pressure on the authorities to make GMO label available on the food they consider to be GMO. Lack of authorized GMO events in a country that imports vast quantities of food and feed creates difficult situation for the authorities that is not unique for B&H. The cost to EU due to asynchronous approvals of rice, maize and soybean events exceeds 1 billion EUR. Enforcement of “zero tolerance” may cause grave financial consequences to B&H economy.

Expression and prognostic relevance of MACC1 in breast cancer cells: A Future perspective

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Abstract

Breast cancer is the most common type of cancer in woman worldwide. The variations in morphologies, differences in metastatic behavior and the response to therapeutic treatments make breast cancers hard to treat. It is necessary to elucidate possible signaling pathway that could serve as potential therapeutic target and at the same time also be a biomarker for early detection no matter which subtype patients are diagnostic with. The newly identified proto-oncogene MACC1 gene (Metastasis-Associated Colon Cancer 1) is a prognostic marker for the detection of metastases in primary tumors of colon cancer. The MACC1 was first identified to be overexpressed in primary and metastatic tumors tissue of colon cancer compared to healthy tissue. MACC1 stimulates proliferation, motility and invasion in colon cancer cells through transcriptional up-regulation of c-MET. Once activated, c-MET can result in activation of several downstream signaling cascades, such as MAPK and PI3K/Akt pathways. MACC1 gene could play a key role in differentiation, migration and invasion of breast cancer cells and serve as potential therapeutic target. There are studies showing that these signaling pathways play a key role in carcinogenesis, cell survival, anti-apoptotic effect, invasion, metastasis and angiogenesis in malignancies including breast cancer it is of interest to elucidate the role of MACC1 gene in this cascade and the correlation with c-MET in breast cancer cells.

Keywords: MACC1, breast cancer, c-MET, biomarker

Micropropagation of vetch (*Vicia sativa* L) cv. Kubilay

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Abstract

The study reports a successful in vitro propagation system for valuable forage legume crop plant vetch (*Vicia sativa* L) cv. Kubilay by in vitro culture of mature cotyledon node explants on agar solidified MS medium containing variants of 0.25 -1 mg/ BAP – 0.5 mg/l NAA with 3% sucrose. The explants induced direct bud break without callus proliferation. High proliferation and average height of shoots was noted on MS medium supplemented with BA (0.25 mg/l) NAA (0.5 mg/l NAA). Rooting of shoots was achieved on MS medium containing 0.5 mg/l IBA. Plantlets were successfully transferred to pots for acclimatisation after they grew more than 4 roots obtaining length of 2-3 cm. The plants flowered in the greenhouse and set seeds.

Keywords: micropropagation, in vitro, rooting, acclimatisation

Preparation of Surface Plasmon Resonance Based 17-B-Estradiol Sensor

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Abstract

Many environmental pollutants, coming from various sources, can act as endocrine disrupting chemicals (EDCs) and may affect the normal functions of the endocrine system causing adverse effects on exposed wildlife and humans. The impacts restricted to individuals may subsequently extend to effects on whole populations and the community. In wildlife, the effects of endocrine disrupters are also mostly related with reproductive and developmental abnormalities. Studies have shown that the natural hormone, 17- β -estradiol (E2), is the most potent estrogens and induce changes in fish reproduction at low concentrations present in some wastewater treatment plants. The aim of this study is preparation of surface plasmon resonance (SPR) biosensor for E2 detection. E2 imprinted SPR chip was prepared in the presence of EGDMA, HEMA, AIBN and MALM-E2 complex. SPR chip was characterized using atomic force microscopy (AFM), ellipsometer, FTIR-ATR and contact angle measurements. E2 solutions with different concentrations were used to determine the adsorption kinetics. In order to determine the selectivity of imprinted chip, E2 at the same concentration, stigmasterol and cholesterol solutions were injected into the SPR system. Imprinted chip detected E2 five more fold than stigmasterol and ten more fold than cholesterol. Solutions which have same concentration E2 were given 10 times to SPR system and the SPR chip has still same detection limit without significant decrease.

Keywords: 17- β -estradiol (E2), surface plasmon resonance, imprinted SPR chip.

Effects of Salicylic Acid on Antioxidative System Components and two Gene Activities (*top2* and *pdh47*) in Pea (*Pisum sativum* L.) Under Chilling and Freezing Stress

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Abstract

High and low temperatures and drought are the most devastating stress factors causing significant yield losses in crop plants worldwide. Pea (*Pisum sativum* L.) seeds are mostly sown at late winter or early spring months during which the plants are frequently exposed to chilling and freezing stresses and complete yield losses are observed when early cold stress is accompanied to late drought stress during seed set period. Some plant growth regulators including salicylic acid take role in signal transduction and defense mechanisms in plants under stress conditions. In this study, anatomical features, relative water content, proline, malonedialdehyde, hydrogen peroxide and chlorophyll contents, ion leakage and SOD enzyme activities were determined and the expression of the genes encoding topoisomerase II (*TOP2*) and DNA helicase 47 (*PDH47*) enzymes were analyzed by semi-quantitative reverse transcription PCR under chilling and freezing stress. While important stress indicators; proline, malonedialdehyde, hydrogen peroxide and ion leakage levels increased under cold and freezing stress, the presence of salicylic acid decreased the levels significantly back to control levels. Salicylic acid also increased relative water content of pea under freezing stress, however did not affect anatomical properties and SOD activities, significantly. The data showed the capability of salicylic acid on the regulation of *TOP2* and *PDH47* genes, which have functions in transcription, translation and DNA/RNA repair and therefore have a potential of taking active role on stress tolerance, that brings about the observed positive effects on antioxidative defense system.

This study was supported by TUBITAK TOVAG, with project number 112O367.

Keywords: Pea, oxidative stres, *Pisum sativum* L., semi-quantitative reverse transcription PCR

Shoot Regeneration from Hypocotyl Explants of Flax (*Linum usitatissimum* L.) on Hormone-Free Growth Medium

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Abstract

Plant regeneration is a serious limiting factor to obtain transgenic plants in biotechnology. Flax (*Linum usitatissimum* L.) is the important crop for its high quality drying oil and fiber. It was reported that flax regenerates more easily from hypocotyl explants and the medium containing 1 mg l⁻¹ BAP and 0.02 mg l⁻¹ NAA has been successfully used for shoot regeneration. In the present study, hypocotyl explants of three flax cultivars were cultured on hormone-free and hormone-enriched MS mediums for shoot regeneration. Seeds of flax (*Linum usitatissimum* L.) cvs. 'Madaras', '1886 Sel.' and 'Clarck' obtained from "Northern Crop Science Laboratories", in North Dakota, USA, were surface sterilized with 40% commercial bleach containing 5% sodium hypochlorite at 10°C for 12 min. with continuous stirring and then were washed three times with sterile distilled water at the same temperature. Sterilized seeds were germinated on a basal medium containing the mineral salts and vitamins of Murashige and Skoog (MS), 3% sucrose and 0.7% agar in Magenta vessels. All cultures were incubated at 25±1°C under cool white fluorescent tubes (27 µmol m⁻² s⁻¹) with a 16 h light/8 h dark photoperiod. The pH of the medium was adjusted to 5.8 prior to autoclaving. Hypocotyl segments in 5 mm length were excised from 7-day-old seedlings and cultured on hormone-free MS medium and MS medium containing 1 mg l⁻¹ BAP and 0.02 mg l⁻¹ NAA. Twenty explants were placed per petri dish at a '1.0 x 1.0' cm culture distance. Four weeks after culture initiation, regeneration percentage, shoot number per explant, weight of explant including shoot, the highest shoot length, total shoot number per petri dish, chlorophyll a, chlorophyll b and total chlorophyll contents were recorded. According to the results, it was observed that flax hypocotyl explants regenerated better on hormone-free medium than hormone-enriched medium.

Keywords: Flax, hormone-free regeneration, hypocotyl

The Effect of Endogenous Plant Hormones on Shoot Regeneration from Hypocotyl Explants of Flax (*Linum usitatissimum* L.)

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Abstract

Tissue culture studies aim to obtain high-frequency shoot regeneration, which is also a prerequisite for an efficient transformation system and a clonal propagation of plants. Plant growth regulators are very important in plant tissue culture. Determination of correct concentrations and combinations of growth regulators in tissue culture media is very important in controlling morphogenesis in plant tissues. It is reported that endogenous plant hormone levels may affect the ability of *in vitro* regeneration. It is also reported that explants which themselves produce or contain sufficient auxins or cytokinins, do not need extra auxins or cytokinins to be added to medium. In the current study, hypocotyl explants of two flax cultivars were cultured on MS medium containing 1 mg l⁻¹ BAP and 0.02 mg l⁻¹ NAA as a control and on MS medium having different concentrations of plant extract. Seeds of flax (*Linum usitatissimum* L.) cvs. 'Madaras' and '1886 Sel.' obtained from "Northern Crop Science Laboratories", in North Dakota, USA, were surface sterilized with 40% commercial bleach containing 5% sodium hypochlorite at 10°C for 12 min. with continuous stirring and then were washed three times with sterile distilled water at the same temperature. Sterilized seeds were germinated on a basal medium containing the mineral salts and vitamins of Murashige and Skoog (MS), 3% sucrose and 0.7% agar in Magenta vessels. All cultures were incubated at 25±1°C under cool white fluorescent tubes (27 µmol m⁻² s⁻¹) with a 16 h light/8 h dark photoperiod. The pH of the medium was adjusted to 5.8 prior to autoclaving. 7-day-old seedlings were used for explant source and for obtaining plant extracts. Above ground parts of sterile seedlings were divided into small parts. Two g of these parts was put into 20 ml sterile distilled water (100 mg ml⁻¹) in a falcon tube of 50 ml and shaken at 24±1°C in a rotary shaker (200 rpm) for 24 h. Then, this was centrifuged at 10 000 rpm for 20 min. and supernatant was harvested. Hypocotyl explants were cultured on MS medium containing 1 mg l⁻¹ BAP and 0.02 mg l⁻¹ NAA, and MS media having three different concentrations (3, 9 and 15 ml l⁻¹) of plant extract (100 mg ml⁻¹). Four weeks after culture initiation, regeneration percentage, shoot number per explant, the highest shoot length and total shoot number per petri dish were recorded. Then, regenerated shoots were transferred to soil. Two weeks later, the number of plants growing in soil was determined. Results showed that flax hypocotyl explants could be regenerated successfully on MS medium having plant extract instead of culturing on MS medium containing 1 mg l⁻¹ BAP and 0.02 mg l⁻¹ NAA.

Keywords: Auxin, cytokinin, endogenous plant hormone, flax, hypocotyl

Comparison of PCR-RFLP and PFGE for determining the genetic diversity of *Salmonella* isolates

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Abstract

The aim of the present study is to investigate genomic heterogeneity of 41 *Salmonella* strains originated from Turkey by using two molecular typing methods; PCR-RFLP and PFGE. 16S rRNA-PCR was performed using *Salmonella* genomic DNA for RFLP. PCR amplicons were cleaved with restriction enzymes *AluI*, *BglII*, *HindIII*, *EcoRI*, *BgIII*. PFGE was carried out using CDC PulseNet protocol for *Salmonella*. Chromosomal DNA was subjected to enzymatic digestion with 20u of *XbaI*. Electrophoresis of DNA fragments was achieved with CHEF DR-III system. *Salmonella* Braenderup strain H9812 was used as marker. Digested 16S rRNA fragment and the pulsotype's digital images were analyzed using NTSYSpc software. Cluster analysis of the Dice similarity indices based on UPGMA was undertaken to generate a dendrogram describing the relationship among isolates. The similarity level in two main branches ranged from 44-100% according to cluster analysis of PCR-RFLP profiles. 92.5% of isolates were obtained by considering homology of about 70%. Cluster analysis of PFGE profiles subtyped 41 strains into 21 different pulsotypes with sizes from 2 kb to 1,135 kb. The similarity level in two main branches ranged from 33-100%. 85% of isolates were obtained by considering homology of about 36%. PFGE-based assay segregated the most of strains into different branches, PCR-RFLP grouped these into a single branch because of had 9 different restriction patterns. These results support that *XbaI*-PFGE fingerprinting is more reliable, useful, superior molecular epidemiological technique in typing of *Salmonella* than PCR-RFLP because of its high discriminatory power to reveal clonal relationships among the isolates.

Keywords: *Salmonella*, PCR-RFLP, PFGE

Analysis of Central Carbon Metabolism of Model Plant *Brachypodium distachyon*, a Member of Poaceae Family

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Abstract

Brachypodium distachyon, an endemic plant in Mediterranean and Middle East region, is a member of grass family (Poaceae) together with a number of cereals, fundamentally important for human diet such as wheat, barley, rye and oat. *B. distachyon*, has all basic requirements to be a model organism, such as having a compact genome (272 Mb), short generation time (~12 weeks), 5 pair of chromosomes (2n=10) and simple growth requirements. These properties and being a close relative to important crops, makes *Brachypodium* an attractive model plant for improvement of cereals. In recent years, despite an increasing interest in genomic and transcriptomic studies on *Brachypodium*, these are shown to be insufficient in understanding metabolic networks composed of biochemical reactions, mainly due to lack of correlation between mRNA and protein levels and the lack of enzymatic activity of proteins upon translation. Despite its importance of being the closest omic level to physiology, studies on metabolomics for *Brachypodium* are at their infancy. In this study, we discuss the results of the targeted metabolome analysis under different growth and stress conditions for central carbon metabolism in model plant *Brachypodium distachyon*. The information obtained from this work is foreseen to fill in an important knowledge gap in understanding the response of plant metabolism to stress conditions.

Keywords: *Brachypodium distachyon*, Central Carbon Metabolism, Abiotic Stress

Determination of Mitochondrial DNA Variations in *Arvicola amphibius* (Mammalia: Rodentia) Populations by PCR-RFLP

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Abstract

Arvicola amphibius, which is widely distributed in Palearctic Region, is a semi aquatic rodent. It lives sluggish streams, lakes, dam lakes, mars areas and around of irrigation channels in Turkey. Genetic differences in the cytochrome *b* region of mitochondrial DNA of 116 samples collected from 23 different localities in Turkey were determined by PCR-RFLP. Approximately 1140 bp length region of cytochrome *b* was amplified and cut by for different restriction enzymes (*Rsa* I, *EcoR* V, *Alu* I ve *Sau3A* I). According to UPGMA dendrogram based on PCR-RFLP results, Eastern Anatolian populations were separated from Thrace, North and West Anatolian and Central Anatolian populations in the cluster. Thrace, Hatay and Kahramanmaraş populations were positioned separately in the same sub cluster in comparison with North and West Anatolian and Central Anatolian. Due to an extra band profile of Sakarya samples in the digestion pattern of *Sau3A* I, Sakarya samples were clustered with the Eastern Anatolian populations. Molecular Variance Analysis showed that all observed variations were within the populations. Principal Coordinate Analysis based on the genetic distance revealed that geographically close populations tend to cluster together. Result of Network analysis including 28 haplotype supported results obtained by other analysis.

Keywords: *Arvicola amphibius*, PCR-RFLP, Turkey

Construction and characterization of a recombinant *Chilo iridescent virus* expressing Green fluorescent protein reporter gene

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Abstract

Chilo iridescent virus (CIV) is the type species of the genus Iridovirus (family Iridoviridae). Here, a recombinant CIV (rCIV- Δ 157L-*gfp*) expressing a green fluorescent protein (GFP) under the viral major capsid protein gene (*mcp*) promoter was constructed. For insertion of the *gfp* into the CIV genome, the position of a putative apoptosis inhibitor gene (157L), previously showed as non-essential for apoptosis inhibition function, was chosen as target. The *gfp*, which fused to the viral *mcp* promoter, was inserted into the 157L gene loci of CIV by homologous recombination. The constructed recombinant virus was analyzed in *Anthonomus grandis*, BRL-AG-3A cells. Recombinant virus was purified by seven successive rounds of plaque purification and it was confirmed by PCR, sequencing and restriction analysis. All of the plaques produced by the purified recombinant virus emitted green fluorescence. One-step growth curves revealed that infection with recombinant and wild-type CIV are similar. In BRL-AG-3A cells, the recombinant virus produced the same cytopathic effects as its parental virus. This study became also a functional analysis work for CIV 157L gene by showing it as a nonessential one for virus replication. Consequently, the CIV 157L gene can be used as a site for insertion and expression of foreign DNA into CIV genome.

Keywords: *Chilo iridescent virus*, green fluorescent protein, recombinant virus, 157L, apoptosis

Antimicrobial Activities of Spirogyra aequinoctialis and S. ellipsozpora

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Abstract

In this study, antimicrobial activity of *Spirogyra aequinoctialis* and *S. ellipsozpora* which were grown in proper culture condition was searched with their extracts. The antimicrobial activity of algal extracts were tested on *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O 157:H7, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, *Candida albicans* ATCC 10239 by using disc diffusion method. *Spirogyra aequinoctialis* extract showed the highest antimicrobial activity against *Escherichia coli*. The extract of *Spirogyra ellipsozpora* showed that the high antimicrobial activity against *Salmonella typhimurium* and *Candida albicans*. According to estimates by analysis of variance, the test results were significant differences between the groups in terms. The differences between the results was regarded as statistically significant ($p < 0.01$).

Keywords: *Chlorophyta*, *Spirogyra aequinoctialis*, *S. ellipsozpora*, Antimicrobial activity

Genotyping of human papillomavirus high-risk types and correlation with potential risk factors

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Abstract

Human papillomavirus (HPV) is one of the most common sexually transmitted disease (STD) worldwide. If diagnosed on time HPV can be successfully treated, however, in some cases it can lead to the development of tumor. Most of cervical tumors contain HPV DNA, and majority of them contain high-risk types HPV16/HPV18. Different risk factors are associated with HPV infection, including behavioral and biological predispositions. Aim of this study is to genotype potentially infected patients on high-risk types HPV DNA and to correlate the results with patient's different biological and lifestyle factors. For this purpose 20 gynecological smear samples were collected from women, previously subjected to the survey. Methodology included DNA extraction and real-time polymerase chain reaction (RT-PCR). Results showed that out of 20 patients five were positive for high risk HPV. Four of five positive patients were positive on HPV16 type of which one had HPV16 together with others high risk types. One of five positive patients was positive on HPV18 type and other high risk types not identified. Final outcome indicates the correlation of potentially endangered patients with specific sexual behavior and lifestyles, and furthermore represent the general consensus and awareness level this disease has on the public.

Keywords: Human papillomavirus (HPV), HPV high risk types, RT-PCR

Fatty Acid Composition of a Solitary Wasp Species, *Sphex flavipennis* (Insecta: Hymenoptera)

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Abstract

Fatty acid composition in head, thorax, and abdomen of male and female specimens of the solitary wasp species *Sphex flavipennis* was investigated. Samples collected from their natural habitats in Tokat province. Total unsaturated fatty acids of body parts of sexes were found: 80.19 % in female head, 75.73 % in male head; 81.57 % in female thorax, 81.43 % in male thorax; 79.03 % in female abdomen, 74.68 % in male abdomen. Total saturated fatty acids of body parts and sexes were found: 19.57 % in female head but 24.67 % in male head; 14.83 % in female thorax, 16.91 % in male thorax; 19.77 % in female abdomen, 22.78 % in male abdomen. The highest of fatty acids are oleic acid and linoleic acid ranging between 27.68 % and 52.65 %, 19.00 % and 37.36 % respectively. Fatty acid levels were found different between sexes as well as their body parts which is due to physiological and metabolic differences between male and female insects.

Keywords: *Sphex flavipennis*, fatty acids, Hymenoptera

Differential replication of wild type and AMV197 (a putative serine/threonine kinase gene) knocked out *Amsacta moorei* Entomopoxvirus

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Abstract

In the current study, a serine/threonine (Ser/Thr; ORF AMV197) protein kinase gene of *Amsacta moorei* entomopoxvirus (AMEV, type species of Betaentomopoxvirus) was characterized via end point dilution assay (EPDA) of progeny virus replication, slot blot hybridization of viral DNA and microarray of whole genome transcription. A recombinant virus (Am Δ PK/*gfp*) was constructed by deleting protein kinase gene from AMEV genome via homolog recombination. The infectious virus particle was determined by using EPDA and found that the virus titer of Am Δ PK/*gfp* was decreased approximately five fold compare to the wild type virus. Slot-blot hybridization analysis of DNA samples taken from Am Δ PK/*gfp* infected Ld652 cells at 0, 6, 12, 24, 36 and 48 hours post infection showed that while the DNA replication of Am Δ PK/*gfp* was detected at 24th hour post infection, the control DNA replication was detected at 36th hour. Whole-genome gene expression microarray showed that the expression levels of 126 genes (55.7 %) were significantly changed in Am Δ PK/*gfp*-infected samples. Of these, 88 (69.84 %) transcripts were up-regulated and 38 (30.15 %) were down-regulated. Results showed that AMV197 deletion in the AMEV genome caused the DNA replication start early however decreased the virus titer and altered transcription of viral genes.

Keywords: Am Δ PK/*gfp*, *Amsacta moorei* Entomopoxvirus (AMEV), protein kinases, RNA microarray, virus replication

Evaluation of Nanoparticle-Biomolecules Interactions

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Abstract

Nanoparticles (NPs) present an excellent platform for a broad range of biological and biomedical applications. These applications are limited with NPs effects on health and environment.

A complete characterization of the different physical chemical properties of nanoparticles (NPs) has to be done to evaluate their impact. Different methods have been developed for characterization of NPs. Methods based on surface properties of NPs are the least developed.

This work present a review of NPs interactions with biomolecules, specially proteins and methods used for NPs-biomolecule interactions characterization.

Keywords: Nanoparticle, nanoparticle-biomolecule interactions, surface properties, protein

3D structure prediction of Histone acetyltransferase (HAC) proteins of the p300/CBP family and their interactome in *Arabidopsis thaliana*

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Abstract

Histone acetylation is an important posttranslational modification correlated with gene activation. In *Arabidopsis thaliana* the histone acetyltransferase (HAC) proteins of the CBP family are homologous to animal p300/CREB (cAMP-responsive element-binding protein)-binding proteins, which are important histone acetyltransferases participating in many physiological processes, including proliferation, differentiation, and apoptosis. In this study the 3-D structure of all HAC protein subunits in *Arabidopsis thaliana*: HAC1, HAC2, HAC4, HAC5 and HAC12 is predicted by homology modeling and confirmed by Ramachandran plot analysis. The amino acid sequences of HAC1 and other HAC members are highly similar to the sequences of the homologous human p300/CREB protein. Conservation of p300/CBP domains among the HAC proteins was examined further by sequence alignment and pattern search. The domains of p300/CBP required for the HAC function, such as PHD, TAZ and ZZ domains, are conserved in all HAC proteins. Subcellular localization and interactions of each HAC protein subunit were examined and confirmed. Interactome analysis revealed that HAC1, HAC5 and HAC12 proteins interact with S-adenosylmethionine-dependent methyltransferase domain-containing protein that shows methyltransferase activity, suggesting an additional function of the HAC proteins. Additionally, HAC5 has a strong interaction value for the putative c-myb-like transcription factor MYB3R-4, which suggests that it also may have a function in regulation of DNA replication.

Association of common genetic variants with markers of Type 2 diabetes in population from Bosnia and Herzegovina

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Abstract

Recently, several novel loci affecting insulin resistance and lipid status in Type 2 diabetes (T2D) were identified in European populations and later confirmed in other ethnic groups. The aim of this study was to investigate an association between several common T2D risk variants and diabetes-related traits in population from Bosnia and Herzegovina (BH). A total number of 268 subjects, 40-65 years old, were recruited in the study (162 T2D patients and 106 nondiabetics) and genotyped by using the MassArray Sequenom iPLEX platform. Strikingly, our results demonstrated a significant difference in genotype frequencies for apolipoprotein B (*APOB*) rs693 between T2D and nondiabetic subjects ($p = 0.011$). This variant was associated with 3-fold decreased T2D risk (adjusted OR = 0.33, 95% CI = 0.14-0.79, $p = 0.013$) and lower fasting plasma insulin levels in T2D patients ($p = 0.003$). Furthermore, our data showed a significant association of cholesteryl ester transfer protein (*CETP*) - 629A>C SNP with increased insulin levels ($p=0.008$) and HOMA-IR ($p=0.014$). On the other side, our data demonstrated that plasma lipid levels were significantly affected with variants of transcription factor 7-like 2 (*TCF7L2*), ATP-binding cassette transporter A1 gene (*ABCA1*), *CETP*, and *TRIB1* genes in this BH population cohort. Particularly, rs7903146 *TCF7L2*, *ABCA1* rs3890182, and *CETP* rs1800775 polymorphisms showed a significant association with lower HDL-cholesterol levels in nondiabetic subjects ($p=0.027$, $p=0.013$, and $p=0.010$, respectively). Interestingly, our data demonstrated an association of *TRIB1* rs17321515 polymorphism with lower LDL ($p=0.012$) and total cholesterol levels ($p=0.007$) in T2D patients. In conclusion, our results indicated that *APOB* and *CETP* variants appear to affect insulin sensitivity and glucose control in normoglycaemic population. Furthermore, a protective effect of *APOB* and *TRIB1* variants in regards to the T2D risk and plasma lipid profile, respectively, was demonstrated by our data. However, *TCF7L2*, *ABCA1*, and *CETP* variants appeared to be associated with decreased HDL-cholesterol levels and thus, could be considered as risk alleles for lipoprotein abnormalities in T2D.

In vitro Multiple Shoot regeneration of aquatic *Lysimachia nummularia* L.

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Abstract

Creeping Jenny or Moneywort (*Lysimachia nummularia* L.) is an aquatic plant belongs to Myrsinaceae family. The plant is native to Europe and is one of the popular plant in aquarium industry. The cultivar Aurea (Golden Creeping Jenny) is popular due to its yellow leaves and lesser agressivity. It is also used as medicinal plant in Traditional Chinese Medicine due to containing number of phenolic acids. The plant is used for healing wounds and for the treatment of stone lin syndrome and painful gout symptoms. In this study, different nodal segments explants (shoot tip, 1st and 2nd nodal segments) were cultured on MS medium provided with different concentrations of 0.25, 0.50, 0.75, 1.0 and 1.25 mg/l BAP. Maximum number of shoots per explant of shoot tip (9.30), 1st nodal segments (8.94) and 2nd nodal segments (8.11) were recorded on 1.25 mg/l BAP containing medium. Contrarily, shorter shoots of 1.47 cm, 1.43 cm and 1.46 cm were obtained from shoot tip, 1st and 2nd nodal segments explants respectively. Successful rooting was done by culturing the shoots on MS rooting medium containing different concentrations of 0.25-1.0 mg/l IBA. Thereafter, rooted plantlets were acclimatized in aquariums successfully.

Keywords: Aquatic plant, Acclimatization, Axillary shoot regeneration, Medicinal plant, *Lysimachia nummularia*

**POSTER PRESENTATION
ABSTRACTS**

Molecular characterisation of a novel *Nonomuraea* sp. K271 isolated from Northern Cyprus soil

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Abstract

Objectives: Members of the genus *Nonomuraea* are aerobic, Gram positive, non-acid-fast, non-motile actinomycetes which form extensively branched substrate and aerial mycelia. The genus is characterized chemotaxonomically by the presence of *meso*-diaminopimelic acid in the cell wall, madurose as a characteristic sugar in whole-cell hydrolysates. The aim of this study was to determine the taxonomic position of the isolate using a polyphasic approach.

Materials and Methods: K271 was picked after 4 weeks of incubation at 28°C on Stevenson's medium No 2 supplemented with cycloheximide (50 µg ml⁻¹), neomycin sulphate (4 µg ml⁻¹) and nystatine (50 µg ml⁻¹). Genomic DNA isolation was performed by Pitcher *et al.* (1989). The 16S rRNA gene was amplified by PCR by using 27f and 1525r universal primers. Phylogenetic analyses were performed by using three treemaking algorithms. DNA-DNA hybridisation was carried out as described by De Ley *et al.* (1970). Sugars in cell wall hydrolysates were analysed by using the methods established by Hasegawa *et al.* (1983). Polar lipids were analysed by using the methods described by Minnikin *et al.* (1984). Isoprenoid quinones were analysed by HPLC as described by Minnikin *et al.* (1984) and Kroppenstedt (1982).

Results: K271 shared highest 16S rRNA gene sequence similarity with *Nonomuraea kuesteri* GW 14-1925^T (98.77 %). The genotypic, chemotaxonomic and phenotypic data showed that strain K271 represents a novel species of the genus *Nonomuraea*.

Keywords: *Nonomuraea*, 16S rRNA gene, Stevenson's medium

Acknowledgements: This study was supported by Ondokuz Mayis University (PYO. FEN. 1901.12.014)

Molecular characterisation of a novel *Streptomyces* sp. S4702 isolated from Black Sea deep sediment

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Abstract

Objectives: The marine environments have a rich source of both biological and chemical diversity and there are several reports that the marine actinomycetes yielded several metabolites such as antibiotics and enzymes. Therefore, definition of novel *Streptomyces* from marine environments is important to search for new bioactive compounds. The aims of the present investigation was to determine the taxonomic position of strain S4702 using molecular approach.

Materials and Methods: *Streptomyces* sp. S4702, isolated from Non-sporulating medium, supplemented with rifampicin (5 µg/ml), and nystatin (50 µg/ml) incubated at 28°C for 30 days. The isolate was tested according to phenotypic properties as biochemical, degradation and nutritional. Genomic DNA extraction and PCR-mediated amplification of the 16S rRNA gene were carried out following Chun and Goodfellow (1995). Phylogenetic analysis were performed by using three different algorithms. DNA-DNA hybridisation was carried out as described by De Ley *et al.* (1970). Sugars in cell wall hydrolysates were analysed by using the methods established by Hasegawa *et al.* (1983). Polar lipids were extracted, examined by using two-dimensional TLC and identified on the basis of procedures described by Minnikin *et al.* (1984). Isoprenoid quinones analysed by HPLC as described by Minnikin *et al.* (1984) and Kroppenstedt (1982).

Results: S4702 shared highest 16S rRNA gene sequence similarity with *Streptomyces qinglanensis* 172205^T (97.82 %) and *S. marinus* DSM 41970^T (97.53 %). The predominant menaquinone was MK-9(H₈) (88 %). Phospholipids detected were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides.

Keywords: Black Sea, *Streptomyces*, 16S rRNA gene

Acknowledgements: This study was supported by Ondokuz Mayıs University (PYO. FEN. 1901.12.014)

Cloning and Expression of Glutathione-S-Transferase Gene from *Klebsiella pneumonia*

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Abstract

Lignin is the most abundant natural aromatic polymer in the biosphere. It comprises 20-30% of woody plant cell walls and by forming a matrix surrounding the cellulose and hemicelluloses, it provides strength and protection. Lignin is highly branched and heterogeneous structure made up of phenylpropanoid units which are interlinked through a variety of different bonds.

In recent studies it is reported that GST breaks the beta-aryl-ether bond of the lignin. There haven't been any study about kraft lignin degradation or biobleaching of pulp in the literature although it is reported that GST's break the lignin side chains.

In order to remedy this shortcoming in the literature within the scope of the study GST screening was carried out among the bacteria that exist in our laboratory. As a result of this screening we determine that *Klebsiella pneumonia* has GST activity. After this the gene was cloned to pET20b vector and expressed in *E. coli* BL21 DE3 PLYS host. After expression and purification partial characterization was carried out. As a result of this study we determine the optimum temperature of the enzyme is 30°C and the optimum pH is 6.

In experiments conducted in this study we determined that GST can degrade kraft lignin. However it didn't cause any change in Kappa number. The reason that the enzyme's inability to break bonds in the backbone of lignin. Only break the bonds in the side branches of the pulp didn't cause decrease in Kappa number. Therefore this enzyme is used as an adjunct to other enzymes in the bleaching of the pulp.

Keywords: Lignin, GST, characterization

Utilization of Biotechnology in Turkey's Some Natural Forest Tree Species

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Abstract

Wood raw material requirements are increasing with rapid population growth in Turkey as in the world. In order to supply deficit for closure of forest products, productivity and quality of production should be improved. Basic ways to increase efficiency in forest production is silvicultural precautions, tree breeding studies. Genetic variation can be increased by utilizing the existing diversity and genetic variation. So new combinations can be obtained and we can raise efficiency using some selection strategies. At this point, biotechnological methods are required to meet genetic material. Studies of forest tree breeding is a slow process due to the size of genome and length of tree life span. Biotechnological applications in forest trees provide many important benefits in terms of the time saving and reducing cost when compared to classical breeding studies. Sustainable forestry practices is gaining rapid acceleration via biotechnology and modern sciences. In this study, the biotechnology methods for some natural forest tree species in Turkey (1-tissue culture and clonal propagation, 2-molecular marker applications, 3- marker assisted selection and breeding, 4-genomic and proteomic studies, 5-genetic modification and genetic engineering applications) was evaluated.

Keywords: tree breeding, biotechnology, sustainable forestry, tissue culture, genomics studies

Production and Characterization of Monoclonal Antibody Against to Diphtheria Toxin

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Abstract

Diphtheria toxin produced by *Corynebacterium diphtheriae* is rapidly developing upper respiratory tract disease and usually occurs in children, but less frequently occurs in older age. Nowadays, diphtheria has very low incidence of disease, but it is kept on the agenda as a potential problem due to the relatively high sensitivity of the adult population or vaccination targets not achieved the desired proportions all over the country. In this study, diphtheria toxoid was injected intraperitoneally to test animals (6-8 weeks old Balb /C mice) at specific time intervals and specific doses. Antibody titers of mice were determined by ELISA method and the mouse with the best immune response was selected for fusion. For the fusion process, spleen cells of mice were isolated by standard methods. These splenocytes were fused in PEG (polyethylene glycol) with myeloma F0. Then, hybrid cells, which produced antibody against diphtheria toxin were selected by indirect ELISA method. These cells were grown in vitro (located in medium) and their produced monoclonal antibodies were isolated from medium by using gel-filtration chromatography method. The produced monoclonal antibodies were characterized by Western-blot, indirect ELISA and neutralization tests. Furthermore, the subtype of monoclonal antibodies was determined.

Keywords: Diphtheriae, Monoclonal antibody, ELISA, Purification

Characterizing Cotton Germplasm with SSR Markers

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Abstract

Cotton is a fiber plant cultivated in nearly 70 countries and it is considered to constitute a source of livelihood of 180 million people. Both the natural fiber and oil obtained from seeds and other by-products have economic importance. Therefore, one of the world's most important agricultural products respectively. Fibers in the textile industry, the oil obtained from the seed as well as in human nutrition has been used in soap making and oil industry.

In this study , with 234 cotton genotypes sampled from different region of Turkey and 19 cotton wild type genotypes were described by the 6 SSR (Simple Sequence Repeats) genetic locus. Using SSR data, filogenetic tree analyzed using NTSYSpc2.1 computer program.

Genetic similarities within population, synonym/homonym cultivars and also DNA identity information of the population have been described according to the genetic findings.

Keywords: *Gossypium* sp. ,SSR, characterization

Relationship Between PAI-1 Gene Variant and Lung Cancer

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Abstract

The aim of this study was to investigate the polymorphism frequency of Plasminogen Activator Inhibitor Type-1 (PAI-1) gene 4G/5G in patients with lung cancer. In this study, 286 genomic DNAs (154 lung cancer patients + 132 subjects without lung cancer) were analyzed. PAI-1 gene 4G/5G polymorphism genotypes was determined using PCR and electrophoresis. The results were statistically analyzed. The frequencies of *PAI-1* gene 4G/5G genotypes were found to be 21% 4G/4G, 16 % 4G/5G, 63 % 5G/5G in the control group and 31.4% 4G/4G, 30.8% 4G/5G, 37.8% 5G/5G in the patient group. It was determined that 5G/5G genotype frequency was high in patients in comparison to other genotypes. There was a statistically significant difference between the groups with respect to genotype distribution. Finally, we can say that *PAI-1* gene 4G/5G polymorphism 5G/5G genotype determination can be used as a marker for lung cancer in the Turkish population.

Keywords: Plasminogen Activator Inhibitor Type-1 gene, *PAI-1* gene, 4G/5G polymorphism, lung cancer

Characterization of A Novel Lytic Murein Transglycosylase from *Anoxybacillus Gonensis* PDF21

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Abstract

Lytic transglycosylases are an important class of bacterial enzymes that act on peptidoglycan with the same substrate specificity as lysozyme. In this study, a novel lytic murein transglycosylase was obtained and identified from the *Anoxybacillus gonensis* PDF21 strain.

The gene encoding lytic murein transglycosylase contained an open reading frame of 636 bp, encoding a 211 amino acid protein with a calculated molecular mass of 23.76 kDa and an isoelectric point of 6.03 (DNASTAR Lasergene 9.1 software). His-tagged recombinant protein was overexpressed in *E. coli* BL21 cells using a pET20b+ expression vector, and the recombinant protein was induced by 0.5 mM isopropyl- β -D-thiogalactopyranoside (IPTG). Expressed recombinant protein was purified by using Ni²⁺ affinity chromatography. The recombinant lytic murein transglycosylase was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The optimal pH, temperature and the antibacterial activities of recombinant protein were determined by turbidimetric assay.

The recombinant protein displayed lytic activities against Gram-positive bacteria *Micrococcus lysodeikticus* and Gram-negative bacteria *Vibrio alginolyticus*, which was confirmed by the inhibition zone assay. Enzyme activity assays under different pH and temperature indicated that the optimum pH and temperature of enzyme were 7.5 and 55 °C, respectively.

Keywords: lytic murein transglycosylase, lytic activity, *Anoxybacillus gonensis* PDF21, optimum pH and temperature

Coronary Artery Disease (CAD) and Plasminogen Activator Inhibitor-1 Gene (PAI-1) 4G/5G Polymorphism

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Abstract

This study was performed on coronary artery disease (CAD) to determine the frequency of the Plasminogen activator inhibitor-1 (PAI-1) gene 4G/5G polymorphism allele frequencies and to examine the role of this polymorphism in CAD development. Genomic DNA obtained from 185 persons (94 CAD patients, 91 controls) was used in the study. PAI-1 gene 4G/5G polymorphism genotypes was determined using PCR and electrophoresis. The results were statistically analyzed. The frequencies of 4G/5G genotypes, in controls 4G/4G 38%, 4G/5G 30% , 5G/5G 32% and in CAD patients 4G/4G 54%, 4G/5G 20% , 5G/5G 26% were found. 4G allele frequency was determined 53% and 64%. (respectively; control and patient). 5G allele frequency was found 47% and 36%. (respectively; control and the patient). There was no significant difference between the control group and the patient groups in genotype frequencies. It was determined that 4G/4G genotype frequency increases significantly in patients according to control group. Finally, we may suggest that there is no association between CAD and PAI-1 gene 4G/5G polymorphism.

Keywords: Coronary artery disease, CAD, PAI-1 gene, 4G/5G polymorphism

Phylogenetic systematics of *Streptomyces* sp. N1119 and *Streptomyces* sp. N4208 isolates based on multilocus genes sequences

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Abstract

Objective: It is clear that 16S rRNA gene is insufficient to distinguish closely related species in bacterial phylogeny. After the proposition ad hoc committee to the combined use of several house keeping genes for re-evaluation of the species definition in bacteriology, multilocus sequence analysis (MLSA) have been used frequently for clarifying the phylogenetic relationships to closely related species. In this study, phylogenetic systematics based on multilocus gene sequence analysis of two *Streptomyces* strains isolated from Nigerian arid soil, was carried out.

Materials and Methods: N1119 and N4208 isolates were picked after 30 days of incubation at 28°C on Starch-casein agar supplemented with cycloheximide (50 µg/ml). Genomic DNA isolations were carried out and PCR-amplification of 16S rRNA, *atpD*, *gyrB*, *recA*, *rpoB* and *trpB* gene regions were performed using related universal primers. Phylogenetic trees were carried out by using the neighbour-joining algorithm, evolutionary distances were calculated by using the Kimura two-parameter (K2P) model.

Results: N1119 and N4208 isolates shared highest 16S rRNA gene sequence similarity with *Streptomyces capoamus* JCM 4734^T, 98.77 % and 97.82 %, respectively. 16S rRNA gene nucleotide similarity between two isolates is 96.69 %. The evolutionary distances between isolates N1119 and N4208 with *Streptomyces capoamus* JCM 4734^T are 0.053 and 0.038, respectively. Evolutionary distance between the isolates based on multilocus sequence analysis is 0.045. According to these results, N1119 and N4208 isolates distinguished both from each other and from *Streptomyces capoamus* JCM 4734^T which is phylogenetically related species.

Keywords: MLSA, *Streptomyces*, 16S rRNA

Antimicrobial activity of *Klasea bornmuelleri* (Azn.) endemic to Malatya

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Abstract

Klasea genus is located in Asteraceae familia which is a quite cosmopolitan familia. *Klasea* genus is represented by 18 taxa in our country. *Klasea bornmuelleri* collected by researchers in the last 100 years ago, again was able to in 2011. The aim of our study is to evaluate the antimicrobial activity of methanol, ethanol and water extract of *Klasea bornmuelleri* by antimicrobial activity methods including agar well-diffusion and minimal inhibitory concentration (MIC) with microdilution assay. In agar well-diffusion assay, Ampicillin (antibacterial) and Ketoconazole (antifungal) as standart antimicrobial agents and %50 DMSO as negative control were used. As a result of agar well-diffusion assay, the methanol and water extracts were found to have mild antimicrobial activity against to some tested bacteria. It was found that methanol extract has antimicrobial activity against to *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *S. aureus* ATCC 25923, and also water extract has antimicrobial activity against to *K. pneumoniae* ATCC 25955. As a result of microdilution assay (MIC), MIC values of methanol extract was found for *E. coli* ATCC 25922 and *E. coli* ATCC 35218 31,25 mg/ml, and for *S. aureus* ATCC 25923 50,00 mg/ml. MIC value of water extract was found for *K. pneumoniae* ATCC 25955 31,25 mg/ml. Compared with the agar well-diffusion assay, the microdilution assay (MIC) values of result was found to be low value, while it was found that the agar well-diffusion a large zone diameter. The results demonstrated that methanolic and water extracts of *Klasea bornmuelleri* has mild antimicrobial effect against to some microorganism and it could be a potential antimicrobial agent source.

Inhibitory Effects of Sulfanilamide Derivatives on Lactoperoxidase

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Abstract

Sulphanamide compounds are biologically important molecules that possess antibacterial, antiglaucoma, diuretic, hypoglycemic and antithyroid activity (1). They used to treat a variety of bacterial diseases in humans and other species (2).

In this study, *In vitro* inhibition effects of sulfanilamide derivatives 6-Aminopyridine-3-sulfonamide, 4'-Amino[1,1'-biphenyl]-4-sulfonamide, 3,4-diamino-benzenesulfonamide on bovine Lactoperoxidase (LPO; E.C. 1.11.1.7) enzyme were investigated. To determine kinetic properties of these sulfanilamide derivatives, both K_i and IC_{50} parameters on bovine LPO were firstly determined. The IC_{50} values of 6-Aminopyridine-3-sulfonamide, 4'-Amino[1,1'-biphenyl]-4-sulfonamide and 3,4-diamino-benzenesulfonamide were indicated 184,76 μ M, 231,05 μ M and 43,31 μ M respectively and the K_i constants for these derivatives were determined 0,09 \pm 0,3 μ M, 77,49 \pm 9,01 μ M and 2,94 \pm 1,1 μ M respectively. 4'-Amino[1,1'-biphenyl]-4-sulfonamide was shown non-competitive inhibition type, 6-Aminopyridine-3-sulfonamide and 3,4-diamino-benzenesulfonamide were shown competitive inhibition.

Keywords: Sulfonamide, Lactoperoxidase, Enzyme Kinetics

Influences of Chemotherapeutic Drugs on NADPH Oxidation Activity of Human Platelet Nitric Oxide Synthase

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Abstract

Nitric oxide synthases (EC 1.14.13.39) NOSs are a family of enzymes (eNOS, nNOS, iNOS) catalyzing the production of nitric oxide (NO) from L-Arginine. NO is an important cellular signaling molecule and it acts as a regulator of numerous processes in the nervous, immune and cardiovascular systems [1, 2]. In this study, we purified NOS from human platelet fractions by using DEAE-Cellulose anion exchange chromatography and 2',5'-ADP Sepharose 4B affinity chromatography with 6,34% yield 1972,34-fold and 1,854 U/mg specific activity. Purified enzyme appeared as a single band (81 kDa) under denaturing condition(SDS-PAGE). The native molecular weight of enzyme estimated (153 kDa) by gel filtration chromatography. In addition, *in vitro* inhibition effects of some chemotherapeutic drugs on NADPH oxidation activity of human platelet NOS. IC50 values were calculated 0.039 mM, 0.0506 mM, 0.217 mM, 0.233 mM, 0.411 mM, 0.434 mM, 0.947 mM, 1.486 mM, 1.988 mM, 15.67 mM for bleomycin, oxaliplatin, vinorelbine, etoposide, carboplatin, paclitaxel, 5-fluorouracil, cyclophosphamide, fludarabine phosphate, gemcitabine respectively from Activity%-[drug concentration] graphs.

Keywords: Nitric oxide synthase, enzyme, inhibition

Determination of Rickettsia species by PCR in hard ticks (Acari: Ixodidae) collected from humans in Tokat (Turkey)

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Abstract

Tick are ectoparasitic animals having important role in transmission of several zoonotic diseases agents to humans and animals. Among these diseases agents *Borrelia*, *Francisella* and *Rickettsia* cause severe diseases in humans. Although, until the 2012, only *Rickettsia conorii* ve *Rickettsia akari* have been documented in Turkey, 25 rickettsia species are recorded by recent studies. In the present study, 1000 hard tick samples collected from humans in Tokat region were tested for the presence of Rickettsia by PCR using gltA and ompA primers. PCR results showed that 60 out of 1000 ticks were rickettsia positive. Sequencing and NCBI BLAST analysis of the rickettsia positive amplicons demonstrated the presence of *Rickettsia aeschlimanii*, *Rickettsia barbariae*, *Rickettsia mongolotimonae*, *Rickettsia raoultii* and *Rickettsia slovaca* in ticks tested. This results indicates the Rickettsia transmission potential of ticks in the region.

Keywords: Ticks, Human, Rickettsia, Tokat

The Importance of Biological Sensors in Bioterrorist Events

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Abstract

Biological terrorism also known as Bioterrorism is a term used for terrorism using biological weapons such as infectious diseases or biological toxins against a community. This biological agents may be used to create an epidemic in community, lead to loss of many lives and also society's moral and economic weakening. Sampling for the diagnosis of infectious diseases and toxins which could be used as a biological weapon is a time consuming and laborious process and must be taken to an appropriate laboratory for detecting. Particularly for threat mitigation, treatment and management of the scene, a quick detection of biological weapons in place is a vital issue. The quicker biological agent type comes up, the sooner authorities takes measures.

Therefore, in case of any biological attack, the necessity of handheld biological sensors are undeniable and ensure detection in place, quickly determine the type and amount of the agent. Such sensors according to the type of the threat, determine biological agent such as in air or water, and yet from infecting humans, identificate the threats and provide immediate ways to take precautions. Biological sensors that are used for detection of biological agents, could be based on the general antigen antibody interactions, mass measurement systems or potentially produced by methods such as the determination of genetic structure. Present sensors are not portable and quick enough for determining and identifying the biological agents. The need for this type of sensors recognized by many developed countries and development efforts are currently underway.

Keywords: Biosensor, Bioterror, Biological agent determination

Detection of Genotoxic Effects of Flumetralin Using *in vitro* Chromosome Aberration and Micronucleus Test in Human Peripheral Blood Lymphocytes

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Abstract

Flumetralin, which is a plant growth regulator with herbicidal activity belonging to the 2,6-dinitroaniline class of chemicals, has been used widely to control axillary bud (sucker) growth on tobacco plants.

The aim of this study was to evaluate the cyto-/genotoxic effects of flumetralin on human peripheral blood lymphocytes (PBLs) using the *in vitro* chromosome aberration (CA), and cytokinesis-block micronucleus (MN) assays. For this aim, the human PBLs were exposed to four different concentrations of flumetralin (125, 250, 500 and 1000 µg/mL) for 24 and 48 hr. Following treatment with flumetralin of PBLs, a statistically significant increase in CA frequency was observed at three high concentrations (250, 500 and 1000 µg/mL) for 24 hr, and at all concentrations (125-1000 µg/mL) for 48 hr when compared with both the negative and the solvent control. In addition, MN formation was significantly induced at higher concentrations (250, 500 and 1000 µg/mL) for 24 hr and at 125 and 500 µg/mL of flumetralin for 48 hr treatment period as compared to the controls. Due to excessive toxicity binuclear cells could not be detected sufficiently in the highest two concentration of flumetralin (500 and 1000 µg/mL) for 48 hr treatment times. Furthermore, flumetralin significantly decreased the mitotic index and nuclear division index for all concentrations and treatment times as compared to control groups.

In conclusion, it could be said that flumetralin had a significant clastogenic and cytotoxic/cytostatic effects at the tested concentrations for the human PBLs. This study presents the first report on cyto-/genotoxic properties of flumetralin.

This study was supported by Mustafa Kemal University Research Fund (project code: 282).

Keywords: Flumetralin; human peripheral blood lymphocytes; chromosome aberration; micronucleus

The effect of naringin on nitric oxide, iNOS, eNOS and arginase activity in small intestine induced ischemia-reperfusion

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Abstract

Object: The present study was aimed to explain the possible role of naringin against induced ischemia-reperfusion (I/R) in small intestine by considering level of nitric oxide, arginase activity, iNOS (inducible nitric oxide synthase) and eNOS (endothelial nitric oxide synthase) *immunoreactivity*.

Methods: 32 animals were divided randomly into four groups, each containing 8 male Wistar albino rats (200-250 g). Group A was the sham control, group B was ischemia by occlusion of the superior mesenteric artery (SMA) for 120 min. Group C was I/R by occlusion of SMA for 120 min. and then reperfusion for 120 min. Group D was treated with naringin (50 mg/kg, i.p) after ischemia and then reperfused for 120 min. Tissue sample was collected from small intestine for biochemical and histopathological evaluation. eNOS and iNOS expression were observed by immunolabelling. Arginase activity and amount of NO and total protein in tissues were measured. Mann-Whitney U was used for statistical analysis and significance level was determined as $p < 0,05$.

Results: Mesenteric I/R caused degeneration of the intestinal mucosa by stimulation of oxidative stress. The amount of NO and arginase activity were decreased ($p < 0,05$) in naringin group when compared with I/R group. The amount of total protein was increased ($p < 0,05$) in ischemia and decreased ($p < 0,05$) in I/R according to control group but there was not any difference between I/R and naringin group. Any significant recovery was not detected morphologically by the effect of naringin. But increasing immunoreactivity of iNOS and eNOS in I/R group was decreased in naringin group ($p < 0,05$).

Conclusion: We observed naringin has significant rehabilitative effect onto biochemical parameters in I/R but it did not reflect into tissue morphology in this application and treatment period. We think that treatment of naringin by different doses should be needed to investigate in I/R period with other parameters in details.

Keywords: Ischemia, reperfusion, naringin, small intestine

Anticancer and anticholinesterase activities of several mushroom species from Turkey

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Abstract

In this study we focused to evaluate anticancer and anticholinesterase activities of methanol extracts of *Pleurotus ostreatus*, *Boletus edulis*, *Tricholoma populinum*, *Helvella queletii*, *Armillaria tabescens*, *Psathyrella candolleana* and *Helvella leucopus* mushroom species. Anticancer activity of mushroom methanol extracts was screened by MTT cytotoxicity assay on cancer (HeLa) and normal epithelium (NRK-52E) cell lines. The IC₅₀ values of the extracts were 1.58-25.11 and 2.05-22.32 mg/mL for HeLa and NRK-52E cells, respectively. To indicate anticholinesterase activity the acetyl- and butyryl-cholinesterase inhibitory activities of the mushroom extracts were studied. None of the methanol extracts of the mushrooms possessed anticholinesterase effect, only the methanol extract of *P. ostreatus* indicated moderate butyrylcholinesterase inhibitory activity (48.21 % inhibition), this is followed by *B. edulis* as 36.60 % and *H. leucopus* as 32.48 % inhibitory activity at 200 µg/mL.

Keywords: Anticancer, anticholinesterase, mushroom

Investigation of Monosodium Glutamate Induced DNA Damage by Using RAPD-PCR in Human Peripheral Lymphocytes

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Abstract

Food additives play a vital role in food industry. Monosodium glutamate (MSG) (E621), the sodium salt of the non-essential amino acid glutamic acid, is well known and widely used flavour enhancer in the World. MSG strengthen flavour characteristics of food, increasing saliva, cause the desire of more frequent and faster eating. We encounter MSG in some kind of chips, fats, bouillons, instant soups, sauces, processed red meat, fish and chickens and spice mixes. Genotoxicity tests are an important way to evaluate hazard of chemicals. The genotoxicity of food additives can be evaluated by several ways; one of them is RAPD-PCR. In the present study, in vitro genotoxic effect of the MSG was investigated by analysing the RAPD-PCR with arbitrary 10-mer primers. Peripheral blood samples obtained from one healthy non-smoking female donor aged 26 years were added to chromosome medium B. The cultures were incubated at 37°C for 72h. MSG was added after 24h and 48h of culture initiation. Human lymphocytes were exposed to different concentrations (250; 500; 1000; 2000; 4000 and 8000 µg/mL) of MSG. A negative (distilled water) and a positive control (mitomycin-C; MMC) were also maintained. The changes occurring in RAPD profiles following MSG treatments include increase or decrease in band intensity and gain or loss of bands. In addition, the results demonstrated that RAPD markers are a useful tool for determining the genotoxicity of MSG.

Keywords: Genotoxicity, flavour enhancers, monosodium glutamate, MSG, RAPD-PCR

Relationships between levels of reactive oxygen species in spermatozoa and GSTM1 polymorphism in infertile men

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Abstract

aim: To examine whether a relationship exists between glutathione S-transferase Mu-1 (GSTM1) gene polymorphism and the susceptibility of spermatozoa from patients with idiopathic infertility to oxidative stress.

Methods: Fifty-two men with idiopathic infertility and 60 healthy fertile men were recruited to this study. GSTM1 gene polymorphism was determined by polymerase chain reaction (PCR) and both the infertile and control individuals were divided into GSTM1 null and GSTM1 positive groups according to their GSTM1 gene structure. ROS formation was followed by chemiluminescence of luminol-fluoresceine-enhanced system. We compared reactive oxygen species (ROS) generation in spermatozoa from infertile patients and controls with respect to GSTM1 genotype.

Results: Significantly higher levels of ROS were found in idiopathic infertile men with the GSTM1 null genotype compared with those with the GSTM1 positive genotype. There was no significant difference in genotype distribution for the GSTM1 variant between the idiopathic infertile subjects and fertile subjects.

Conclusion: Our results suggest that the susceptibility of spermatozoa to oxidative stress is significantly greater in idiopathic infertile men with the GSTM1 null genotype compared with those possessing the gene. Therefore, in patients with idiopathic infertility, GSTM1 polymorphism might be an important source of variation in susceptibility of spermatozoa to oxidative damage

Keywords: GSTM1 polymorphism, Reactive Oxygen Species, Spermatozoa

Investigation of Efficient Regeneration Protocol for Different Flax (*Linum usitatissimum* L.) Cultivars

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Abstract

In this work which aimed to developed *in vitro* shoot regeneration of different flax cultivars, hypocotyl, stem and radicula segments derived from Omega and McGregor cultivars cultured on MS media containing different combinations as 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) phytohormones. It was observed that hypocotyl was the explant to prove the highest shoot regeneration and Omega cultivar had the highest shoot regeneration with 60%, total shoot number per petri dish (33,67) and shoot number per explant (3,03) on MS containing 0,5 mg/l BAP + 0,02 mg/l NAA. The highest callus induction (100%) and the highest callus weight (0,391g) were observed on MS containing 1 mg/l BAP + 0,2 mg/l NAA.

In cv. ' McGregor ', it was observed that callus induction was 100% as the highest. The highest callus weight (0.383 g), shoot regeneration (36.67%), shoot number per explant (1,20) and total shoot number per petri dish (12) were recorded from hypocotyl explants cultured on MS medium with 1 mg/l BAP + 0,04 mg/l NAA. Differences among genotype, hormone combinations and explant types were also observed, and it was proved that shoot regeneration was affected by them.

Keywords: Callus induction, *Linum usitatissimum* L., Shoot regeneration

Interaction of β -galactosidase from *Escherichia Coli* with polycationic polymers

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Abstract

Biocatalyst immobilization has gained increased attention for the synthesis of several industrial bioproducts. β -galactosidase (EC 3.2.1.23) catalyzes lactose hydrolysis as a forward reaction and oligosaccharide synthesis as a reverse reaction. β -galactosidase preparations that are thermally stable and exhibit high transgalactosylation activity need to be developed for efficient oligosaccharide synthesis. Polyethyleneimine (PEI) has been an essential ingredient in many enzyme immobilization procedures, where it serves to coat an inert support such as porous glass microbeads, polymeric membranes and cotton clothes etc. PEI, a highly branched cationic chain polymer, has many applications in biochemistry due to its ability electrostatically interact with negatively charged species. In this research, the effect of PEI-enzyme interaction on the activity was investigated and the effects of pH and temperature on the immobilization yield was also studied.

Keywords: Enzyme immobilization, polyethyleneimine, β -galactosidase, *Escherichia coli*

Characterization of Extracellular lipases Isolated from *Acinetobacter psychrotolerans* strains

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Abstract

Lipases were obtained from animals, plants and especially microorganisms. They are the major industrial enzymes and were used extensively. In this study, lipolytic activities of *Acinetobacter psychrotolerans* Xg1 and Xg2 strains were qualitatively determined in Tween 20, Tween 80 and Tributirin Agar. For lipase activite test was used Rhodamine B agar medium. Both strains gave positive results in this medium. Lipase activities using p-nitrophenyl palmitate was quantitatively measured by a spectrophotometer.

Next, extracellular lipases of *Acinetobacter psychrotolerans* Xg1 and Xg2 strains were characterized. Both enzymes exhibited maximum activity at pH 8 and 30 °C. The enzyme exhibited the highest stability in the presence of various organic solvents such as hexane, ethyl acetate, chloroform and N,N dietil formamid, but it was determined reducing at organic solvents isopropanol, asetonitril and bütan-1-ol. The lipase of Xg1 strain was inhibited in the presence FeCl₃, CuCl₂ ve ZnCl₂, but the lipase of Xg2 strain was inhibited in the presence CuCl₂ ve ZnCl₂. When in presence EDTA, the lipase activities of Xg1 and Xg2 strains was inhibited. In presence SDS, they was exactly inhibited. In culture supernatants obtained from Xg1 and Xg2 strains were performed ultrafiltration, gel filtration chromatography, SDS-PAGE and activite tests, respectively.

Keywords: *Acinetobacter psychrotolerans*, Lipase, Partial Characterization

Isolation And Identification of *Rhizobium* Spp. Bacteria of Wild Leguminous Plants Collected from Kırşehir

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Abstract

In our study used wild leguminous plants Kırşehir belongs to 1094 -1379 altitude regions (Akçakent, Akpınar, Boztepe, Çiçekdağı, Kaman, Mucur) were collected during the months of May, June and July. Nodules were obtained from this plant were sterilized, YMA plates were streaked and petri dishes 28 +1 °C were incubated for 3-5 days . Colonies appear after incubation typically constitute (white , clear or slightly opaque , mucosity , round , raised) 30 isolates were selected and transferred to tubes and refrigerated YMA were stored at +4 0C . For this purpose isolates ; YMA containing Bromothymol blue, Congo red and Litmus milk medium reproduction, gram stain reaction , movement and subjected to catalase and oxidase tests were evaluated. The cytological and biochemical analysis of results showed that 30 of 21 strains belonged to *Rhizobium* spp.

Keywords: *Rhizobium* spp., leguminous, nodule

Investigation of effects against pathogenic bacteria of novel Pt(IV) complexes

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Abstract

The coordination chemistry of transition metal complexes with azomethine ligands has been widely studied, partly due to the use of such compounds as antibacterial drugs in the field of medicine. In particular, titanium (IV), platinum (II) and silver (I) complexes have been used in the treatment of numerous diseases. The aim of this work was to investigate the antibacterial activities of new Pt(IV) complexes. Four new Pt(IV) complexes have been examined for antibacterial activity against pathogenic strains *Listeria monocytogenes* 4b ATCC19115, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC1280, *Salmonella typhi* H NCTC 901.8394, *Brucella abortus* (A.99, UK-1995) RSKK03026, *Staphylococcus epidermis* sp., *Micrococcus luteus* ATCC93419, and *Shigella dysenteriae* typ 10 NCTC 9351, *Enterobacter aerogenes* sp., *Bacillus cereus* sp.,

As a result of the evaluation made in fused six carbons thiophene Schiff base complexes containing fluorine atom in the aromatic ring derivative is more effective against pathogenic species was observed.

Keywords: Aminothiophene, Schiff bases, Pt(IV), Antibacterial

Interaction takes place between 337L and 295L Proteins belong to Chilo Iridescent Virus

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Abstract

Chilo Iridescent Virus (CIV) belongs to Iridoviridae family and has large host spectrum because it infects insects belong to Lepidoptera, Diptera, Coleoptera, Hymenoptera and Orthoptera. After the entry of CIV virions to host cells via endocytosis, viral genome is directed to nucleus. Among 468 open reading frames (ORF) of CIV, 337L (412 amino acids) encodes glycoprotein beta hormones signal and 295L (1343 amino acids) encodes bipartite nuclear localization signals. Study of homologous of 337L protein belong to ascoviruses showed that encoded protein facilitate attachment of virus and beginning of viral infection. Another study on homolog of 295L protein in Frog Virus 3 (FV3) showed that expressed protein plays a role for delivering of viral genome to nucleus. Interaction of these proteins is important in frame of making the viral infection quick and efficient.

In this study, 337L and 295L were expressed in Sf9 cells using Bac-to-Bac system with HA-taq and His-taq respectively. Sf9 cells were infected with bacmids separately that carry 337L and 295L genes. SDS-PAGE and Western Blot analysis showed that 337L and 295L encoded proteins about 46 kDa and 95kDa, respectively. For the Pull-Down Assay, Sf9 cell lysates which were infected with 337L and 295L bacmids, were mixed at 4°C for 4 hours. 295L protein included His-taq that is 95kDa were purified with using Magne-His protein purification kits from the mix. Purified protein analysed with Western Blot using anti HA antibody and the band was that 46 kDa obtained. This resulted, 337L protein also purified with binding to purified 295L and thus protein interaction between 337L and 295L gene products was demonstrated.

Keywords: CIV, Pull-Down Assay, 337L,295L, Gene Expression

Cavern Bacteria and Their Potential in Biotechnology

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Abstract

Investigation of biological diversity in the world of modern science is very important not only due to its scientific importance but also the great industrial potential. In particular, microbial diversity also offers a marvelous metabolic variance for biotechnology. Extremophile isolates from some compelling environments are quite proficient in producing organic ingredients, enzymes and proteins etc. on the exceptional qualities. Therefore, caves where have one or more compelling factors are important industrial sources.

The caves, as part of the biosphere, have different properties in ventilation, light, organic material inlet, and water/moisture content in terms. Thus, the caves have a special biological diversity because these differences often create restricted and unusual ecosystems. In Turkey where contains many caves due to its geographical and geological properties, this diversity has not been investigated enough. In the manner of biological diversity, especially bacteria draw more attention than higher plant and animal species because of the limiting factors.

These bacterial species with many capabilities such as determined temperature requests, growing abilities restricted with nutrition presence, precipitation capabilities for various substances together with calcium and etc., are very important because of their biotechnological potentials.

Keywords: Cave, microbial diversity, biotechnological potential, bacteria

Acknowledgements: This study was supported by Scientific Research Projects of Atatürk University

Applications of Bacteriocins as Biopreservatives in Food

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Abstract

Bacteriocins are ribosomally synthesized antimicrobial peptides and proteins, which are produced by a wide variety of bacteria. Of all the bacteriocins known, only a very few of them are actually allowed to be used as a preservative in the food industry. The bacteriocins offer several desirable properties that make them suitable for food preservation: (i) are generally recognised as safe substances, (ii) are not active and nontoxic on eukaryotic cells, (iii) become inactivated by digestive proteases, having little influence on the gut microbiota, (iv) are usually pH and heat-tolerant, (v) they have a relatively broad antimicrobial spectrum, against many food-borne pathogenic and spoilage bacteria. These biopreservatives can be used in a number of ways in food systems: (i) the use of the bacteriocin-producing strains directly in food as starter or protection cultures, or (ii) the use of concentrated bacteriocin preparations as food additives in food systems. Undoubtedly, the most extensively studied bacteriocin is nisin, which has gained widespread application in the food industry. This Food and Drug Administrations-approved bacteriocin is produced by the generally recognized as safe (GRAS) organism, *Lactococcus lactis*, and used as a food additive in at least 48 countries, particularly in processed milk and milk products, canned foods, fish and meat products, wine manufacture, liquid egg and confectionery. This biopreservative was also added to the European food additive list where it was assigned the number E234 from European Economic Community, EEC in 1983.

Keywords: Bacteriocin, biopreservative, food

Characterization of Polyphenoloxidase Isolated from *Portulaca oleracea*

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Abstract

Polyphenol oxidases in a group called oxidoreductases are enzymes that catalyze the oxidation of phenolic compounds into qinone using molecular oxygen. The ability of polyphenol oxidases to act on phenolic compounds makes them highly useful biocatalysts for various biotechnological applications. They are commonly found in animals, plants and fungi. Polyphenol oxidases are also widespread in bacterial species.

In this study, polyphenol oxidase of purslane (*Portulaca oleracea*) was partially purified and some of its kinetic parametres were investigated. Polyphenol oxidase of purslane (*Portulaca oleracea*) was partially isolated and spesific activity of partially purified enzyme using catechol as a substrate, was measured by spectrophotometer method. The optimum temperature and pH of polyphenol oxidase for catechol found to be 50 °C and 7.0, respectively. Moreover, the effects of some inhibitors on enzyme activity were investigated.

Keywords: Purslane (*Portulaca oleracea*) polyphenoloxidase, optimum pH and temperature, inhibitors

Assessment of The Influence of Diabetes and Body Mass Index (BMI) on DNA Damage by Comet Assay in Hemodialysis Patients

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Abstract

Patients with chronic kidney failure (CKF) are a major public health concern. Whether dialysis treatment to patients lead to genome damage is a controversial issue. However, diabetes is the most important cause that leads to CKF and increased body mass index (BMI) is associated with CKF. The purpose of this study is to investigate possible genetic damage in hemodialysis patients with CKF and the relationships between DNA damage and diabetes and BMI levels. Therefore, the possible genetic damage was investigated by comet assay in 22 diabetic and 31 non-diabetic hemodialysis patients with CKF. In addition, DNA damage in 20 CKF patients with BMI levels higher than 25 (kg/m²) was compared to 33 CKF patients with BMI levels less than 25 (kg/m²) by comet assay. According to the results, there was no significant difference between diabetic and non-diabetic patients in terms of DNA damage. Whereas, the comet tail intensity (p=0.023) and tail moment (p=0.046) in patients with BMI levels higher than 25 (kg/m²) significantly increased compared to those of less than 25 (kg/m²). These results suggest that diabetes did not effect DNA damage in hemodialysis patients with CKF and greater BMI increased comet tail intensity and tail moment.

Keywords: Chronic kidney failure, hemodialysis, diabetes, body mass index, comet assay

Investigation of Antimicrobial, Antioxidant and Cytotoxic Effects of *Achillea teretifolia* Willd. Extracts

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Abstract

In this study, it is aimed to investigate antimicrobial, antioxidant and cytotoxic effects of *Achillea teretifolia* Willd. water extract (AWE) and methanol extract (AME). On this way, the extracts were analysed for antimicrobial and antioxidant activity by agar well diffusion, minimum inhibition concentration (MIC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and phenolic content detection with Folin-Ciocalteu reactive. Cytotoxic effects of the extracts were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method on HeLa (Cervix cancer), MCF-7 (breast cancer), DU145, PC-3 (prostate cancer) and human gingival fibroblast (HGF) cells and also by propidium iodide (PI), 4',6-diamidino-2-phenylindole (DAPI) and 3,3'-diethylthiopyranine iodide (DiOC6) fluorescence dyes. Active caspase-9 and poly ADP ribose polymerase (PARP) protein levels were investigated by western blotting in cytotoxic extracts. According to antimicrobial results, only AME had antimicrobial effect against some bacteria. In antioxidant result, AME had higher DPPH scavenging effect and phenolic content. Although the extracts at 0,001-25 µg/ml concentration range had no cytotoxic effects on PC-3 and DU145 in 24 hours, they were effective on MCF-7 (IC₅₀>100µg/ml) and HeLa (AME IC₅₀: 0,0055±0,0040 µg/ml, AWE IC₅₀: 0,10±0,05 µg/ml). They were also cytotoxic on PC-3 (AWE IC₅₀:1,30±0,03 mg/ml, AME IC₅₀:0,35±0,06 mg/ml) and DU145 cells (AWE IC₅₀:1,30±0,05 mg/ml, AME IC₅₀:0,40±0,05 mg/ml) at higher concentration range (0,1-1mg/ml). The extracts had no cytotoxic effect on HGF. HeLa was the most sensitive cancer type against the extracts. As a consequence, AME is more effective in all of the experiments and induces apoptosis activating PARP and caspase-9 enzymes on HeLa cell line.

Keywords : *Achillea teretifolia* Willd., antimicrobial, antioxidant, cytotoxic effect

Intraspecific comparison of *Scorzonera pygmaea* (Asteraceae) based on ITS and *trnT-L+trnL-F* intergenic spacer

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Abstract

S. pygmaea subsp. *pygmaea* and *S. pygmaea* subsp. *nutans* were described firstly from Turkey based on morphological data. We aimed to compare these subspecies based on molecular data. Plant materials used in this study were collected from the wild habitat during field study in Turkey. Total genomic DNA was extracted from silica-dried materials. ITS and cpDNA *trnTL+trnLF* intergenic spacer from these subspecies, as well as 9 other species from the family, were sequenced. nrDNA ITS and cpDNA *trnTL+trnLF* intergenic spacer were amplified using universal primers and sequenced by Macrogen Inc. The phylogenetic analysis was carried out by selecting the Maximum Likelihood (ML) criterion of MEGA 6.02 software. ITS sequences and two chloroplast regions of all populations of *S. pygmaea* subsp. *pygmaea* and *S. pygmaea* subsp. *nutans* were identical. These results reveal that *S. pygmaea* subsp. *pygmaea* and *S. pygmaea* subsp. *nutans* which are quite similar to the morphological characteristics should be treated under *S. pygmaea* according to the molecular data.

Keywords: cpDNA, nrDNA, *Scorzonera pygmaea*, Turkey

This project was supported by TÜBİTAK (TBAG-109T972)

eNOS Gene Polymorphisms in the Paraffin-Embedded Tissues of Prostate Cancer Patients

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Abstract

Molecular genetic studies have demonstrated that a gene on the q arm of human chromosome 7 plays an important role in prostate cancer progression. The eNOS gene is located on chromosome 7q35-36, spans 21 kb and comprises 26 exons. In our study, eNOS gene polymorphisms (T-786C promoter region, G894T ve Intron 4 VNTR (4a/b)) were examined at extracted DNAs from formalin-fixed paraffin-embedded tissues of prostate cancer patients (CaP) (n=50). Molecular variants genotyping was performed by PCR-RFLP technique. For T-786C polymorphism, we found that TC genotype was associated to CaP risk (OR:3.325, CI: 1.350-8.188, p=0.008). The eNOS G894T polymorphism was significantly associated with CaP patients compared with healthy controls. eNOS 894T allele frequency was significantly associated with CaP patients. No association was identified on intron 4 VNTR polymorphism in CaP patients in this study.

In conclusion, we found significant differences genotypic and allelic frequencies between CaP patients and controls for eNOS T-786C and G894T polymorphisms. The presence of T-786C genotype and 894T allele in carriers increased the risk of CaP. No association was found between intron 4 VNTR polymorphism and CaP patients.

Keywords: Polymorphism, Prostate cancer, eNOS gene, Turkish Population

Classification of Cell Death

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Abstract

In 1972 Kerr and colleagues collect the cell death under two headings. Although cell death is known as apoptosis ve necrosis, molecular studies add to morphological studies and according to this, this classification is extended. In 2009 NCCD classifies it entosis, mitotic collapse, necrosis, necroptosis and pyroptosis by using terminological words. In addition to these there are different types of cell death in terms of signal pathway. These are autophagic cell death, cornification cell death, netoz cell death, partanatoz cell death, anoikis cell death. In this article these types of cell death is noticed in detail.

Keywords: Mitotic catastrophe, pyroptosis, cornification, parthanatos, anoikis

Determining of RAPD Profiles of *Camellia sinensis* to Generate SCAR Markers

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Abstract

Tea is one the important breeding plants in Turkey. For half a century in Turkey, many clonal tea cultivars have been bred to improve the yield and quality of tea production. These cultivars are used to provide tea cultivation and production. Due to the production of tea by seeds, tea-growing areas are in different quality in terms of efficiency. Up to now, Rize Tea Research Institute determined ten different cultivars. The distribution rates of these cultivars in the field of tea production are unknown. In this study, it is aimed to determine the RAPD (Random Amplified Polymorphic DNA) profiles for ten cultivars defined by Rize Tea Research Institute that can be used for the development of the Sequence Characterized Amplified Region (SCAR) markers. Total of 18 samples (10 cultivars and 8 different samples were collected from 8 different parts of Rize) were selected to perform the work. *Camellia olifer* was used as external group. 88 RAPD primers were used for RAPD amplifications and despite to most primers generated monomorphic patterns some primers are suitable to generate SCAR markers. Especially, BIO-02, BIO-16, BIO-28, OPC-02, OPC-11, OPC-15, S-202, S-207, S-208, S-211, S-216, S-224, S-234, S-251, S-279, OPA-9, OPA-12, OPB-10, OPV-12, OPQ-13, OPQ-5, SC10-12, SC10-19, SC10-97, OPH-09, OPI-04, OPJ-05, OPQ-10, OP-03, OP-05, OPB-03, OPV-14, OPV-20, OPW-02 and BIO-03 RAPD primers were determined as a candidate for SCAR markers in repetitive experiments. Each primer is suitable for improving SCAR marker for one or more of cultivar based on the band profiles. Each of the primers described in this study can be used to develop SCAR marker for tea cultivars to distinguish them from each other.

Keywords: *Camellia sinensis*, RAPD, SCAR Marker

The Somatic Incompatibility in *Trametes versicolor* (L.) Lyod.

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Abstract

The somatic incompatibility in *Trametes versicolor* (L.) Lyod. was studied using ten wild strains. The samples were collected at Black Sea Region especially Ordu, Giresun Samsun locations. Two different types of somatic incompatible interactions were observed lightly or heavily pigmented lines developing between the two isolates. All isolates were examined with help of both light microscopy and scanning electron microscopy (SEM). The width of the compatible hyphae is 2.25 μ m and incompatible hyphae are 1.08-1.20 μ m.; 960nm-1.36 μ m. This financial support by Kırıkkale University SRP Coordination Unit via grant numbered 2011-09.

Keywords: *Trametes versicolor*, Somatic Incompatibility, Mycelium interactions

Algae-Photobioreactors

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Abstract

In our world, which constitutes an important source of biomass algae, In recent years, food, medicine, cosmetics, industrial and energy fields are attracting great interest. Because of this interest, rapid algal production in axenic conditions has become imperative. Established for this purpose a special laboratory culture, cultural collections and algae photobioreactors; has become a need for developing the scientific world. In this study, in order to produce some algal species was designed algae- photobioreactors are included.

Keywords: Algae, Algae-photobioreactors, Algal production

Determination of Telomerase mRNA Ekspression (DogTERT) in Dogs

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Abstract

In this study, we aimed to evaluate quantitative determination of dogTERT mRNA expression and to analyze the correlation between the expression level of dogTERT mRNA and different age and race. The level of dogTERT mRNA was analyzed in 38 different age and race dogs by qRT-PCR.

The levels of dogTERT mRNA expression in group 1 (2,5-18 month) were significantly higher than group 2 (3-6 age) dogs. Our preliminary results show that qPCR measurement of dogTERT mRNA in peripheral blood discriminates the first study of all about dog telomere studies.

However, a further study with long-term follow up in a larger number of patients is required to confirm the clinical application of this molecular marker.

Keywords: Telomere, Telomerase, dogTERT, qRT-PCR, Dog

Shark Cartilage and Liver Oil Using Possibilities Against to the Cancer Formation

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Abstract

Cancer has been seen rarely or none in sharks, compare to the other organisms, and it is important to understand what the relationship of the shark's cancer-preventing immune system is that? A lot of different suggestions were made by the scientists. One of the important main explanation in that, the sharks cartilage and liver oil effections' against to the cancer formation.

There are a lot of reasons to being cancer. But mainly, depends on the blood vessel developing status. One of the main differences between sharks and higher vertebrates; is that a shark's skeleton is made of cartilage. Cartilage is different from other types of tissue, meaning it does not contain any blood vessels. Recent research indicates there is a relation between the lack of blood vessels in shark cartilage and sharks anti cancer mechanism. It was determined that shark cartilage is a powerful inhibitor of tumor growth.

Another important differences in sharks that their almost 10 times higher amount of liver oil content, compare to the other animals. The effective parts of shark liver oil have been determined as a group of ether-linked glycerols or in other saying alkylglycerols. The activation of protein kinase C, having a role in cell proliferation, can be prevented by alkylglycerols. Different studies indicated that, alkylglycerols have a multifunctional role and shark liver oil can be used in treatment of several types of cancer.

Keywords: shark, cartilage, liver oil, cancer

Screening of Morphological and Anatomical Features of *Mitrophora semilibera* (DC.) Lev. from Turkey

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Abstract

In this study, the morphological and anatomical features of *Mitrophora semilibera* (DC.) Lev. spores and mycelium were examined. In our country; they show a wide distribution in temperate periods. Cap is reach diameters up to 2-5 cm, brownish, conical and irregular brownish ribs. Stalk is whitish, about 9-10 cm long and hollow. The mushroom samples were collected from Kırıkkale region at Turkey and were brought to the laboratory. Tissue fragments were taken and they were cultured on the potato dextrose agar (PDA). They were incubated in the dark for 10 days, in 24°C. In the during incubation period, the development of mycelium were measured on a daily and the radial growth rates were taken as criteria. During the development, the mycelium has cottony to the agar medium surface and they were so quickly developed. There were air hyphae. No pigmentation was observed at the mycelium. The spores and mycelium of *M. semilibera* were investigated with help of both light microscopy and scanning electron microscopy (SEM). The spores of *M. semilibera* are smooth, with dimensions of 22-28 x 11-16 µm. Generally, they shaped like elliptical. This financial support by The Scientific and Technological Research Council of Turkey (TUBITAK) via grant numbered TUBITAK 210T083.

Keywords: *Mitrophora semilibera*, Turkey mycobiota, Kırıkkale macrofungus, Mycelium development

Antiproliferative effect of *Thymus cherlerioides* Vis. Var. *cherlerioides* water extract on prostate cancer cell lines

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Abstract

Genus of *Thymus* which belongs to Labiatae family is one of the important genus including medical and aromatic plants. It is known that this genus is used as a folk medicine in treatment of diabetes mellitus, regulation of blood circulation, overcoming of common cold, shortness of breath in asthma, appetizer and also contain antiseptic, carminative, antimicrobial and antioxidant properties. *Thymus cherlerioides* Vis. var. *cherlerioides* belonging to this genus is an endemic species spreading rocky and pebbly areas, named as “yer kekiđi”. In this study it is aimed to investigate antiproliferative effect of this species on DU145 and PC-3 prostat cancer cell lines. For this purpose, prostate cancer and normal gingival fibroblast cells were treated with water extract at 0.2-0.3-0.4-0.5-0.6-0.7-0.8-0.9-1 mg/ml doses within 24 and 48 hours. The effect of cancer and normal cell viability of the extract were detected by 3-(4,5-dimetilthiazol-2-yl)-2, 5 difeniltetrazolium bromid (MTT) method. Although the cell viability in normal cells wasn't changed significantly ($p < 0,05$) by treatment of *Thymus cherlerioides* Vis. var. *cherlerioides* water extract according to concentration and time (24 or 48 hours), prostate cancer cell viability was decreased ($p < 0,05$). It was detected that effect of the extract on cell viability of DU145 cells derived from hormone resistance epithelial carcinoma (IC50: 0.58 ± 0.50 mg/ml) was higher than PC-3 derived from adenocarcinoma (IC50: 0.90 ± 0.20 mg/ml) on 24 hours, significantly ($p < 0,05$). According to these results, it was determined that *Thymus cherlerioides* Vis. var. *cherlerioides* water extract had an antiproliferative effect, which was more distinctive on hormone resistant prostatic epithelial carcinoma cells. Mechanisms of this antiproliferative effect should be investigated in molecular level and also be endorsed by in vivo studies.

Keywords: *Thymus cherlerioides* Vis. var. *cherlerioides*, prostat cancer, cytotoxicity, MTT, gingival fibroblast

Evaluation of SNPs prior to genotyping studies

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Abstract

Prostate cancer is the fourth most common cancer type in men throughout the world. It is the second cause of death because of cancer among men in Turkey. Prostate cancer is often asymptomatic at the early stages of disease and occurrence of symptoms indicates the progression of disease or metastasis formation. A direct cause of prostate cancer has not been identified, but a number of risk factors such as age, hereditary predisposition, diet, race and genetical factors have been identified. Single nucleotide polymorphisms (SNP) have been proposed as ideal markers in disease association studies. Genome-wide association studies (GWAS) have identified multiple SNPs associated with prostate cancer. It is known that different populations may have different sets of risk alleles, so SNP profiles of each population must be defined, separately. In association studies, Hardy Weinberg Equilibrium (HWE) of each SNP is generally tested before analyses of disease association. If a population is in Hardy-Weinberg equilibrium, allele frequencies will not change from generation to generation and genotype frequencies can be predicted from allele frequencies. It is also known that many human SNP's are in HWE. Traditionally, SNPs that depart significantly from HWE are excluded before further analyses. In this study, genotyping of 67 SNP was performed with hME/iPLEX method in study group which consists of prostate cancer patients and healthy controls (total 175 individual). HWE was tested for each of 67 SNPs by comparing the observed genotype counts in a sample with those expected under HWE. The results showed that rs11135910, rs11902236, rs445114, rs721048, rs6497287 ve rs6983267 genotype frequencies departed from HWE ($p \leq 0,05$).

Keywords: Prostate cancer, single nucleotide polymorphisms (SNP), Hardy Weinberg equilibrium, SNP genotyping

Multiple SNP genotyping of intracranial aneurysm patients by MassARRAY iPLEX platform to be used for risk assessment of the disease

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Abstract

Intracranial aneurysm, also called as cerebral aneurysm, is a major public health problem with high mortality and morbidity which affects %5-10 of general population. It is a cerebrovascular disorder characterized by localized structural deterioration of the arterial wall and its rupture can lead to a subarachnoid hemorrhage. The pathogenesis of intracranial aneurysm is not well known and usually bleeding is seen as the first symptom of the disease. Therefore it is very important to diagnose intracranial aneurysm early since it causes disability or death when it bleeds. It is known that genetic factors has role on pathogenesis of intracranial aneurysm. Recently, genome wide association studies are one of the most highlighted area in genetics which typically focus on associations between single-nucleotide polymorphisms (SNPs). SNP genotyping technologies have changed from labor intensive and expensive processes to the most automated, efficient, and relatively cost effective methods. In this study it is aimed to genotype some SNPs related with intracranial aneurysm using MassARRAY iPLEX platform. It is shown that system is one of the simplest, most reproducible and relatively cost effective method available for SNP analysis. Efficiency of the reaction was improved by the powerful combination of the homogeneous reaction format and the standardized assay conditions. Benefit of using a single extension primer to detect both alleles also improved result quality and reduced the total number of reactions proceeded.

Keywords: Intracranial aneurysm, single nucleotide polymorphism (SNP) genotyping, mass spectroscopy, risk assessment, population study

An important Question for Phytoremediation technology: Why did plants evolve hyperaccumulation of heavy metals?

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Abstract

Heavy metal pollution of soils is increasingly becoming a global problem with the development of industry, mining activity, irrigation of wastewater etc. Heavy metal accumulation in soils has an important influence not only on the fertility of soils and functions of ecosystem but also on the health of animals and human beings via food chains. Today, the removal of toxic metals from the environment using plants (phytoremediation-green technology) has become more important issue.

Many metals such as Zn, Mn, Ni and Cu are essential micronutrients. In common non-accumulator plants, accumulation of these micronutrients does not exceed their metabolic needs. In contrast, metal hyperaccumulator plants can accumulate exceptionally high amounts of metals. Recent studies have shown that metal accumulation in the foliage may allow hyperaccumulator species to evade predators including caterpillars, fungi and bacteria.

The discovery of a class of plants that concentrate exceptionally high amounts of normally toxic heavy metals in leaves has attracted considerable interest, and challenged biologists to find reasons for this unusual behaviour by providing answers to the question: why do some plants do it? In other words: what functions does hyperaccumulation perform in these plants and what are the benefits and the adaptive values of metal hyperaccumulation. This *study* is *aimed* to find the answers of these question by explaining the two hypotheses (“elemental defence” and “joint effects” hypothesis) associated with them.

Keywords: Heavy metal, hyperaccumulation, phytoremediation, elemental defence

Antioxidant and Cytotoxic Activities of *Heliotropium dolosum* De Not. (Boraginaceae) on Brine Shrimps and Human Non-small Cell Lung Cancer Cell Line H1299

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Abstract

Heliotropium is a genus of flowering plants in the Boraginaceae family. There are 250 to 300 species in this genus, which are commonly known as heliotropes. In Turkey, this genus is represented with about 17 species. *Heliotropium* species constitute a rich source of pyrrolizidine alkaloids, some of which have antitumor and hepatotoxic activities.

Identifying plants with therapeutic properties is a great purpose of novel researches. In this study, antioxidant activities of various solvent extracts (methanol, ethanol, chloroform and water) obtained from aerial parts of *Heliotropium dolosum* were determined. Antioxidant properties were evaluated by using DPPH (2,2-diphenyl-1-picrylhydrazyl) and β -carotene-linoleic acid assays. In addition, total phenolic contents in all the extracts of *H. dolosum* were determined as gallic acid equivalents. Brine shrimp (*Artemia salina*) lethality bioassay was used for the preliminary screening for cytotoxicity of the extracts, as this assay has shown a good correlation with cytotoxicity assays with human cell lines. The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic properties.

Conventional anticancer drug discovery and development have focused on the cytotoxic agents. In this investigation, our results indicate that the crude extract of *H. dolosum* has antioxidant properties and cytotoxic effects on Brine Shrimps and Human Non-small Cell Lung Cancer Cell Line H1299 cells and that this cytotoxic effect comes from probably pyrrolizidine alkaloids.

Keywords: Antioxidant activity, brine shrimp, cancer cell line, *Heliotropium dolosum*

Differential Expression of miRNAs in colorectal cancer tissues using RT-PCR

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Abstract

Colorectal cancer (CRC) develops and progresses through a systematic selection for (epi) genetic alterations that drive the transformation from normal colon epithelium to adenocarcinoma. Recent studies have demonstrated a link between the aberrant expression of MicroRNAs (miRNAs) to the pathogenesis of cancer. miRNAs are small non-coding RNAs as 18-25 nucleotides in length that downregulate or upregulate gene expression during various in cellular process such as cell-cycle regulation, differantiation, apoptosis and metastasis.

The aim of this study was analyse the state of art of gene expression profile in CRC using quantitative reverse transcription-real time PCR (RT-PCR) to explore some perspectives in this research field. The biopsy materials of tumoral and normal tissues were taken from the Gaziantep University Research Hospital. Total RNA from tissue samples extracted using Qiagen miRNeasy mini kit. Six mature miRNAs (miRNA-92, miRNA-21, miRNA-143, miRNA-145, miRNA-155 and miRNA-192) were selected for experimental validation using RT-PCR protocol. Isolated miRNAs were reverse transcribed into cDNA by using miscript II RT Kit. qRT-PCR reactions were performed in RotorGene Q (Qiagen) multiwell plates. The $\Delta\Delta C_t$ method was used for calculating the relative expression of a given miRNA between a normal and tumor samples. It was found that some of miRNAs were up or down regulated in CRC cells compared to non tumor cells. The upregulated oncomiRNA-21 was found in all tested tumor samples. Meanwhile tumor supressor miRNA-143 was down regulated in a most of samples as compared to non tumor samples.

Keywords: miRNAs, Colon cancer, Microarray

Ethic number : 74059997.050.01.04/020

Antimicrobial activity of *Streptomyces* sp. Ork12 isolated from orchid root

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Abstract

Objectives: Orchids are ubiquitous; there are epiphytes, terrestrial plants with underground roots and bulbs, and even mycotrophic species. Actinomycetes are bacteria known to constitute a large part of the rhizosphere microbiota. Endophytic actinomycetes were increasingly important because of some actinomycete strains have the ability to produce the bioactive compounds inhibiting some of the pathogenic fungi and bacteria. The aims of the present investigation was to determine antimicrobial activity, cultural characteristics and isolation of strain Ork12 isolated from *Ophrys sphegodes* root.

Materials and Methods: Root sample were taken during growing season from terrestrial. The root were aseptically sliced into 2-5 mm-length segments, which were ground into a sterile mortar with a small amount of water into a suspension that was then successively ten-fold diluted. 0.05 ml of each dilution was plated on modified Czapek's agar supplemented with nystatin (50 µg/ml) and incubated at 28°C for 10 days. Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene were carried out following Chun and Goodfellow (1995). Phylogenetic analysis were performed by using three different algorithms. Antimicrobial activity of strain Ork12 to inhibit the growth of twenty one microorganisms like Gram (+) and Gram (-) bacteria, fungi was observed using an overlay technique described by Williams *et al.* (1983).

Results: Ork12 shared highest 16S rRNA gene sequence similarity with *Streptomyces hygroscopicus* subsp. *glebosus* AB184479^T (98.98 %) and *S.caniferus* AB184640^T (98.97 %). Ork12 isolate exhibited activity against some pathogenic microorganisms tested.

Keywords: *Streptomyces*, Orchid, 16S rRNA gene, Antimicrobial activity

Acknowledgements: This study was supported by Ondokuz Mayıs University (PYO. FEN. 1901.12.014)

Polyphasic taxonomic characterization of a novel *Streptomonospora* sp. BN506, isolated from the Salt lake (Tuz gölü) in Turkey

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Abstract

Objectives: The genus *Streptomonospora* is Gram-positive, aerobic organisms with branching hyphae, which formed non-fragmenting substrate mycelium. The aerial mycelium forms short chains of non-motile spores; spores in short chains are oval- to rod-shaped with wrinkled surfaces. The aim of the present polyphasic study was to clarify the taxonomic position of the novel strain BN506 of the genus *Streptomonospora*, isolated from the Salt Lake, in Turkey.

Materials and Methods: *Streptomonospora* sp. BN506, was picked after 6 weeks of incubation at 28°C on Modified Bennett's Agar containing 15 % (w/v) NaCl. Cultural characteristics were determined after incubation for 4 weeks according to Shirling & Gottlieb (1966). Genomic DNA isolation was performed by Pitcher *et al.* (1989). The 16S rRNA gene was amplified by PCR using universal primers 27f and 1525r. Phylogenetic analyses were performed by using three different algorithms. DNA-DNA hybridisation was carried out as described by De Ley *et al.* (1970). Sugars in cell wall hydrolysates, polar lipids and isoprenoid quinones were determined using the described procedures by Hasegawa *et al.* (1983), Minnikin *et al.* (1984), Minnikin *et al.* (1984) & Kroppenstedt (1982), respectively.

Results: BN506 shared highest 16S rRNA gene sequence similarity with *Streptomonospora halophila* YIM 91355^T (98.14 %). MK-10(H₈) and MK-10(H₆) were the predominant menaquinone. The whole-cell sugars mainly comprised ribose, glucose and galactose.

Keywords: Salt lake, *Streptomonospora*, 16S rRNA gene, Sequence

Acknowledgements: This study was supported by Ondokuz Mayıs University (PYO. FEN. 1901.11.011)

Cloning, Expression and Characterizations of The *Acidithiobacillus ferrooxidans* M1 Wild Type and Mutant Iron Oxidases

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Abstract

The use of microbes to extract metals from ores is simply the harnessing of a natural process for commercial purposes. Microbes have participated in the deposition and solubilization of heavy metals in the earth's crust since geologically ancient times. Most of this activity is linked to the iron and sulfur cycles. The use of microbes in ore processing has some distinct advantages over the traditional physicochemical methods. Microbial extraction procedures are more environmentally friendly. They do not require the high amounts of energy used during roasting or smelting and do not produce environmentally harmful gaseous emissions. One of the most studied bacteria used in biomining is *Acidithiobacillus ferrooxidans*. It is characterized as chemolithoautotroph that obtains energy for its growth mainly from the oxidation of ferrous iron (Fe²⁺) or reduced sulfur compounds. The Iro protein was proposed to be the first electron acceptor in several alternative models of electron transfer chain between Fe²⁺ and oxygen. We cloned the *iro* gene of *Acidithiobacillus ferrooxidans*M1 and performed two mutations in conserved amino acids.

*Acidithiobacillus ferrooxidans*M1 was isolated from a copper mine in Murgul, Artvin. The genomic DNA of the bacterium was isolated and the *iro* gene amplified with special primers in PCR. The gene cloned to a expression vector (pET28(a)+) with NdeI-HindIII restriction sites and expressed in *E.coli* BL21 (DE3).

For determining the effect of three conserved amino acids on the protein structure and the stability, Q9F and S21Y/V22D mutations were performed by site-directed mutagenesis techniques on cloned iron oxidase. The wild type enzyme and mutant enzymes were expressed and purified. The activity of the enzymes determined with calorimetric method by using α -phenanthroline.

Keywords: *Acidithiobacillus ferrooxidans*, iron oxidase, chemolithoautotroph

New Molecular Methods for Detection of Bioterrorism Agents

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Abstract

Bioterrorism is a term used for terrorism using biological agents, such as infectious diseases or biological toxins. Biological agents act like a natural outbreak so the time and type of the strike cannot be predicted. However people have learned to live with this risk and work for protecting themselves against such disease outbreaks. For surveillance programs in place, we have the necessary tools to detect and act against many biological agents that may be used by terrorists. We have issued mostly about two important agents which are anthrax and botulinum caused by *Bacillus anthracis* and *Clostridium botulinum* respectively. Both can live in all kinds of animals, including the animals people consume. These agent form spores that are difficult to kill and can easily breed in food. For that reason these agents are at the top of the list of most dangerous diseases. Because of the increased global demand to have preventive tools for possible terror attacks, this study is about determining anthrax and botulism, as well as several other potential biological agents. New determination methods based on Immunoassay and PCR are also discussed. This review deals with methods to detect biological agents.

It can be foreseen that the introduction of new methods, not only in the field of bioterrorism, but also in the fields of public human health, animal health and diagnostic tools used by hospitals will help mitigation of such terrorist attacks and determining biological agents.

Keywords: Bioterror, *Bacillus anthracis*, *Clostridium botulinum*, Immunoassay and PCR based determination methods

Genetic Nature of Plasmids Derived from Multiple Drug Resistant *Salmonella* Strains

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Abstract

The main role of plasmids encoding MDR is to confer a survival ability against antimicrobial compounds for *Salmonella* strains in hosts. The aim of this study is to examine plasmids of eight *Salmonella* spp. of food origin for the presence and the relationship of antibiotic resistance genes and class 1 integrons. MDR *Salmonella* isolates's plasmid DNA was extracted by Kodo-Liu alkaline lysis method and plasmids were separated by electrophoresis in 0.7% agarose gel with Supercoiled DNA Ladder. Each of plasmids were purified from agarose gel using gel extraction kit. PCR amplifications were performed in order to detect whether strains include *aphA1*, *blaTEM*, *tetA*, *parC* and *gyrA* genes. Plasmid DNAs were amplified by using class 1 integron primer pairs and resistance genes examined on integron. *Salmonella* isolates contained plasmids which were varied in number from 1 to 7 and in different molecular sizes (0.4-150 kb). The PCR analysis showed that all isolated plasmids were found to have *parC*, *aphA1* and *blaTEM* genes and to carry 12% *tetA* resistance gene, 36% *gyrA* resistance gene. Class 1 integron was also detected on 120 kb plasmid of one strain. *blaTEM* and *gyrA* genes were detected on 1200 bp integron. High prevalence of plasmids against to integrons demonstrate that these genetic elements are common among in epidemiologically unrelated isolates and associate with reduced susceptibility to antimicrobial drugs. Plasmids can mediate to transfer of antibiotic resistance genes to other bacteria present in food chain. This issue suggests that *Salmonella* infections aren't only clinical.

Keywords: *Salmonella*, plasmid, antibiotic resistance, class 1 integron

Efficient regeneration system from rye leaf base segments

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Abstract

Rye is extremely recalcitrant to tissue culture. Efficient plant regeneration system from leaf base segments of rye (*Secale cereale* L.) was developed. The factors affecting the callus formation and regeneration capacity of leaf segments of diploid and tetraploid *Secale cereale* plants were investigated. The highest callus formation rate (10.4%) and shoot formation (4.5%) were achieved in the first segments. Two different media type, N6 and MS medium were also investigated. The highest callus (15.78%) and shoot (6.7%) formation were observed in MS medium. Different carbohydrate sources which were 20 gr/L sucrose and 20 gr /L maltose were assessed to determine the effect on callus and shoot formation. The highest callus formation rate (11.72 %) was observed in medium containing 20 gr/L Maltose. Based on shoot formation, sucrose (5.9%) performed better than maltose (3.13%).

Keywords: auxins, cytokinins, regeneration, carbohydrates

Determination of Lipids and Anticancer Activities of *Cyclotrichium niveum*

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Abstract

Cancer is one of today's most important disease is the main reason a large portion of human death. Modern anti-cancer drug development for the treatment of cancer studies before II. World War began (He and Liu, 2007). Anticancer drugs are produced of plant or animal sources or by direct chemical synthesis. More than 60% of anti-cancer agents are of natural origin. For this purpose, anticancer activity of a many of plant extracts were examined in cancer cell lines. In this present study, essential and fixed oils extracted from leaf of *C. niveum* which was collected from Isparta province were determined. Anticancer activity of essential oils of the plant were also determined on C6, HeLa and HT29 cell lines as in vitro. Lipids extracted from the leaves by the method Blig& Dyer (1959). According to gas chromatography results, docosahexaenoic acid (23.62%), gammalinolenic acid (11,81%), palmitic acid (10.14%), arachidonic acid (8.15%), stearic acid (8.67%), caprylic acid (7,17%), nervonik acid (6,77%), linoleic acid (6,54%), oleic acid (6.51%), alpha-linolenic acid (3.75%), eicosapentaenoic acid (2,67%) were determined as major fatty acids. Essential oil compenents obtained from the leaves were determined isomenthone (42%) and pulegone (47%). These essential oils followed limonene, menthone and limonene as essential oils in less amounts. Antiprolative activity of the essential oils was examined against to HeLa, C6 and HT29 cell lines by using Cell Proliferative ELISA in 5µg/ml to 100µg/ml concentrations. The essential oils have antiproliferative activity on HT29 cell line, but not on HeLa and C6 cell lines. The essential oil was more antiproliferative at high concentrations on C6 and HeLa cells indicating cell specific activity. Anticancer drug potential of essential oil of *C. niveum* leaf needs further investigation.

Keywords: Anticancer activity, Cell lines, *Cyclotrichium niveum*

Serum Fatty Acids and Colon Cancer

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Abstract

Cancer is a common disease in the world is the variety. Specific abnormalities in lipid metabolism have been reported in cancer patients. Essential polyunsaturated fatty acids and their derivatives contain important biological activities. In this study, colon cancer samples have investigated the relationship between serum fatty acid composition. Blood samples were collected in the outpatient clinic General Surgery 10 subjects diagnosed with colon cancer and non-cancer, were taken from 10 healthy subjects. Total lipids were extracted from serum examples with the use of modification of the Bligh & Dyer method. Fatty acid methyl esters were prepared by direct transesterification from total lipid extracts. Fatty acid methyl esters were analyzed by a gas-chromatography. Samples taken to the vials of fatty acid type and% abundance is determined. According to results of gas-chromatography, fatty acids of colon cancer and control samples were determined from 16C to 20C. In both groups, palmitic acid (16:0), palmitoleic acid (16:1), margaric acid (17:0), stearic acid (18:0), oleic acid (18:1), elaidic acid (18:1 trans), linoleic acid (18:2), dihomo-gamma-linolenic acid (DGLA 20:3) and arachidonic acid (20:4) was determined. Palmitic, steari and linoleic acid were major fatty acids in both groups. Differences in patient and control groups is made according to t test for independent samples, $p < 0.05$ significance value . Palmitic, palmitoleic, oleic, elaidic acid and dihomo-gamma-linolenic acid in $p < 0.05$ significance level was determined differences.

Keywords: Cancer, lipid metabolism, saturated faat acids, omega-3

Genotoxicity of Sodium Propionate in Isolated Human Lymphocytes Assessed by Comet Assay

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Abstract

Food additives, especially preservatives, are in widespread use in food industry. Sodium propionate (SP) (E 281) is a preservative widely used in the whole world. It is sodium salt of propionic acid. It inhibits the growth of mold and bacteria, thus prolonging the shelf life of bread and baked goods. The present study was planned to determine the genotoxic effect of SP by comet assay in isolated human lymphocytes. The comet or single cell gel electrophoresis assay (SCGE) is a commonly used technique for the measurement and analysis of DNA damage in individual cells. Peripheral blood was obtained from three healthy (1 male and 2 female), non-smoking donors (24-26 years old) without a history of exposure to genotoxic agents. Lymphocytes were isolated using Biocoll separating solution. The isolated human lymphocytes were incubated with six different concentrations of SP (15.62; 31.25; 62.50; 125; 250 and 500 µg/mL) for 1 hour. A negative (distilled water) and a positive control (hydrogen peroxide; H₂O₂) were also used. The tail length (µm), tail intensity (%) and tail moment of 100 comets on each slide (a total of 300 comets per concentration) were determined using specialized Image Analysis System ("Comet Assay IV", Perceptive Instruments, United Kingdom). SP significantly increased the comet tail length (r=0,80), tail intensity (except 15,62 µg/mL) (r=0,91) and tail moment (except 15,62 µg/mL) (r=0,96) in all the concentrations compared the control indicating that SP induced primer DNA damage in isolated human lymphocytes. However, our results concern only in vitro experiments with human lymphocytes. So, additional animal and human studies should be performed before to assess genotoxic potential of sodium propionate.

Keywords: Food additives, genotoxicity, sodium propionate, comet assay

Histoprotective Effect of Vitamin D against Carbon tetrachloride (CCl₄) Nephrotoxicity in Rats

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Abstract

Carbon tetrachloride (CCl₄) is a biologically dangerous agent. It induces acute and chronic renal injuries. Vitamin D is endogenously synthesized in the proximal tubule cells. The aim of this study was to examine the protective effect of vitamin D treatment on CCl₄-induced nephrotoxicity in rats using histopathological parameters. In this study, adult male Wistar albino rats weighing 200-220 g were used. The rats were divided into 4 groups (control, 10 week CCl₄ group, 10 week CCl₄ + vitamin D group and 12 week CCl₄ + vitamin D group). There were 6 rats in each experimental group with a total of 24 rats. 1 ml/kg dose of CCl₄ was injected subcutaneously twice a week. 0.5 mg/kg dose of Vitamin D was intraperitoneally administrated every day. Kidney tissues were processed for light microscopy and embedded in paraffin. Sections were stained by hematoxylin-eosin and Periodic Acid Schiff and examined by light microscopy. In CCl₄ treated rats tubular and glomerular degeneration were detected in the kidney tissues. There was dilatation and vacuolization within the tubules and hemorrhage in the inter-tubular region. In the kidney glomeruli, congestion, atrophy and adhesion to parietal layer were observed. Tissue disorganization and aggregation of Bowman's capsules were noted. In addition, mononuclear cell infiltration was observed between the glomeruli and the tubules. Results of Vitamin D treated group were similar to the controls. In conclusion, this study suggests that Vitamin D treatment could reduce renal damage.

Keywords: Kidney, Carbon tetrachloride, Nephrotoxicity, Vitamin D

Nuclear Translocation of Nrf-2 and NFκB in Streptozotocin Induced Diabetic Liver

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Abstract

Nuclear factor erythroid 2-related factor (Nrf2) functions as a key mediator of the redox homeostatic gene regulatory network under conditions of oxidative and electrophilic stress. Nrf2 signaling pathway is activated to enhance the expression of multiple antioxidant and phase II enzymes. Furthermore, depletion of cellular antioxidant defense mechanisms and the generation of oxygen free radicals results in the activation of nuclear factor kappa B (NFκB), an inducible transcription factor, and thus promotes the expression of NFκB-regulated genes, which thereby contributes to the further enhancement of defense against oxidative stress. Under normal oxidative conditions, Nrf2 and NFκB are present in the cytoplasm and unable to operate if there is any signal, such as those induced by changes in the redox potential. When the redox status altered, these transcription factors move into the cell nucleus and impinges on the promoter regions of genes encoding phase II detoxification enzymes and antioxidant proteins. Therefore, present study was designed to demonstrate the oxidative stress observed in diabetes alter the redox homeostasis and modulate the nuclear translocation of Nrf2 and NFκB in the liver tissues. Diabetic rat models were produced with streptozotocin and liver tissues were homogenized to separate nuclear and cytoplasmic fractions. Western blot analysis of Nrf2 and NFκB from both cytoplasmic and nuclear fractions indicated that diabetes induces nuclear translocation of both transcription factors which is revealed from significant increase in the nuclear to cytoplasmic protein ratios in the liver tissues.

Keywords: Nuclear factor erythroid 2-related factor (Nrf2), Nuclear factor kappa B (NFκB), Oxidative stress, Diabetes

The Effects of Various Concentrations of Boric Acid and 24-Epibrassinolide on Germination, Growth and Pigment Contents in *Arabidopsis thaliana* (L.)

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Abstract

Brassinosteroids (BRs), an important plant hormone group regulating the growth and development of the plants are studied in recent years with respect to their ameliorating effects against some environmental stresses. Boron, while essential at trace amounts, can be toxic at increasing levels. In this study, the effects of boron and BRs were studied on the germination of *Arabidopsis thaliana* plants. A number of different concentrations for 24-epibrassinolide (EBL) (0, 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 μM) and boric acid (0, 100, 200, 300, 400, 500, 600, 700 and 800 ppm) were selected in the in vitro tests of the preliminary study. It was found that 1.5 μM EBL treatments gave the best results as compared to other EBL concentrations. 800 ppm boric acid was determined as the lethal dose. Up to this value of 500 ppm (500 ppm was chosen as the highest concentration in observing the development of the plants following germination) all the boric acid concentrations were tested in combination with all of the EBL concentrations shown above to the level of 1.5 μM . The seeds were germinated in the semi-solid $\frac{1}{2}$ MS medium containing either BA or EBL or both. The growth parameters (percent germination, leaf numbers, root length, root formation, fresh and dry weights) and pigment contents (total chlorophyll and carotenoid) were determined and statistically analysed.

This study is a first in investigating the response exerted by 24-EBL under boron stress during germination of the *A. thaliana* plants.

Keywords: *Arabidopsis thaliana* (L.), boric acid, 24-epibrassinolide, germination and growth

Characterization of Enzyme Produced Isolates From Fecal Samples

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Abstract

Production of extracellular enzymes such as catalase, protease, lipase are increased because of industrial applications. These enzymes have got a wide application areas like chemistry, waste treatment, pharmaceutical.

In this study, a variety of enzymes (protease, lipase, catalase) produced strains of infant (only breastfeeding baby) fecal samples were isolated and these isolates were selected and analyzed under the light microscope. It was found that all the isolated strains were Gram positive and *diplococcus* shaped. None of the isolates showed catalase activity. The isolates could be identified *streptococcus*. Physiological and biochemical characterizations are continued. Molecular identification was based on the polymerase chain reaction of 16S rRNA gene region. PCR amplification products were sent to sequencing. All of the results will be included in poster presentation.

Keywords: Gaita, microbial, purification, phenotype, genotype

Co-Immobilization of Glucoamylase, Pullulanase and Glucose Isomerase as Combined Cross-Linked Enzyme Aggregates (Combi-CLEA) and Its Use in Production of Fructose From Starch at One Step

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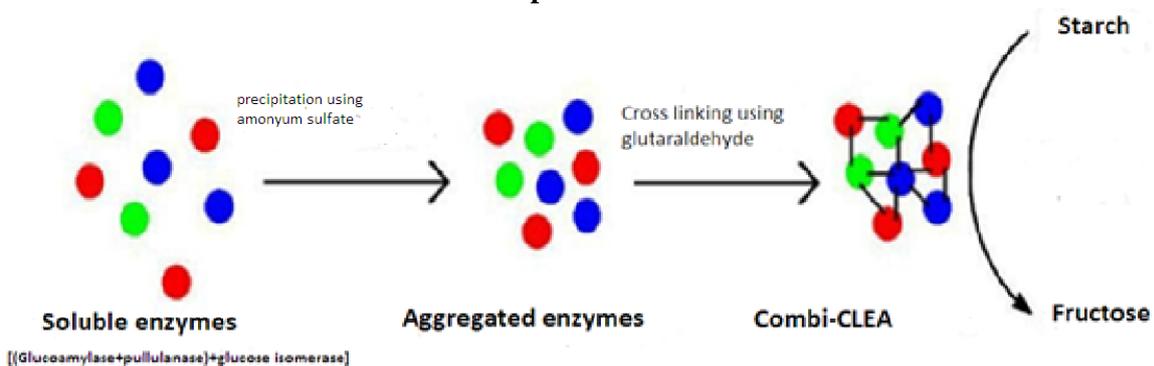
Abstract

Co-immobilized enzymes can be used in multi step enzymatic process without need of separation of intermediates. Such 'telescoping' of multi-step process into a one-pot catalytic process has several benefits such as fewer unit operations, less solvent, small reactor volume, shorter cycle times, higher volumetric and space time yields and less waste generation (1)

In this study, (Glucoamylase (GA)+ pullulanase (PUL)) and glucose isomerase (GI) were co-immobilized as cross linked enzyme aggregate (combi-CLEA) and used to obtain fructose from starch. For the determination of fructose, the prescribed method by Chen et al. was used(2). Combi-CLEA's $V_{max,app}$ and $K_{m,app}$ were found as 12 U/g combi-CLEA and 0.1157 mg/ml respectively, at optimum conditions. The residual activity of combi-CLEA was 69% of its initial activity after 50 reuses and combi-CLEA protected 44% and 29% of its initial activity after 24 h preincubation at 60°C and 80°C, respectively. After 30 days storage time, combi-CLEA protected 56% of its initial activity at 5°C and 13% of its initial activity at 25°C, respectively.

Keywords: Glucoamylase, Pullulanase, Glucose Isomerase, Combi-CLEA, Immobilization

Graphical abstract



Isolation and characterization of thermostable, alkaline, detergent and H₂O₂ resistant Cellulase (CMCase) from a novel strain *Bacillus sp.* CY-8 isolate

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Abstract

A thermostable carboxymethyl cellulase producing *Bacillus sp.* CY-8 was isolated from soil and biocompost samples. The enzyme was optimally produced in a medium containing CMC.

Partially purified enzyme showed a single band with molecular weight of 49.4 kDa in SDS-PAGE analysis. The enzyme showed its optimal activity at pH 10.0 and 80°C. More than %78 original activity of the enzyme was observed after pre-incubation at pH 7.0-12.0 at room temperature for 24 h between. It conserved more than 90% of its original activity after pre-incubation at temperature ranging from 30 to 110°C for 60 min. The enzyme exhibited up to 123%, 121%, 122%, 125%, 130%, 126%, 133% and 135% of original activity at 3%, 5%, 7.5%, 10%, 15%, 20%, 25% and 30% concentrations of NaCl, respectively. In the presence of 5mM EDTA, CaCl₂, ZnCl₂, MgCl₂ and 1,10-Fenantrolin, 3mM PMSF, %0.1 Tween-20 and Tween-80, %1 SDS, %1Triton X-100 and %1β-Mercaptoethanol, %0.1 H₂O₂, 8M urea, it remained %63, 76, 69, 67, 68, 70, 60, 68, 75, 67, 83, 97, 65 of its original activity, respectively. According to TLC analysis, CMC was hydrolyzed into maltose, glucose and other oligosaccharides by CY8 CMCase.

High thermostability, pH stability, detergent stability and antioxidant stability of *Bacillus sp.* CY8 CMCase make this enzyme very useful in textile, laundry and in other industrial applications.

Keywords: *Bacillus sp.*, Thermostable, CMCase, textile, H₂O₂

Evaluation of The Relationship Between DNA Damage And Parathormone Levels in Chronic Kidney Failure Patients By Comet Assay

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Abstract

Today, the incidence of chronic kidney failure (CKF) is a disease that is increasing rapidly. The incidence of cancer in chronic kidney disease found to be higher when compared to general population. On the other hand, high level of parathormone (PTH) is associated with vitamin D deficiency. According to the recent studies, reductions in the level of vitamin D increased the incidence of cancer. Nowadays, the comet assay is widely used to determine the genotoxic, mutagenic and carcinogenic potential. In this study, we aimed to determine DNA damage in CKF in hemodialysis patients by using comet assay. Moreover, the effect of increased PTH value on primary DNA damage was determined in these patients. For this purpose, we evaluated 53 hemodialysis patients with CKF (33 patients with PTH \leq 300; 20 patients with PTH $>$ 300) and 24 healthy individuals. The results showed that, comet tail length ($p<0.001$) and tail moment ($p=0.008$) were significantly higher in CKF patients than the control group. In addition, the comet tail length significantly increased ($p=0.003$) in patients with PTH levels higher than 300 pg/ml compared to the patients with PTH levels less than 300 pg/ml. There was a positive correlation between PTH and the comet tail length ($r=0.437$, $p=0.001$).

These findings showed that DNA damage increased in CKF in hemodialysis patients compared to control. The increase in PTH value increased comet tail length further.

Keywords: Chronic kidney failure, hemodialysis, DNA damage, comet assay, parathormone

Identification of Clinical and Environmental Based Strains with Biomerieux Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometer and Typing The Strains With Multiple-Locus Variable Number Tandem Repeat Analysis

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Abstract

In this study, it was aimed to compare two different findings. First of all, it was intended to compare the identification results (16 human, 24 animal and 12 drinking water strains) of 52 *Aeroomonas spp* strain identified with BD Phoenix automated microbiology system), the same isolates with the identification results acquired with BioMerieux Matrix Assisted Laser Desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Second of all, typing results of the same isolates acquired previously with pulsed-field gel electrophoresis (PFGE) were aimed to be compared with the typology results acquired with Multiple- Locus Variable- Number Tandem Repeat (MLVA) in this study. Following the comparison of identification results, 11 identification results were found out to be different. These strains were identified again with 16S rRNA gen sequence analysis. 16S rRNA gene sequence and re-identification results of 11 strains were found out as compatible with the results of MALDI-TOF method. In previous typing with PFGE, 44 separate PFGE patterns were acquired, 2 strains were found out to have identic patterns; however PFGE pattern of 7 strains weren't acquired. Typing of all strains were carried out with MLVA method and 46 patterns were acquired. Six different identic patterns were found out, each of them included two different isolates. As a result of the study, Biomerieux MALDI-TOF method was found out to be less reliable in the identification of environmental isolates. When two different molecular typing results were compared, separation power of PFGE method was found out to be more and MLVA method was found be more sensitive about feasibility.

Intracellular trafficking of Diphtheria Toxin Mutant CRM197

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Abstract

Diphtheria toxin (DTx) is a well-defined bacterial pathogen. The catalytic domain, fragment A (FA) of diphtheria toxin ADP-ribosylates eukaryotic elongation factor 2 in the presence of NAD⁺ and protein synthesis halts subsequently. Diphtheria toxin mutant CRM197 is non-toxic due to single amino acid substitution (glycine 52/glutamic acid) in catalytic domain. It has been observed in our previous studies that the depolymerization of the filamentous actin (F-actin) occurs right after the infection of the endothelial cells with either DTx or its mutant form CRM197 via the interaction between fragment A and F-actin. Besides the cellular effect of FA, the intracellular transport of CRM197 has not been fully elucidated yet. The delivery of toxin to the cytosol is a sequential process. The toxin binds to the cell surface via R-domain and it is internalized by clathrin-mediated endocytosis. FA becomes active following disulfide bond reduction and chaperone-dependent refolding. In this study it is aimed to investigate toxin trafficking through early and late endosomes. Human umbilical vein endothelial cells (HUVEC) were cultured and treated with CRM197 (0,75 nM) for 15 minutes. F-actin, endosomes and FA were detected by immunofluorescence microscopy. FA was spotted on Western blotting from endosome fraction of cell lysate. Immunofluorescence staining revealed that CRM197 loaded endosomes were overlapped with actin cytoskeleton and in endosome fraction Hsp90 was present. It has been concluded that the delivery of C-domain of CRM197 to the cytosol follow similar process as DTx.

Keywords: Diphtheria toxin, CRM197, F-actin, Endosome

***In vitro* testing for iron oxide nanoparticle toxicity**

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Abstract

With the development of nanotechnology and the wide use of iron oxide nanoparticles, it has become necessary to assess the potential biological adverse effects of magnetite. This study investigated the cytotoxicity, genotoxicity and oxidative damage of different concentrations of magnetite (0 to 1000 mg/L) in human whole blood cultures. After supplementation of magnetite, the blood samples were incubated for 72 hours. Then, cell viability was detected by [3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyltetrazolium bromide] (MTT) and lactate dehydrogenase (LDH) release assays, while total antioxidant capacity (TAC) and total oxidative stress (TOS) levels were determined to evaluate the oxidative injury. The DNA damage was also analyzed by sister chromatid exchange (SCE), micronuclei (MN) assay, chromosome aberration (CA) assays and 8-oxo-2-deoxyguanosine (8-OH-dG) levels as indicators of genotoxicity. The results of MTT and LDH assays showed that the higher concentrations of magnetite (100, 150, 300, 500 and 1000 mg/L) decreased cell viability. Also, concentrations higher than 10 mg/L of magnetite increased TOS levels and decreased TAC levels in human blood cells. On the basis of increasing concentrations, the magnetite caused significant increases of MN, SCE and CA rates and 8-OH-dG levels as compared to control culture. In conclusion, the obtained results showed that magnetite had dose dependent effects on oxidative damage, genotoxicity and cytotoxicity in human blood cells.

Keywords: Cytotoxicity, Genotoxicity, Iron oxide, Lymphocyte, Oxidative stress

Studies on the Antioxidant, Cytotoxic and Genotoxic Activities of Carvone Using *In Vitro* Models

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Abstract

Carvone (CVN; p-mentha-6,8-dien-2-one), a naturally occurring monoterpene, exhibits antitumor, antinociceptive, anti-inflammatory, antifungal and antibacterial effects. However, there are limited data in the literature on nature and/or biological roles of CVN. The aim of this study was to investigate the genetic, oxidative and cytotoxic effects of CVN in cultured human blood cells (CHBCs) (n=5) for the first time. Human blood cells were treated with CVN (0 to 200 mg/L) for 24 and 48 h, and then cytotoxicity was detected by lactate dehydrogenase (LDH) release and [3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyltetrazolium bromide] (MTT) assay, while DNA damage was also analyzed by micronucleus (MN) assay, chromosomal aberration (CA) assay and 8-oxo-2-deoxyguanosine (8-OH-dG) assay. In addition, biochemical measurements (total antioxidant capacity [TAC] and total oxidative stress [TOS]) were investigated to detect oxidative effects. In our *in vitro* test systems, it was observed that CVN had no mutagenic effects on human lymphocytes. On the other hand, CVN (at 25, 50, 75 and 100 mg/L) treatment caused statistically important ($P > 0.05$) increases of TAC levels and increases of TOS levels (at 150 and 200 mg/L) on CHBCs. According to the results of LDH assay, while CVN did not lead to cytotoxicity on CHBCs at 24 h, CVN induced cytotoxicity on CHBCs in a dose-dependent manner at 48 h. Also, MTT assays showed that CVN (at concentrations above than 100 mg/L) decreased cell viability. In conclusion, these findings suggest that CVN may be an important source of therapeutic antioxidants.

Keywords: Carvone, Cytotoxicity, Human lymphocytes, Genotoxicity, Oxidative effects

The Determination of the Glycoconjugates in the Epidermal Mucous Cells of the Red Californian Earthworm (*Eisenia foetida*) by Lectin Histochemistry

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Abstract

The glycoconjugates, which are essential components of the mucous of many cell types, are taken part in many functions including osmoregulation, cell to cell recognition, binding of hormones, protection of cells from phagocytosis and dehydration, differentiation, defence and ion transport. The aim of this study was to characterize lectin histochemically the nature and regional distribution of the glycoconjugates secreted by epidermal mucous cells in *Eisenia foetida* (Annelida, Oligochaeta).

This study was carried out in 20 adult specimens of *Eisenia foetida*. The specimens were divided into six parts from anterior to posterior (I, II, III, IV, V, VI) and samples were fixed in Bouin's fluid. For the lectin histochemical study, the sections were incubated with Horseradish peroxidase-conjugated *Glycine max* (SBA), *Datura stramonium* (DSA), *Dolichos biflorus* (DBA), *Bandeiraea simplicifolia* (BSA-I-B₄), *Helix pomatia* (HPA), *Ulex europaeus* (UEA-I), *Arachis hypogaea* (PNA) and *Canavalia ensiformis* (Con A) lectins.

There were no BSA I-B₄ and Con A positive glycoconjugate in the mucus cells of all region. While the SBA-positive mucus cells were few in I. and II. regions, number of them showed an increase in V. region and there were no this reactivity in III., IV. and V. regions. There were very weak reaction in only second region by DBA, in second and third regions by DSA and in first, second and sixth regions by UEA-I. It was detected that a large number of mucus cells contained very intense HPA positive glycoconjugate in the all regions.

Keywords: Glycoconjugate, mucus, lectin histochemistry, epidermis, earthworm

The Determination of Glycoconjugates in Sheep (*Ovis aries*) Submandibular Salivary Gland by Lectin Histochemistry

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Abstract

Glandula submandibularis, called as submandibular salivary gland, creates 60 to 70% of saliva produced per day and secretes sero-mucous secretion. Saliva consists of water, electrolytes and mucus. Water and mucus serve as lubrication of the nutrients to facilitate the chewing and swallowing of nutrients. Mucus is the name of given to the viscous secretions containing musin glycoproteins, water and inorganic salts suspended in water. In this study, it was aimed to determine the glycoconjugates in sheep (*Ovis aries*) submandibular salivary gland by lectin histochemistry.

The samples taken from submandibular salivary glands of adult sheep were used as material in this research. They were fixed in Bouin's fluid for 18-24 hours. After fixation the samples were passed through the tissue following procedure and embedded in paraffin. For the lectin histochemistry, the sections were incubated Horseradish peroxidase-conjugated (HRP) Con A (*Canavalia ensiformis*), UEA-I (*Ulex europaeus*) and BSA-I-B₄ (*Bandeiraea simplicifolia*) lectins.

As a result of the investigations, it was detected that UEA-I and Con A reactions were moderate in serous cells of Giannuzzi demilune and weak in mucous cells of gland and epithelial cells of eluent channel. There were detected that the moderate BSA I- B₄, strong Con A and moderate and strong UEA-I reactions in the cell surface of eluent channel.

Keywords: Submandibular salivary gland, glycoconjugate, lectin histochemistry, sheep

Removal of uranium(IV) from aqueous medium using immobilized micro algae *C. reinhardtii*

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Abstract

Uranium is one such heavy metal having both radiochemical and toxicological effects. It is usually present in a hexavalent form which is toxic to living beings. Uranium is considered carcinogenic, leading to bone cancer. So, the removal and pre-concentration of uranium from wastewater is important. In general, the hazardous materials from wastewater can be removed by physical, chemical and biological methods such as biosorption, flocculation, coagulation, precipitation, membrane filtration and electrochemical techniques. Among them, biosorption is becoming one of the more attractive alternative methods for the removal of radioactive ions from wastewater.

In this work, biosorption of uranium from aqueous solution onto the free and immobilized *Chlamydomonas reinhardtii* in carboxymethyl cellulose (CMC) beads was investigated in a batch system using bare CMC beads as a control system. CMC can be a potential natural biosorbent for radionuclide removal as it contains carboxyl groups. The biosorbent preparations were characterized by swelling tests, FTIR, and surface area studies. The effects of pH, temperature, ionic strength, biosorbent dosage, and initial uranium concentrations on uranium biosorption were investigated. Free algae exhibited the highest uranium uptake capacity with an initial uranium ion concentration of 1000 mg/L at pH of 4.5 and at 25 °C. The removal of U(VI) ion from the aqueous solution with all the tested biosorbents increased as the initial concentration of U(VI) ion increased in the medium. Maximum biosorption capacities for free algal cells, immobilized cells, and bare CMC beads were found to be 337.2, 196.8, and 153.4 mg U(VI)/g, respectively. The algal cells entrapped beads were regenerated using 10 mM HNO₃, with up to 94% recovery. Algal cells entrapped CMC beads is a low cost and a potential composite biosorbent with high biosorption capacity for the removal of U(VI) from waters.

Keywords: Uranium(VI); *C. reinhardtii*; Immobilization; Biosorption

Effects of Apelin on C-FOS Expression in Epilepsy in Male Rats

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Abstract

Studies have demonstrated a widespread distribution of apelin-synthesizing neurons, but little is known about the physiological role of apelin in brain functions. Expression of c-fos (an immediate early gene, IGEs) has been commonly used as a marker for neuronal activation in the brain. Changes in seizure-induced c-fos expression has been shown in many experimental studies. The present study was designed to investigate the changes of c-fos expression after intracerebroventricular (icv) infusion of Apelin-13 on penicillin-induced epileptiform activity in rats. Adult male rats were divided into control and experimental groups. Epileptic activity was induced by intracortical injection of penicillin (penicillin-G-potassium, 500 IU/2,5 µl) in the left somatomotor cortex. Apelin-13 (50 µg/1 µl) was icv given 30 min after application of penicillin to the experimental group while saline was icv infused to the control group. Epileptic activity was recorded "online" by Powerlab data acquisition unit for a period of two hours. c-fos expression in the left and right hemisphere cortices was evaluated by immunohistochemistry. One-Way ANOVA test was used for statistical analysis of results. It was found that apelin-13 significantly reduced the spike frequency values of epileptiform activity ($P < 0.05$). In the immunohistochemical evaluation; c-fos expression was found to be significantly different between experimental and control groups as well as between right and left hemispheres. Expression of c-fos in the left hemisphere of both control and Apelin-13 group was found to be significantly higher than the right hemisphere ($P < 0.05$). In addition c-fos expression was significantly decreased in the Apelin-13 group in the left hemisphere compared to the control group ($P < 0.05$). In conclusion, these findings suggest that apelin-13 may have anticonvulsant properties in epileptic seizures by changing the activation of c-fos expression in the cortex.

Keywords: Apelin, c-fos, epilepsy

Karyotype of *Apodemus flavicollis* in Giresun, Turkey

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Abstract

In this study, total 8 specimens that three of them were females, one of them was male and three males and female samples were born at the laboratory, which were taken with the active traps from the three different localities (Keşap, Yağlıdere, Merkez) in the land and were taken into the laboratory were used in the karyologic studies. According to Ford and Hamerton (1956) their karyotype analysis were done by using the technique of “Colchicinehypotonic citrate”. The diploid number of chromosomes (2n), the fundamental number (NF) and the number of autosomal arms (NFa) were determined by examining approximately 30 metaphase cells of the each sample with the immersion objective of x100 from the karyotype preparations which were prepared; and the photograph and the ideogram of the metaphase plaques which were looked well were given.

It has been determined that in the getting thin karyotypes of the *A. flavicollis*, the diploid number of chromosomes was $2n=48$, the fundamental number was $NF=48$ and the number of autosomal arms was $NFa=46$. It has been determined that all autosomal chromosomes were acrocentric and in the different sizes, X chromosome was in the form of big acrocentric and Y chromosome was in the form of small acrocentric. In addition to this, it is determined that there was not a different between the field specimens and the laboratory specimens.

Keywords: *Apodemus flavicollis*, karyotype, Giresun

Phylogenetic Diversity of the Methane oxidizing bacteria in petroleum reservoirs of Southeast Anatolia region in Turkey

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Abstract

Methane (CH₄) is the second most important greenhouse gas which is on a molar base 20 to 24 times more effective to CO₂ in absorbing infrared radiation. It is produced in nature biogenically as the end product of microbial metabolism or thermogenically at greater depths under high temperature and pressure. Petroleum reservoirs also contain various gases such as CH₄ and CO₂. It has been shown that petroleum reservoirs harbor many different groups of aerobic and anaerobic microorganisms, among which also methanogenic archaea and methanotrophic bacteria (MOB). The latter can utilize methane as a sole carbon and energy source and can thereby contribute to mitigation of methane that is typically released with exploitation of oil wells.

This study aimed at assessing the most common types of methanotrophs in a petroleum production well of Southeast Anatolia region in Turkey.

Petroleum samples (oil/water/gas mixture) were obtained from oil fields (Raman, Camurlu, Garzan, Oyuktas) in the Southeast Anatolia region. Total genomic DNA was extracted from the water phase and the *pmoA* (particulate methane-monooxygenase) gene (specific for methanotrophic bacteria) was PCR amplified. From the purified PCR products clone libraries were created and sequenced. *pmoA* gene sequences were compared with the sequences available in public databases, using the BLAST software from the National Center of Biotechnology Information, to determine their phylogenetically closest relatives.

The sequences obtained were most closely related to environmental clones, obtained from soda lakes and littoral wetlands distantly related to *Methylomonas* and *Methylocystis* species. This indicates that classical proteobacterial methanotrophs are present in these oil wells, which are most related to MOB from aquatic habitats.

In petroleum microbiology, an increasing application of culture-independent techniques has allowed a more complete characterization of microbial communities in petroleum reservoirs. Our current knowledge of the diversity and in situ activities of the microorganisms present in these particular subsurface ecosystems is still limited.

Assessment of the Toxic Effect of Insecticide Chlorantraniliprole on Growth Parameters and Proline Content of *Zea mays* L.

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Abstract

Chlorantraniliprole is the ISO approved common name for 3-bromo-N-[4-chloro-2-methyl-6(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide). Chlorantraniliprole is an insecticide that operates by a highly specific biochemical mode of action. It binds and activates ryanodine receptors, resulting in depletion of intracellular calcium stores and leading to muscle paralysis and death. But no more data is available about the plant growth responses to chlorantraniliprole. For this reason, we investigated germination percentage, radicle and coleoptile lengths, radicle number and proline content of maize seedlings with various levels of chlorantraniliprole in the present study.

Commercial variety of maize (*Zea mays* L.) seeds were used as the test plant. Treatment concentrations were prepared using control (distilled water) and 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5 ppm of chlorantraniliprole insecticide solution. Germination percentage, radicle and coleoptile lengths, radicle numbers of seedlings were measured as growth parameters. Also proline content was compared all of the concentrations.

The results showed that frequency of seed germination, rate of radicle and coleoptile growth were found to be affected by chlorantraniliprole concentrations. The most toxic effect of chlorantraniliprole on growth parameters was observed in concentration of 0.5 ppm. And also, the proline content showed a significant increase with increasing concentration of chlorantraniliprole.

We concluded that higher concentrations of chlorantraniliprole have harmful effects on various growth parameters of maize plants. Also, proline content increased with high concentrations of chlorantraniliprole.

Keywords: Chlorantraniliprole, *Zea mays* L., Growth parameter, Proline

The Growth Responses of Maize Seedlings to exposed to Deltamethrin, a synthetic pyrethroid insecticide

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Abstract

Deltamethrin, a synthetic pyrethroid is used to control the pests of various agricultural crops, used in public health programme and protection of stored crops. As pyrethroids are widely used as insecticides, they are present in the environment in considerable amounts. However, to date, no information has been available in the literature on maize morphological (germination percentage; radicula and coleoptile lengths; radikula number) responses to deltamethrin stress. Therefore, we investigated growth responses and proline content of maize seedlings grown with various levels of deltamethrin in the present study.

Treatment concentrations were prepared using control (distilled water) and 0.01, 0.05, 0.1 and 0.5 of original insecticide solution and seeds were pre-treated for 72 throughout hours. Seeds of maize were sown in petri dishes with 10 ml of the distilled water for 7 days. Seeds were considered to be germinated with emergence of the radicle. At the end of the 7th day, the radicle and coleoptile lengths of seedlings were measured with a millimetric ruler. Also, proline content was compared all of the concentrations.

There was a clear propensity that the growth of plant decreased with increasing dosage level of deltamethrin. Statistical analysis showed that the germination and seedling growth of the seeds treated with deltamethrin was rather different from the control group ($P < 0.05$). The germination percentage of maize seeds treated by 0.01, 0.05, 0.1 and 0.5 ppm of deltamethrin were decreased by 1%, 16%, 48% and 92% respectively. Plants were highly damaged at the concentration of 0.5 ppm. The elongation of radicula was depended on the used deltamethrin concentration. The highest of its dose almost totally inhibited the radicula length. Similiar, effects of deltamethrin on coleoptile length and radicula number were observed. Proline content showed a significant increase with increasing concentration of deltamethrin as compared to the control group

Keywords: Pesticide, Deltamethrin, Stress, Germination, Maize

Optimization of medium components for phytase production from *Aspergillus niger* FM-20

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Abstract

Aspergillus niger FM-20, an isolate from agricultural soil, which was detected for molecular characterization by 18S rRNA gene sequence. This strain was evaluated for production of extracellular phytase in medium containing various agricultural residues: wheat bran, maize bran, rice bran and rice husk under submerged fermentation conditions (SmF). The culture conditions were optimized for maximum enzyme production. The maximum production of phytase by this isolated was obtained on thirteenth day. The best carbon and nitrogen sources for maximum phytase production were 1% lactose and 1% yeast extract, respectively. The enzyme was stable between the pH 2.0 and 6.0 but the optimal pH was found to be 5.5. The enzyme was also stable between temperature ranges 20 °C and 60 °C but the best temperature for enzyme activity was found to be 40 °C. Furthermore, inoculum amount 2% (v/v) of spore suspension (5×10^6 spores /ml) and agitation rate 200 rpm was found.

As a result, maximum phytase activity was found 426 U/mL in rice husk medium. Thus, phytase can be a potential candidate in animal nutrition due to its high activity and stability.

Keywords: Phytase, *Aspergillus niger*, Submerged Fermentation

Characterization and Production of Phytase from *Aspergillus niger* FM-7

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Abstract

Aspergillus niger FM-7 isolated from agricultural soil, which was detected for molecular characterization by 18S rRNA gene sequence. Isolation of phytase producers was performed by the agar plate of method. Extracellular phytase was produced under submerged fermentation conditions at 30 °C in a medium containing cornstarch(2.8%), glucose(0.5%), meat peptone(1.8%) and Na-phytate (0.1%). Maximum phytase activity (0.45 FTU at pH 4.6) was obtained on the twelfth day. The molecular weight determined from the SDS-PAGE was 100 kDa, the monomer being 83 kDa. Phytase had optimal pH at 4.6 with temperature optimal at 70 °C respectively. The enzyme activity retained 84% after 10 minute at 90 °C.

Most of metal ions such as Ca^{2+} , Mg^{2+} , Zn^{2+} , Mn^{2+} stimulated phytase activity, while Hg^{2+} , Co^{2+} and Fe^{2+} were inhibited. Anionic detergent (SDS) and non- ionic detergents like Tween-80 and Triton-X-100 inhibited the enzyme. The enzyme displayed slightly higher activity when assayed with the ATP and AMP as opposed to sodium salt of phytic acid, and displayed lesser activity when assayed using non- phytate- based phosphorylated substrates. The enzyme proved to be fairly specific for phytate, and the kinetic parameters for hydrolysis of sodium phytate were K_m 17.9 mM and V_{max} 5332.7 U/mL. As a result, the present enzyme can be potential application in animal nutrition.

Keywords: Phytase, *Aspergillus niger*, Submerged Fermentation

Impact of Pyriproxyfen on Plant Growth Parameters and Proline Content in Maize Seedlings

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Abstract

In agricultural fields, considerable amount of pesticides are being used to increase the agricultural production, by controlling insect pests, diseases and weeds as these chemicals act on pests that are detrimental to agricultural output. In recent years, the uses of pesticides have increased tremendously in crops and a number of them have been shown to cause decreased or increased nodulations and directly affect the plant growth.

In agriculture the pesticide pyriproxyfen [4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether] is a juvenile hormone analog, preventing larvae from developing into adulthood and thus rendering them unable to reproduce. It has registered uses for the control of scale, whitefly, aphids and fire ants. It is used extensively worldwide, particularly in developing countries, but there is no information about growth parameters and proline content on maize seedlings.

Uniform-sized seeds of a commercial variety of maize (*Zea mays* L.) were used as the test plant. Treatment concentrations were prepared using control (distilled water) and 0.1, 0.2, 0.4 and 0.6 ppm of original insecticide solution of pyriproxyfen. At the end of the 7th day, germination percentage, radicula and coleoptile lengths, radicula numbers of seedlings were measured for growth parameters. Also proline content was compared all of the concentrations. In general, pyriproxyfen had a detrimental effect on growth parameters. The most toxic effect of pyriproxyfen on germination percentage, radicula length, coleoptile length and radicula number was observed in concentration of 0.6 ppm. And also, the proline content showed a significant increase with increasing concentration of pyriproxyfen as compared to the control group.

In conclusion, high concentrations of pyriproxyfen caused varying degree of toxicity on maize seedlings. The increasing accumulation of proline in response to phyto-toxicity of insecticide create stress on maize, which causes both yield and quality losses.

Keywords: Maize, Pesticide, Proline, Pyriproxyfen, Toxicity

Elucidation of antifungal activity of novel Pt(II) and Pt(IV) complexes containing cycloalkylthiophene-Schiff bases

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Abstract

Schiff bases and their metal complexes have been widely studied due to their import antiparasitic, fungicidal-bactericidal, and anticancer properties. Substituted 2-aminothiophenes have had wide applications in agrochemicals, dyes and pharmacologically active compounds, etc. However, few studies have been performed on Schiff bases and the metal complexes derived from aminothiophenes, including cycloalkylthiophene.

The aim of this work was to investigate the antifungal activities of new Pt(II) and Pt(IV) complexes. Eight new Pt(II) and Pt(IV) complexes have been examined and antifungal activity against *Candida albicans* (Y-1200-NIH, Tokyo) and *Aspergillus fumigatus*. The antifungal test results indicated that Pt(II) complexes have better activity Pt(IV) complexes.

Keywords: Aminothiophene, Schiff bases, Pt(II), Pt(IV), Antifungal

Investigations into Structural and Biochemical Properties of Nuclear Lamins

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Abstract

The nuclear lamins are type V intermediate filament proteins which are important structural elements for the nuclear envelope. The lamins bind the chromatin domains to the nuclear periphery and localize some of the nuclear envelope proteins. In addition, they are related with the regulation of nuclear processes including chromatin organization, DNA replication, transcription and cellular differentiation. It is suggested that lamins may regulate nuclear functions by directly interacting with the chromatins and controlling the position of chromosomes within the nuclear space.

Interest in the nuclear lamina has rapidly increased. This is due to many devastating diseases caused by more than 400 distinct mutations in the LMNA gene; diseases such as Emery–Dreifuss muscular dystrophy (EDMD), dilated cardiomyopathy type 1A, the segmental premature ageing diseases Hutchinson–Gilford progeria syndrome (HGPS) and atypical Werner’s progeria. Different lamin expressions have also been reported in various cancers. The increase in mechanical stress resulting from high lamin levels and the alterations in lamin expressions could modulate cell proliferation, differentiation and migration, each of which is an important step in cancer progression. Different hypotheses have been proposed to explain the molecular mechanism underlying these diverse diseases. Although our knowledge on the functions of nuclear lamins has increased, additional studies are needed to understand its molecular mechanisms. These studies could shed a light on the diagnosis and treatment of the diseases.

Keywords: Nuclear Lamins, LMNA gene, progeria syndrome

Investigating Genotoxic Effects of Sitagliptin the Active Ingredient of an Antidiabetic Drug by Micronucleus Assay

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Abstract

Diabetes mellitus is one of the most significant metabolic syndromes. It is commonly seen type 2 diabetes and was treated with oral anti-diabetic agents. The aim of this study is to examine genotoxic effect of Sitagliptin on human peripheral lymphocytes by using micronucleus assay. Experiments were performed using human peripheral blood lymphocytes from three healthy volunteers, two men and a woman. Human lymphocytes were treated with 31,25; 62,50; 125,00; 250,00; 500,00 and 1000,00 µg/ml concentrations of Sitagliptin at 48h. In this study a negative (distilled water), a solvent (DMSO) and a positive control (MMC) were also included In the MN test, a total of 3000 well-spread binucleated cells (1000 binucleated cells per donor) were analyzed for each concentration. Cell proliferation was evaluated using the nuclear division index (NDI). 500 lymphocytes (total: 1500 lymphocytes for each concentration) were evaluated for the percentage of cells with 1-3 nucleus. This study showed that Sitagliptin significantly increased MN frequency at the three highest concentrations (250,00; 500,00; 1000,00 µg/m) compared to the negative control; however, only the highest concentration (1000,00 µg/ml) significantly increased MN frequency compared to solvent control. On the other hand, significant difference was not observed in the nuclear division index. The results of this study suggest that Sitagliptin significantly increased the frequency of micronucleus in human lymphocytes. However, some other genotoxicity tests should also be performed before a general evaluation.

Keywords: Sitagliptin, Anti-diabetic drug, Human lymphocytes, Genotoxicity, Micronucleus

The Determination of Possible Genetic Damage by Chromosomal Aberrations and Comet Assays in Women Undergoing *In Vitro* Fertilisation with Male Infertility Factor

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Abstract

In this study, it was aimed to determine possible genetic damage at women undergoing *in vitro* fertilization (IVF) due to male infertility factor. Two different genotoxicity tests were used in human lymphocytes; chromosomal aberration (CA) and comet. Especially chromosomal aberration test is crucial in determination of cancer risk; because it was determined that risk of cancer is related with chromosomal aberration rate. Comet is a sensitive technique for detection of DNA damage and repair at the level of a single cell. For CA test, 32 IVF treated and 20 healthy women and for comet test, 31 IVF treated and 18 healthy women were included in the study. As the result, there was no statistically significant increase in chromosomal aberration and DNA damage in IVF treated women compared with the control group. Moreover, there was no significant difference between IVF treated and control group in terms of mitotic (MI) index. Our results showed that *in vitro* fertilization treatments have no risk at genetic level in human lymphocytes.

Keywords: Genotoxic effects, In vitro fertilization treatment, Chromosomal aberrations, Comet assay

Biofilm Condition Formations Methicillin-Resistant *Staphylococcus aureus* (Mrsa) Strains

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Abstract

Idea: Biofilms, from within and protects microorganisms innutrition and such as the inhibitör, antibiotics, and disinfectant chemicals, drought, heat and to variations in pH, by phagocytosis, toxic compounds, virus protects from attacks. In this study, obtained from Denizli State Hospital MRSA (methicillin resistant *Staphylococcus aureus*) 's conditions of biofilm formation was investigated.

Material and method: Incubation time (12th hour, 1, 2, 3, 4, 5 and day 6), pH (6, 7, 8, 9, 10), carbon sources (fructose, glucose, mannitol, sucrose) effects for biofilm formation were studied. Biofilm formation, at specific time intervals are determined using microplate reader at 630nm.

Forming biofilms of MRSA strains was determined by scanning in medium containing Congo red. MRSA 10, MRSA 16, MRSA 18 and MRSA 20 are biofim possitive. MRSA10 at 4.day, MRSA 16 at 6.day, MRSA 18 at 4.day and MRSA 20 at 4.day have maximum biofilm formations. Top ranking MRSA 10; at pH 8> pH 6> pH 10> pH 7> pH 9, MRSA 16; at pH 6> pH 8> pH 7> pH 10> pH9, MRSA 18; at pH 8> pH 9> pH 6> pH10> pH7 and MRSA 20; at pH8 > pH 6> pH9> pH 7> pH10. Maximum biofilms of MRSA 10 in the presence of glucose, MRSA 16, MRSA 18 and MRSA 20's maximum biofilms formed mannitol environment.

Results: MRSA is known to cause major health problems of the biofilm forming conditions is detected, the fight against this type of bacteria is thought to be easier.

Keywords: Biofilm, MRSA, Optimization.

Thanks giving: Master Thesis Project (2013FBE026) supports the PAU would like to thank Scientific Research Projects Coordination Unit.

Genotoxic Effect of Copper Oxide Nanoparticles on Human Lymphocytes by Comet Assay

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Abstract

With the development of nanotechnology, copper oxide nanoparticles (CuO NPs) has been increasingly used in many products, including antimicrobial agents, heat transfer fluids, semiconductors, healthcare products, wood preservation and antifouling paints. The aim of this study was to investigate genotoxic effects of CuO NPs by using alkaline comet assay in human peripheral lymphocytes. First of all, shape, size and aggregation behavior of CuO NPs (Aldrich, <50 nm) in deionized water were analyzed using transmission electron microscopy (TEM) and dynamic light scattering (DLS). CuO NPs were in different shapes (spherical and cubic), formed agglomeration and ranged between 9.75-823.75 nm by EM. The average hydrodynamic diameter and zeta potential of CuO NPs were found to be 612 ± 40 nm and -21 ± 4 mV, respectively. For comet assay, lymphocytes obtained from three healthy donors incubated with 25, 50, 75 and 100 $\mu\text{g/ml}$ concentrations of CuO NPs for 2h and 3 h. A negative, a positive (H_2O_2) and a solvent (PBS) control were also maintained. To assess DNA damage, the tail length (μm), tail intensity (%) and tail moment of the randomly selected 300 cells (100 cells from each of the two replicate slides) were analyzed per treatment using Comet Assay IV, Perceptive Instruments Ltd., UK. The results showed significant increase in DNA damage at all the concentrations at both treatment times compared to the control. Therefore, it is possible that CuO NPs could pose a risk to human health after frequent exposure and necessary precautions may need to be taken when handling these NPs.

Keywords: CuO nanoparticles, genotoxicity, comet assay, human lymphocytes

Analysis of Aflatoxine Contents in Various Food Materials with HPLC Method

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Abstract

In this study, various food materials like nuts, pistachio, almond, dried apricots, dried raisin, dried figs, pepper, red capsicum, corn and moldy cheese which were obtained from several open market places in Karaman and bread materials which were obtained from markets 7 days long, were analysed through AOCA 991.31 method on aflatoxine B1, B2, G1, G2 contents. Four seasons long specimen were collected and from each specimen 2 analysis were obtained. 90 analysis were evaluated from total 45 specimen. Moldy cheese and bread specimen were evaluated only one season, other specimen 4 seasons long. In 40 analysis (% 88,9) aflatoxine B1, B2, G1, G2 were detected. According to Turkish Food Codex and EU Standards in 4 specimen (red capsicum) aflatoxine content were over the allowed limits.

As a result in dried food materials (except red capsicum) aflatoxine contents were under and in allowed limits. The red capsicum specimen obtained four seasons long had an average aflatoxine B1 content of 13,44 ppb. This amount exceeds the allowed limit and could be hazardous to human health. This fact will be reported to related official departments of the Government.

Keywords: Mycotoxin, Aflatoxin, Food Material, HPLC, Derivatization

In Vitro* Propagation and Improvement of Bulb Formation and Rooting with JA and 2iP in Endemic *Hyacinthus orientalis* ssp. *chionophyllus

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Abstract

In this study *in vitro* plant regeneration and bulb formation by means of embryo culture of *Hyacinthus orientalis* ssp. *chionophyllus* which is an endemic subspecies to certain regions of Turkey as well as Kahramanmaraş Province were investigated. The pods with seeds were collected from the plants grown in nature, and after sterilization they were cultured in MS medium containing BA 1.0 mg/l + GA₃ 0.1 mg/l + IAA 0.05 mg/l. The germinating seeds were then transferred into MS media with different sucrose concentrations ((30, 60 ve 90 g/l)) or with two growth regulators (BA 2.0 mg/l + IAA 0.5 mg/l). In the media tested the plantlets with bulbs were developed. In the different sucrose concentration tests, two subcultures were done at 45 days interval and mean plantlet number/explant, diameter of bulbs, rooting percentages and roots number/ plantlet were determined. The highest plantlet number/explant (16.2) and highest root number/plantlet (3.7) and the best bulb size (11 mm) were obtained in MS medium which contained 90 g/l sucrose. The plantlets with average bulb diameters between 5.0 and 5.5 mm were then cultured in eight MS media containing either three concentrations of JA (0.0, 1.0 ve 2.0 mg/l)) or two concentrations of 2iP (0.0, 1.0 ve 2.0 mg/l). The addition of JA and 2iP into MS media positively affected bulb development. The addition of JA 2.0 mg/l into the medium increased the root number/plantlet to 19.1 and bulb diameter to 7.9 mm.

Keywords: Endemic *Hyacinth*, *in vitro*, bulb improvement

A facile method for detection of *E. coli* and *S. enterica* from food samples using immuno-magnetic and multiplex PCR systems

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Abstract

Food-borne diseases are defined as illnesses caused by pathogens that are ingested in contaminated food. Among the major food-borne diseases are salmonellosis and infections caused by enterohaemorrhagic *Escherichia coli*. Salmonellosis is caused by *Salmonella* with symptoms like fever, headache, nausea, vomiting, abdominal pain and diarrhea. *Escherichia coli* is one of the main bacterial species of the mammalian intestines. The detection of *Escherichia coli* in food samples signals the potential presence of pathogens originating from intestinal tract of mammals. The pathogen *Escherichia coli* O157 causes intestinal bleeding infections with relatively low incidence, but severe health consequences. Classical bacteriological tests for the detection and identification of pathogenic bacteria involve methods of analysis that require long assay time and highly qualified scientific personnel *C. reinhardtii*. In this study, an immunomagnetic system was developed by the immobilization of pathogen-specific antibodies on the magnetic beads. For immuno-magnetic separation (IMS) of target bacterial cells from others, antibodies for *E. coli* and *S. enterica* cells were immobilized on the magnetic beads via glutaraldehyde coupling reaction. Our IMS system successfully separated *Salmonella* cells when the concentrations of target (i.e., *Salmonella*) and interfering (i.e., *Escherichia coli*) cells were at the same level. Polymerase chain reaction (PCR) assays amplifying the *rfb/rfbE* region of the *Escherichia coli* genome and a 647 bp fragment of the *invA* region of *Salmonella* were performed as the specific selection to accurately confirm the presence of *Escherichia coli* and *Salmonella*, respectively. IMS and multiplex-PCR methods can be used for specific and quantitative detection of pathogens from food samples. Thus, this study developed a reliable and direct system for rapid detection of *Salmonella* and *Escherichia coli* in food samples. In addition, IMS method could be easily adapted to detect other pathogens by selecting the pertinent antibody.

Keywords: Immuno-magnetic separation; Multiplex-PCR; Food-borne diseases; *Salmonella*; *Escherichia coli*.

Fabrication of glucose-sensor ferrogel-film based on covalently immobilized glucose oxidase for rapid measurement of glucose

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Abstract

Glucose oxidase (GOD), a homo-dimer flavoprotein containing two active sites per molecule, catalyses the oxidation of D-glucose to gluconic acid, concomitant with the reduction of oxygen to hydrogen peroxide. It has been immobilized to use for various biochemical and biotechnological applications, and is the most commonly immobilized in the construction of biosensors for glucose assay development. Diabetic patients currently test blood glucose levels using finger-prick sampling of capillary blood so that hypo- and hyperglycemia can be detected and therapy can be adjusted accordingly there is an urgent need in diabetes care for the development of minimally. There is an urgent need in diabetes care for the development of minimally invasive technology for continuous in vivo glucose sensing new glucose-sensing approaches are being explored.

For this purpose, magnetic poly(2-hydroxyethyl methacrylate-glycidyl methacrylate), p(HEMA-GMA), ferrogels were synthesized in the film form with two sequential step. In the first the film was prepared in the presence of FeCl₃ by UV-induced photo-polymerization. After thermal magnetization with FeCl₂, magnetic ferrogel samples were modified with epichlorohydrin (ECH). Then, GOD was covalent immobilized by bonding via epichlorohydrin coupling. The polarities and the surface free energies of the ferrogel films were determined by contact angle measurement. The resulting immobilized GOD had higher optimum temperature compared with those of free enzyme and exhibited better thermal, broader pH stability and excellent reusability. The K_m values were found to be 5.7 and 8.1 mM for free and immobilized enzymes, respectively, and the V_{max} values were 0.078 and 0.061 mM/min for free and immobilized enzymes. The optimum pHs for the free and bound enzyme were determined to be 5 and 6, respectively. Prepared GOD electrodes exhibit very high operational stabilities. GOD electrodes were used for the determination of glucose in orange juice and in human serum.

Immobilization of laccase on the newly synthesized polymeric support for enzymatic degradation of textile dye in a reactor

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Abstract

A promising biological treatment is the use of white rot fungi. Their extracellular enzymes including peroxidases and laccases have decolorized dyes in liquid cultures. Immobilization of these enzymes is potentially more cost-effective as it would allow their re-use and may improve enzyme stability. Although purified fungal laccases have been shown to decolorize different textile dyes, most studies using immobilized laccase have evaluated their ability to remove pollutants such as pesticides and phenols from synthetic wastewaters.

In this study, the decolorization and detoxification of textile dyes by fungal laccase immobilized on the aggregates of the hydroxypropyl-chitosan, polyvinylalcohol, polylactic acid/polyglycolic acid were studied. The optimized enzyme aggregates with the highest enzyme activity was studied in batch reactor for degradation of Reactive Yellow-2 and Bisphenol A. These results demonstrate the potential and limitations of using immobilized laccase to enzymatically decolorize a range of different dye classes and reduce dye toxicity in a single step. The present study demonstrated the limitations of using *T. versicolor* laccase immobilized on the different polymer composite to decolorize and detoxify a range of dye classes. Decolorization was by enzymatic degradation for all the tested dyes and endrocrin disturbant which inactivated laccase. Although Bisphenol A was decomposed most rapidly, than the tested other compounds. The laccase immobilized aggregate and its free counterpart can be compared and discussed with the earlier studies in the literature with respect to the thermal stability, operational stability, storage stability etc.

Keywords: Immobilized enzyme, Chitosan, Hydroxypropyl chitosan, Dye removal

Acknowledgment: We would like to thank Gazi University for granting us to conduct this study [BAP-05/2012-78].

Investigations on anti-microbial and anti-cancer capacities of some plant extracts

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Abstract

Many phytochemicals from different plant species have potency in treatment and prevention of cancer. Currently, substantial research have being carried out in many laboratories the world over to discover new plant extracts with high anti-cancer and/or anti-microbial activities. In this study, seven different plant species *Glaucium flavum*, *Euphorbia falcata*, *Conyza canadensis*, *Chenopodium botrys*, *Catalpha sp.*, *Quercus coccifera*, *Crataegus monogyna* from Mugla region were examined for their anti-microbial and anti-cancer capacities. Disc diffusion method was used to study anti-microbial potency of ethanol extracts against Gram-positive and Gram-negative bacteria. Anti-tumor activities of the plant extracts were tested against the six different cancer cell lines using the MTT assay. The fruit extract of *Q. coccifera* was found to be the most effective extract (13-15 20 μ L⁻¹ inhibition zone) against *S. Albus*, *M. Luteus* and *S.aureus*. In addition, *C. canadensis* exhibited anti-microbial activity only against *E.coli* and *B. subtilies* (10-14 20 μ L⁻¹ inhibition zone). Based on MTT assays, all of the plant extracts resulted in different degrees of anti-cancer activity on six different cancer cell lines. The most effective extracts were from *C. botrys*, *G. flavum*, *Catalpha sp* and *Q. coccifera* which caused more than threefold decrease in the cell proliferation of K562, PC-3 and MCF-7 cell lines. In conclusion, among the seven tested plant extracts, only *Q. Coccifera* and *C. canadensis* showed anti-microbial capacity. On the other hand, 4 plant extracts have displayed high anti-cancer potency. Future studies related with cell death will elicit the mechanisms of anti-tumor activities of the plant extracts.

Keywords: Plant extracts, Anti-microbial activity, Anti-cancer activity, Cancer cell lines

Synthesis and antimicrobial studies of thiazole-containing Schiff bases and their metal complexes

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Abstract

Thiazolyl and benzothiazolyl groups are of importance in biological systems as anti-inflammatory, analgesic agents and inhibitors on lipoxygenase activities [1, 2]. The effective role of the azomethine linkage in certain biological reactions [3] is well documented. This group of compounds is characterized by great biological activity; they play an important role in biological systems [4]. Two novel bidentate Schiff base ligands, 4-Methoxy-2-{{[4-(3-methyl-3-phenyl-*p*-xylyl/mesityl/cyclobutyl)-thiazol-2-yl]-hydrazonomethyl} phenol (L¹H, L²H), and their transition metal complexes have been prepared and characterised by elemental analysis, electronic, IR, magnetic susceptibility measurement, ¹H NMR, ¹³C NMR and thermal studies. Antimicrobial activities of the ligands and their complexes have been tested against two gram-positive (*Bacillus megaterium* and *Staphylococcus aureus*), two gram-negative bacteria (*Klebsiella Pneumonia* and *Escherichia coli.*) and a yeast-like fungi *Candida albicans*, and some of the complexes were found to be active against some of the microorganisms studied.

Acknowledgement: We would like to thank to the Scientific and Technological Research Council of Turkey (TUBITAK Grant Number 112T339) for financial support of this work

Synthesis and Carbonic Anhydrase Inhibitory Effects of Novel Sulfamides Derived from 1-Aminoindanes and Anilines

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Abstract

Sulfamides are interesting compounds in the field of pharmaceutical chemistry.1 Carbonic anhydrases (CA, EC. 4.2.1.1) catalyse the interconversion between carbon dioxide (CO₂) and bicarbonate (HCO₃⁻) with formation of protons (H⁺).2 Three 1-aminoindanes, four anilines and BnOH/*t*-BuOH were reacted with chlorosulfonyl isocyanate (CSI) to give sulfamoyl carbamates. Pd-C catalysed hydrogenolysis reactions of carbamates or deprotection of Boc group of the carbamates with CH₃CO₂H afforded seven novel sulfamides. CA I, and CA II were purified from fresh human blood erythrocyte with one-step affinity chromatography. The tested novel sulfamide carbamates and sulfamides derived from 1-aminoindanes and anilines effectively inhibited hCA I, and II competitively in the nanomolar range. One of these compounds showed potent inhibitory effect against CA I (K_i: 153.88±04.01 nM). Also, another novel sulfamide was observed that have marked inhibitory effect against CA II (K_i: 117.80±17.87 nM).

Keywords: Carbonic anhydrase; Aniline; Sulfamoyl carbamate; Enzyme inhibition

The phylogenetic position of *Tragopogon subacaulis* O. Schwarz (Asteraceae) endemic to Turkey based on ITS data

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Abstract

T. subacaulis is a scapigerous perennial, plant with purple ligules. It is one of seven endemic taxa of *Tragopogon* grown in Turkey. It was only known from first gathering (Spil Mountain, Manisa) and never subsequently reported or collected up to now. During the revisional study of *Tragopogon* grown in Turkey, this taxon was rediscovered at both from its type locality and Nif Mountain (İzmir). Molecular analysis was applied to determine whether *T. subacaulis* is a distinct species or not and what is its closest relatives among the examined *Tragopogon* taxa. So, ITS regions of *T. subacaulis*, as well as eight taxa belong to *Tragopogon* and four closely related genera belong to Asteraceae was sequenced. As a result of sequence analysis, it was found that *T. subacaulis* has two SNPs different from all the other examined *Tragopogon* taxa at the 322nd and 333rd position of 644 aligned sequence data. Additionally, the trees obtained from MP and NJ analysis revealed that although *T. subacaulis* has purple flowers, it formed a little group with *T. dubius* Scop. and *T. pratensis* L. which have yellow flowers.

Keywords: Endemic, nrDNA, *Tragopogon*, Turkey

Acknowledgement: This study is supported by TUBITAK with the number of 110T954

Bacteria from Warehouses in Trabzon: Isolation, Characterization and Insecticidal Activity

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Abstract

Introduction: The genus of *Bacillus* is a spore-forming and Gram + bacteria that used mostly in biological control. In addition, they originate the basis of microbiological insecticide.

Material and Methods: In this study, samples were obtained from warehouses in Trabzon to determined *Bacillus thuringiensis* strains with new toxin combinations and the bacteria were isolated from the samples. Morphological, physiological and biochemical characterization of these bacterial isolates were accomplished and determined the insecticidal activity of them on warehouse pests. It was obtained 26 bacterial isolates belonging *Bacillus* sp. These isolates were named as B1-5, F2-7, N1-10 and Bg1-5. The colonial, cellular, physiological and biochemical properties of these bacterial isolates were analyzed by the light microscopy, manual tests and API kit, respectively. For the molecular characterization, 16S rDNA sequence and *cry* gene contents were detected.

Results: Isolated bacteria were named as B1-5, F2-7, N1-10 and Bg1-5. As a result of studies, bacterial isolates were recorded as *Bacillus thuringiensis*, *B. pumilus*, *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. atrophaeus*, *B. megaterium* ve *Lysinibacillus sphaericus*. As a result of molecular characterization, it was detected that the isolate Bg5 has a *cry1* gene, N6 has a *cry3* gene. The toxic effects of the isolates were determined by the bioassay using third instar larvae of *Plodia interpunctella* (Indianmeal moth), *Ephestia kuehniella* (Mediterranean flour moth) and adults of *Sitophilus granarius* (Wheat weevil). As a consequence of bioassay, the mortality of Bg5 against *P. interpunctella* was 100%.

Conclusion: It is proposed that highly effective insecticide Bg5 (*Bacillus thuringiensis* subsp. *kurstaki*) may be an important biological control agent in the control with Lepidopteran pests in warehouse.

Keywords: *Bacillus thuringiensis*, warehouse, biological control

Thanks to: This work was supported by KTU-BAP (Project Number: 8642).

Determination of Bacterial Control Agents of *Ricania simulans*

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Abstract

Ricania simulans damages on many vegetables and fruits, including tea. *R. simulans* is widespread through China, Japan and Taiwan in the world and in East Black Sea Region of Turkey. It damages the plants by sucking the nectar. Up to now, it has been controlled by mechanical methods only. This method is not sufficient enough to control *R. simulans*. In order to find a more effective and safer control agent against this pest, first of all, we determined 16 bacterial isolates from *R. simulans* and identified these isolates based on morphological, physiological, biochemical and molecular characteristics. Based on the identification tests, bacterial isolates were identified at species level for nine and at genus level for seven. According to these results, they were identified as *Pseudomonas oleovorans* (Rs1), *Pseudomonas parafulva* (Rs2, Rs3 and Rs6), *Pseudomonas* sp. (Rs4, Rs8, Rs10 and Rs13), *Pantoea* sp. (Rs5 and Rs6), *Microbacterium paraoxydans* (Rs9), *Bacillus* sp. (Rs11), *Bacillus safensis* (Rs12), *Chryseobacterium indoltheticum* (Rs14), *Bacillus thuringiensis* (Rs15 and Rs16). The insecticidal activities of these isolates were performed against both nims and adults of *R. simulans*. According to test results, the highest insecticidal activity was 82% for Rs4 isolate on nymphal stage and 86% for Rs16 isolate on adult stage of *R. simulans*. These results indicate that Rs4 and Rs16 isolates may be valuable as potential biological control agents for the control of *R. simulans*.

Keywords: *Bacillus thuringiensis*, bacterial flora, microbial control, *Ricania simulans*

Production of bio diesel from micro algae biomass *Scenedesmus quadricauda* by using immobilized *Candida rugosa* lipase

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Abstract

Bioenergy resources must replace fossil energy resources, both to meet the target of the limitations of global warming and to substitute fossil fuel when this resource is exhausted. However, land area limits the production capacity, and the increasing world population limits this possibility. The utilization of algae can be introduced as a possibility. In this work, the influence of growth conditions of a micro algae *Scenedesmus quadricauda* based on the biodiesel yield was investigated in a batch reactor. This study has four different processes: algal biomass production, lipid extraction, lipase immobilization, and enzymatic biodiesel production. In the first step, the growth patterns of a fresh water algae i.e. *Scenedesmus quadricauda* was studied in a batch reactor under different experimental conditions. Secondly, the oil was extracted from the algae biomass using hexane as a solvent and simultaneous extraction was also studied using different solvents. Thirdly, acid and plasma treated diatom-biosilica particles, were modified with 3-aminopropyl triethoxysilane (APTES), and activated with glutaraldehyde. Finally, lipase was immobilized onto the pre-activated biosilica by covalent bonding. The biosilica properties were determined using SEM, and FTIR. The enzyme system has been characterized as a function of pH, temperature and substrate concentration. Then, transesterification reaction was realized with immobilized lipase. The systematic characterization of algae biomass, algae oil and algae biodiesel was carried out to establish the potential of microalgae for biodiesel production. The biodiesel production has been studied by means of immobilized lipase in a batch system. The obtained biosilica with immobilized lipase was conveniently applied for biodiesel production via enzymatic transesterification reaction. According to our knowledge, until now, there has not been any report concerned with preparation of biosilica material for lipase immobilization and biodiesel production.

Keywords: Algal biomass, *Scenedesmus quadricauda*, Diatoms, Enzyme immobilization, Lipase, Transesterification, Biodiesel.

Acknowledgment: We would like to thank Hacettepe University for granting us to conduct this study [BAP-013D06604002].

Purification of trypsin from bovine pancreas using *p*-Amino-benzamidine ligand attached magnetic beads

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Abstract

Affinity interactions are characterized by a highly specific interaction between the ligand and the target molecule, whereas the functional groups on the adsorbent and the target molecule should have opposite charge for an ion exchange process. Since affinity chromatography exploits selective interactions between a ligand and a target protein molecule, it can therefore be used to simplify purification schemes and one-step purification procedures are commonly obtained. Most biospecific ligands in affinity chromatography, such as proteins and enzymes, are expensive and unstable in chromatographic systems while small ligand molecules (such as *p*-amino-benzamidine, histidine, phenylalanine, glutamic acid and various dyes molecules) have been reported to be selective for the purification of proteins.

Trypsin (EC 3.4.21.4) is a serine protease found in the digestive system, where it breaks down proteins. Trypsin specifically hydrolyzes peptide bonds at the carboxyl side of lysine and arginine residues. It is used for numerous biotechnological processes such as protein primary structure analysis, to breakdown casein in milk for baby food and to resuspend cells adherent to the cell culture dish wall during the process of harvesting cells [31].

In this study, the preparation of the affinity magnetic beads were described for purification of the trypsin from crude pancreas extract. For this purpose, magnetic beads were decorated with *p*-amino-benzamidine for specific purification of trypsin. Trypsin adsorption experiments were investigated under different experimental conditions (i.e. medium pH, initial trypsin concentration, temperature, and ionic strength) in a batch system. Maximum trypsin adsorption capacity was found to be 75.9 ± 2.6 mg/g beads. Adsorbed trypsin was eluted by using (0.1 M acetate buffer, pH 3.0) with a 97% recovery. The purification factor of trypsin from crude pancreas extract was 8.7 folds. The purity of the eluted trypsin from *p*-amino-benzamidine functionalized magnetic beads was determined as 86% by HPLC. The method developed in this report was successfully applied for purification of the trypsin from crude pancreas extract in a magnetically stabilized fluidized bed reactor.

Keywords: *p*-Amino-benzamidine; Purification; Trypsin

Antibiofilm Effect of Two Propolis Samples from Turkey

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Abstract

Biofilms are structured communities of bacteria, which are adhered to a surface and embedded in a self- produced matrix of extracellular polymeric substances. Since biofilms are resistant to antimicrobial agents, they are at the basis of health problems. Propolis has attracted increased interest due to its antimicrobial activity against pathogenic microorganisms.

We investigated the antibiofilm potencies of Turkish ethanol extract propolis (EEP) against some bacteria (*Listeria monocytogenes* ATCC7644, *Staphylococcus aureus* ATCC29213, *S. aureus* ATCC 33862, MRSA-20 (clinical isolate), *Pseudomonas fluorescens* ATCC55241, *Micrococcus luteus* NRRL-B1013, *Enterococcus faecalis* ATCC19433) known to form biofilms.

Firstly, the antibacterial activity of EEP from Manisa-Salihli (P4) and Izmir-Foa (P10) collected in 2013 was evaluated according to Agar Well Diffusion method. Secondly, we tested with a microplate biofilms assay both the effect of antibiofilm and inhibition of biofilms of EEP.

The EEP samples exhibited good antibiofilms activity against bacteria. The maximum antibiofilms activity percentage of P4 ranged from 85% for *L. monocytogenes* to 68% for *S. aureus* ATCC 29213. Also, the activity percentage of P10 ranged from 79% for *L. monocytogenes* to 48% for MRSA20. In addition, we showed that EEP samples were very effective on tested bacteria biofilms (up to 50% biofilms inhibition percentage).

Keywords: Antibiofilms, Propolis

Acknowledgement: We thanks to PA BAP (2013BSP025) and TUBITAK-BIDEP 2209.

Simple, Fast and Economical Detection of Aminoacids Simultaneously by Liquid Chromatography-Potentiometry Hybrid Analysis System

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Abstract

In the present study, a new type aminoacid sensitive potentiometric biosensors were developed by using micro-sized composite pH and NH₄ selective sensors. Microliters dead volume flow cells of these biosensors were prepared. The flow cells were used as potentiometric detector in liquid chromatography to simultaneous detection of aminoacids. Separation of free aminoacids were performed by 0.1 M sodium hydroxide and 0.3 M sodium acetate mobile phase with flow rate of 1 ml/min. Retention time of each aminoacids injected were calculated from chromatograms (Fig.1) obtained under optimal conditions. Free aminoacids detection limits were calculated from chromatograms obtained by aminoacid injections at different concentrations. The liquid chromatography-potentiometric hybrid method is applied successfully for the detection of free aminoacids in foods and biological liquids.

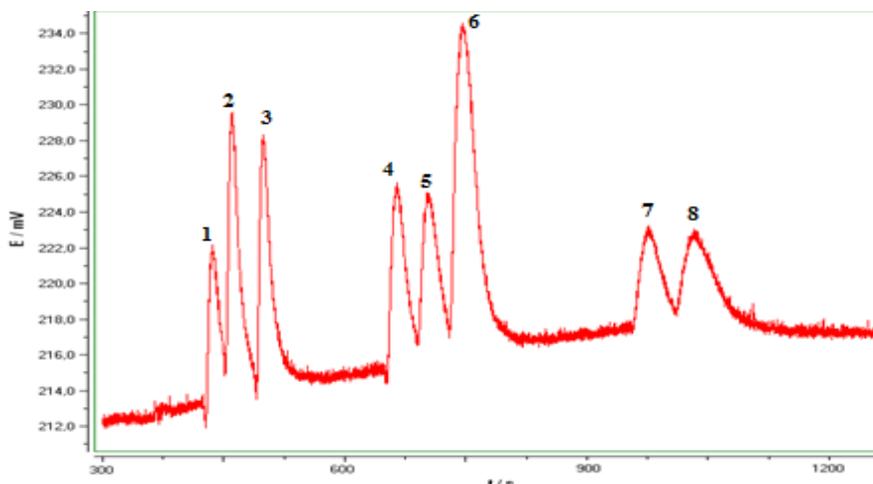


Figure. 1. Chromatogram of 2.5×10^{-4} M aminoacid mix. (Mobile phase: 0.1 M NaOH + 0.3 M sodium acetate; Flow rate: 1 ml/min; Injection volume: 20 μ L; 1. Alanine, 2. Glycine, 3. Valine, 4. isoleucine, 5. Tryptophan, 6. Fenil alanine, 7. Cystine, 8. Tyrosine)

*This work has been supported by the Turkish scientific and technological council with the Project (TBAG 110T793)

Isolation and Optimization of Aldose Reductase from Kidney; Immobilized with Gelatin-Glutaraldehyde System

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Abstract

Aldose reductase (AR) is NADPH-dependent cytosolic enzyme that belongs to a superfamily of aldo-keto reductases. It catalyzes the reduction of glucose to sorbitol. Aldose reductase involved lots of pathological processes that have become major threats to human health. Such pathologies are a number of cardiac disorders, inflammation, mood disorders, renal insufficiency and ovarian abnormalities. To stabilize the enzymes and to use them repeatedly, they are immobilized on a wide range of carriers. The immobilized enzymes, have several application areas in various industrial processes. This biocatalytic processes are environmentally friendly, sustainable and cost-effective. In the last thirty decades, immobilized enzymes are preferred as reusable reagents in industrial processes. Although AR purified from various sources and their mechanism and properties are widely studied, there is no immobilization studies with this enzyme. In this study, AR was isolated from bovine kidney and immobilized onto gelatin-glutaraldehyde system by crosslinking method. The immobilized aldose reductase was characterized for several parameters. The immobilized enzyme showed optimum activity at pH 7.5, while soluble form was 6.5. On the other side, immobilization does not affect the optimum temperature range i.e., both, soluble and immobilized AR exhibited maximum activity at 60°C. The immobilized AR retained more than 95% of its initial activity when stored at 4°C for 20 days and retained 56% of its initial activity after 15 successive repeated-use cycles. In conclusion, the study can be used as base for the immobilization of aldose reductase.

Keywords: Aldose reductase, immobilization, gelatin, cross-linking agents

Creating chimeras of different xylanases isolated from two *Geobacillus* species with DNA shuffling

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Abstract

Xylan is a major component of hemicellulose and is known to be the second-most abundant renewable resource in nature. Xylanases are hemicellulolytic enzymes, which are responsible for the degradation of the heteroxylans constituting the lignocellulosic plant cell wall. The major potential application of xylanases involves the pulp and paper industry, bioconversion of lignocellulose material to fermentation products, clarification of juices, improvement of the consistency of beer and production of acidic xylooligosaccharides having potential pharmacological benefits.

Xylanase genes of *Geobacillus sp. 71* and *Geobacillus sp. TF-16* are cloned into pET20b(+) expression vector and expressed in *E. coli* BL21 (DE3 PlysS) host strain. Protein bands were visualized by SDS-PAGE and activity was determined with zymogram analysis.

To obtain more efficient enzymes in terms of pH and temperature, we use DNA shuffling based engineering method and we obtain four chimeric genes from these two xylanase genes. The two of these four chimeric genes were cloned into pET20b(+) expression vector and expressed in *E. coli* BL21 (DE3 PlysS) host strain. After expression and characterization the chimeric xylanases were compared with wild type enzymes. Based on these data, some properties of the chimeric proteins was found to be better than the wild type.

Keywords: DNA-shuffling, xylanase, chimeric

Cloning and Expression of the Laccase Gene from *Bacillus megaterium*

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Abstract

Due to the increasing development of enzyme technology, variety of product application areas and very high economic value, researches related industrial enzymes in the field of biotechnology becomes even more important. Laccases are an interesting group of multi copper enzymes, which have received much attention of researchers in last decades due to their ability to oxidize both phenolic and non-phenolic lignin related compounds as well as highly recalcitrant environmental pollutants. This makes these biocatalysts very useful for their application in several biotechnological processes. Such applications include the detoxification of industrial effluents, mostly from the paper and pulp, textile and petrochemical industries.

We cloned *Bacillus megaterium* laccase gene into pET28 expression vector system, that have been isolated from soil samples. Recombinant enzyme was expressed and purified for characterization. The optimum temprature of the enzyme was determined as 60°C and the optimum pH was 4.5. The thermal and pH stability of the enzyme is good enough for future analysis. Laccase is treated with paper pulp at optimum conditions. After this process we determine that the enzyme decrease the Kappa number of the paper pulp about %0.8. Based on these data, laccase thought to be suitable for paper pulp industry.

Keywords: Laccase, *Bacillus megaterium*, characterization

Morphological and Molecular Characterizations of Some Selected Genotypes of Cv Tahar Apple (*Malus sylvestris spp. orientalis*)

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Abstract

This study was conducted on ‘Tahar’ apple (*Malus sylvestris spp. orientalis*) that is grown locally in Ürgüp District of Nevşehir Province of Turkey in order to determine its characteristics. Tahar apples which is now under extinction represents a high possibility to use as a dwarfing rootstock for standart apple cultivars. Although Tahar apples are traditionally propagated by vegetative means, some variations in the population were observed. In this study the spesifications of seventeen pre-selected genotypes were determined by morphological and molecular characterization studies. The selecekted genotypes were propagated by layering in stool bed method and then planted in a plot. Morphological characterization were done according to UPOV criteria on each genotype. Also, a molecular analysis was done by AFLP (Amplified Fragment Length Polymorphism) after isolating genomic DNA’s in order to determine the genetic relativeness.

Some of the morphological characteristics of the genotypes were resembled to those of M 9 apple clone which is a popular dwarfing rootstock for apple in the world, however, when all characteristics were considered together, these genotypes were found different from M 9 rootstock as well as from each other. Genotypes were analyzed by UPGMA (Unweighed Pair Group Method of Aritmetic Averages) for grouping and they were fell into four phylogenetical groups. The genetical differences between genotypes and M 9 rootstock ranged from 11 % to 78 %. There were distinct differences among the individual genotypes.

Keywords: Breeding, Genetic, Rootstock

Influences of Chemotherapeutic Drugs on NADPH Oxidation Activity of Human Platelet Nitric Oxide Synthase

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Abstract

Nitric oxide synthases (EC 1.14.13.39) NOSs are a family of enzymes (eNOS, nNOS, iNOS) catalyzing the production of nitric oxide (NO) from L-Arginine. NO is an important cellular signaling molecule and it acts as a regulator of numerous processes in the nervous, immune and cardiovascular systems [1, 2]. In this study, we purified NOS from human platelet fractions by using DEAE-Cellulose anion exchange chromatography and 2',5'-ADP Sepharose 4B affinity chromatography with 6,34% yield 1972,34-fold and 1,854 U/mg specific activity. Purified enzyme appeared as a single band (81 kDa) under denaturing condition(SDS-PAGE). The native molecular weight of enzyme estimated (153 kDa) by gel filtration chromatography. In addition, *in vitro* inhibition effects of some chemotherapeutic drugs on NADPH oxidation activity of human platelet NOS. IC50 values were calculated 0.039 mM, 0.0506 mM, 0.217 mM, 0.233 mM, 0.411 mM, 0.434 mM, 0.947 mM, 1.486 mM, 1.988 mM, 15.67 mM for bleomycin, oxaliplatin, vinorelbine, etoposide, carboplatin, paclitaxel, 5-fluorouracil, cyclophosphamide, fludarabine phosphate, gemcitabine respectively from Activity%-[drug concentration] graphs.

Keywords: Nitric oxide synthase, enzyme, inhibition

Determination of The Effects of Hydrogen Peroxide on Expression Levels of Some Photosynthetic Genes in Maize Seedlings Under Osmotic Stress Conditions

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Abstract

To investigate the effects of hydrogen peroxide (H₂O₂), a signal molecule on photosynthesis in maize (*Zea mays* L.) seedlings under osmotic stress, 10 mM of H₂O₂ was applied exogenously. The seedlings were then treated with PEG-6000 for 12 hours to form osmotic stress. After stress treatment, total RNA was isolated and microarray analysis was performed. According to the microarray, expression levels of some of the genes involved in the photosynthetic system were found to be up or down regulated by H₂O₂ pretreatment. For example, the expression level of chlorophyll a/b binding apoprotein CP26 precursor was up regulated by pretreatment with H₂O₂ under drought conditions. It was 8.2 times more than control group. Expression levels of some other genes induced by H₂O₂ compared to the control were as follow. Light harvesting chlorophyll a/b binding protein 6 was 6.4 times, photosystem II subunit 29 was 6.2 times, photosystem II 11 kD protein was 4.5 times, chlorophyll a-b binding protein 6A was 3.3 times, RUBISCO activase 1 was 3.1 times, ATP synthase gamma subunit 1 was 2.9 times, and glyceraldehyde-3-phosphate dehydrogenase 1 was 2.8 times more than control. On the other hand after H₂O₂ pretreatment, expression of level of Aconitas 2 was found to be 7.5-times lower than control. In addition ferredoxin-dependent glutamate synthase and glucose-6-phosphate/phosphatetranslocat 1 were a 2.2 and 1.1 times reduced compared to control, respectively. In the light of these data, it was concluded that photosynthetic activity was induced by exogenous H₂O₂ under osmotic stress conditions.

Keywords: Hydrogen peroxide, Photosynthesis, Microarray, Osmotic stress, *Zea mays*

Chemical modification of silica gel with 4,4'-((1Z,8Z)-2,5,8-triazanona-1,8-diene-1,9-diyl)diphenol and applications to chromium Cr(VI) ions in industrial wastewaters

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Abstract

This study describes synthesis of a new resin through immobilization of the 4,4'-((1Z,8Z)-2,5,8-triazanona-1,8-diene-1,9-diyl)diphenol (TRI) onto silica gel modified with 3-chloropropyltrimethoxy silane (CPTS) and its application for the removal of chromium(VI) ions from aqueous solution as well as from industrial wastewater. The same applications were also made for industrial wastewater vapor. The newly synthesized Si-TRI is characterized with scanning electron microscope (SEM) and elemental analysis and Cr(VI) heavy metal ions were used as sorbate. The sorption of Cr(VI) ion was evaluated with using batch methods. The value of adsorption of Cr(VI) ion was detected with an atomic absorption spectrometer(AAS). The influences of concentration, temperature, amount of metal ions, contact time and pH to sorption on the Si-TRI were also investigated. The maximum adsorption capacities and isotherm parameters were calculated from the Langmuir, Freundlich and Dubinin-Radushkevich (D-R) isotherm equations. Thermodynamic parameters such as free energy (ΔG°), entropy (ΔS°) and enthalpy (ΔH°) were also calculated from the sorption results. The modified structure used as adsorbent was successfully employed in the removal of Cr(VI) ions on the samples of industrial wastewater.

Keywords: Adsorption, Immobilization, Silica gel, Wastewater, Chromium

Expressions of TRPM6 and TRPM7 and Histopathological Evaluation of Tissues in Ischemia Reperfusion Performed Rats

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Abstract

Aim

Ischemia and reperfusion (I / R) is a common clinical event in many areas of medicine. Calcium is vital essential element for humans and all mammalian cells. However, accumulation of calcium overload in cells, causes excessive activation of the signaling processes that lead to cell death. However, the mechanism of cytotoxic activity of calcium is still a controversy. Although there are several different ways involved the entry of calcium in cells, TRPM channels is one of the most important ones. There exists not much work on the expression of TRPM6/7 in ischemia reperfusion models. In the present study, expression levels of these channel proteins were revealed after modifying the reperfusion duration to 48 hours to investigate pathophysiological changes in renal tissues.

Materials and Methods

For the current study numbers of 20 Wistar albino rats were divided into 2 groups equally. Group I: control group, Group II = I/R group (60 min ischemia +48 hours reperfusion). For the mRNA analysis, right kidneys of I/R group was used as reference in order to eliminate genetic differences. The left renal artery (I / R generated part) of I/R area was removed from all rats in the second group. Likewise, normal tissues of right renal artery were removed from all rats. Histopathologic scoring of the tissue samples were achieved semi-quantitatively according to normal tissue composition. Statistical analysis of resulting data was used One-way ANOVA test. The degrees of significance of differences between groups were assessed by LSD test. All statistical tests were two-tailed and $P < 0.05$ was considered as significant.

Results and Discussion

Both TRPM6 and TRPM7 expression levels were decreased in all groups relative to control group yet these changes were not statistically significant ($p > 0.05$). Additionally, these results are confirmed the results of correlation analysis. In order to investigate pathological changes in renal tissues after ischemia-reperfusion applications, histopathologic examination of tissue samples were stained with Hematoxylin-eosin and scored semi-quantitatively according to overall tissue composition. Taken together, our study results revealed that expressions of TRPM6 and TRPM7 channel proteins were found to be close to that of initial levels after 48 hours of reperfusion. This result suggests that levels of these protein channels have increased and after 48 hours of reperfusion decreased to normal levels in cells. Lastly, tissue damages may also be resulted from oxidative effects as reported previously.

Keywords: Ischemia/reperfusion, TRPM channels, Calcium, Magnesium

Antifungal Activities of *Ailanthus altissima* Swingle and *Juglans regia* L. Against Some Cereal Fungi

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Abstract

In live stock farming, crop growing and industry, the toxic microfungi found in cereals is a very grave problem for centuries. The fungi produce toxins that cause chronic and acute toxications when their productive value of grain is decreased. Because of their susceptible nature to microbial contamination, cereals can be contaminated easily by filamentous fungi. In literatures; some *Fusarium*, *Gibberella* and *Cladosporium* species was given as field fungi and; some *Aspergillus*, *Penicillium* and *Trichothecium* species as storage fungi. The fungus species used in this study were *Aspergillus niger*, *Aspergillus parasiticus*, *Cladosporium cladosporioides*, *Fusarium sporotrichioides*, *Fusarium oxysporum*, *Gibberella fujikuroi*, *Penicillium griseofulvum*, *Penicillium brevicompactum* and *Trichothecium roseum*. This study was undertaken to investigate the antifungal activity of *Juglans regia* L. (walnut) and *Ailanthus altissima* Swingle (tree of heaven) leaves against these mycotoxin and spoilage producing fungus. The powder of the leaves from *J. regia* L. and *A. altissima* Swingle (10 % v/w) showed the maximum antifungal activity against *C. cladosporioides* (71±2.0% for *J. regia* L and 51±1.52% for *A. altissima* Swingle). The minimum inhibitory concentration value of *J. regia* L. methanolic extracts against *C. cladosporioides* was lower (0.625 mg/mL) than *A. altissima* Swingle. Ethanol and methanol extracts of *J. regia* L. completely inhibited the spore germination at 5.0 and 10 mg/mL, and ethanol and methanol extracts of *A. altissima* Swingle at 10 mg/mL. This study demonstrates that *J. regia* L. extracts have a potential to control the fungal contamination caused by *C. cladosporioides*.

Keywords: Antifungal activity, plant extracts, *Cladosporium cladosporioides*

The Effect of *Nigella sativa* L. on the Caspase-3, Caspase-9, Erk, akt Protein Expression and DNA Damage in Rats with Carbon Tetrachloride (CCl₄)-Induced Lung Damage

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Abstract

Introduction: In recent years, studies that on the potential use as medicaments of plant materials were increased, of them black seed (*Nigella sativa* L.) which were found to be particularly powerful antioxidant effects of was reported to be of a healing effect on the lungs.

Material and Methods: Four groups were created in this study; groups: negative control, positive control, CCl₄ group (1.5 ml / kg ip. biweekly for 4 weeks), CCl₄ + *Nigella sativa* group. Lung damage formed in rats with CCl₄. *Nigella sativa* extract added in drinking water (10%), Erk, Akt, Bax and Bcl-2 protein expression and DNA damage was investigated with western blotting and agarose gel electrophoresis. Additionally, tissues and plasma MDA levels were determined.

Results and Discussion: As a result of this study, in CCl₄ + *Nigella sativa* group; MDA level decreased, DNA damage decreased, apoptotic protein synthesis increased in comparison to the CCl₄ group. This data showed that *Nigella sativa* was reduce the lung tissue damage in rats. From these data, making use of these plants is expected to show similar effects on humans.

Keywords: Lung, DNA damage, *Nigella sativa*, apoptosis

Acknowledgements: This work was supported by Firat University Scientific Research Projects Commission (FUBAP) with F.F.13.18 project number.

***In vitro* Assesment of The Probiotic Potential of 7 Strains Orginated From Human**

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Abstract

Probiotics whose names are derived from the Greek ‘for life’ have been used for about 100 years because of their benefits on health. Many strains of lactic acid bacteria (LAB) in both fermented foods and in the gastrointestinal considered as Probiotics. 7 bacteria that endowed with antimicrobial activity were isolated by the feaces that belong to people at different age groups. In regard to biochemical tests and 16S rDNA sequence analyzes within seven isolates, 4 isolates were described as *Lactobacillus casei*, 2 isolates were described as *Lactobacillus plantarum* and one isolate was described as *Pediococcus pentosaceus*. The resistance levels of human origin LAB against the applications of low levels of pH values (pH 3) and pH-pepsin were found as defined probiotic. In contrast to this, *Lactobacillus casei* BH96 and BH101 were not found as suitable for the probiotic selection criteria due to their sensitives to pancreatic and bile salt applications. γ -hemolytic strains were found high resistance levels against the antibiotics which especially used for therapy. The bacteriocin (1600 AU/mL) produced by *Pediococcus pentosaceus* BH105 were characterized as pediocin like bacteriocin with high pH and heat stability. Molecular mass of *Pediococcus pentosaceus* BH105 bacteriocin was found as 5.0 kDa by the tricin sodium dodesil sulfate-polyacrylamide (Tricin-SDS-PAGE) system. The adhesion ratio of BH105 to the Caco-2 cells was determined as 10.12% (± 2.4). In competition tests; BH105 was reduced *E. coli* LMG 3083 adhesion at the rates of 88.72 % (± 5.53) and *S. Typhimurium* SL1344, adhesion at the rates 60.64 % (± 10.97).

Keywords: Probiotic, Human, *Pediococcus pentosaceus*, Bacteriocin

Innovations in Probiotic Researches

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Abstract

Probiotics, defined as 'live microbial feed supplements that beneficially affect the host by improving the intestinal microflora balance', have been used for their therapeutic or prophylactic effects since Nobel Prize winner scientist Elie Metchnikoff. Therapeutic or prophylactic effects are production of antimicrobial metabolites, immunomodulation, maintenance of mucosal intestinal resistance to infectious diseases, prevention of antibiotic-associated diarrhea and surgical wound infections, metal detoxification and decrease of allergic diseases, management of dermatitis, regression of tumors and reduction in carcinogen and mutagen production. Despite the health-promoting benefits of probiotics, there are some significant physiological limitations in clinical applications of probiotics. Therefore, genetically improving properties of a wild probiotic strain such as stress tolerance and the survive in foods prior to ingestion lead the researchers to create new genetically manipulated (GM) probiotic strains. Various genetic systems such as plasmid vectors, chromosome modification and homologous or heterologous gene expression systems have been developed and introduced to modify lactic acid bacteria. Temperature and water availability are the most common stresses encountered during the production of probiotic foods and/or tablet formulations. Equipping probiotic strain with genetic elements, encoding protective compounds which help to stabilize protein structure and function at low temperatures and low a_w conditions such as betaine, carnitine and proline, prevent to reductions in probiotic numbers during manufacture and storage. The other approaches of the GM probiotics aim to positively affect the therapeutic efficiency of the probiotic strain by improving host colonization and blocking crucial ligand-receptor interactions between host cell and invasive pathogens.

Keywords: Probiotic, Genetically manipulated, Therapeutic/ prophylactic effect

The Effects of Citric Acid and Sodium Benzoate on Liver and Kidney Tissue

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Abstract

Object: The effects of sodium benzoate and citric acid which are used as additives on foods on liver and kidney in rats and the changes on total sialic acid (TSA) levels had been examined.

Methods: 30 Wistar albino male rats (200-250 g.) had been divided in three groups (n=10). Control group, Citric acid group and Na-benzoate group had been feed on by gavage once a day for 10 days with tap water, Citric acid (576 mg/kg body weight) and Na-benzoate (2442 mg/kg body weight) respectively.

Blood, liver and kidney of animals were removed at the end of the experiment. Tissues were stained with Hematoxylin-Eosin after routine follow up procedures and analysed in photomicroscope. TSA had been measured by spectrophotometre. The results had been evaluated with the biostatistical methods.

Result: It had been found that the serum TSA amount had significantly decreased ($p < 0.05$) in sodium benzoate group, and no significant change had happened in citric acid group. It had been determined that in sodium benzoate group, sialic acid amount in both liver and kidney had decreased significantly ($p < 0.0001$). The level of sialic acid increased significantly ($p < 0.0001$) in kidney but not changed in liver in citric acid group.

The control group had been observed in their normal appearance by light microscope.

In the citric acid and the sodium benzoate groups, deteriorations on the structure of liver cell string, vacuolization on the hepatocytes, pyknotic nucleus, loss of nucleus, hypertrophy, invagination in the nucleus membrane had been observed.

In both experimental groups, pyknotic nucleus and membrane damage in the apical surfaces of tubule cells, invagination on the nucleus membrane, damage on the basal membranes, mononuclear cell infiltration had been observed.

Discussion: We believe that more detailed studies should be made regarding those additives on foods because the effects of sodium benzoate and citric acid on TSA consider with morphological degenerations in liver and kidney.

Keywords: sialic acid, citric acid, sodium benzoate, liver, kidney.

Investigation of Effect of Naringin on Antioxidant Capacity in Intestinal Ischemia Reperfusion Injury

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Abstract

Objective: The aim of this study was to investigate the effects of naringin on morphologic changes, antioxidant enzyme activities and amount of malondialdehyde (MDA) related with ischemia reperfusion (I/R) injury in small intestine of rats.

Material and Method: 21 male Wistar albino rats were divided to three groups (n=7) as follows:
Group A-sham group.

Group B-Ischemia-reperfusion group. Ligated superior mesenteric artery (SMA) was opened 120 min after ligation and reperfusion was applied for 2 hours.

Group C-Naringin group. 50 mg/kg of naringin was administered intraperitoneally just after SMA was opened.

Intestinal tissues of the animals were removed at the end of the experiment. Amounts of tissue superoxide dismutase (SOD), glutathion peroxidase (GPx), catalase (CAT) and MDA were analysed and all results were evaluated with biostatistical methods.

For light microscopy examination, removed tissues were stained with Hematoxylin-Eosin after routine follow up procedures and analysed in photomicroscope. Light microscopy findings were evaluated with Chiu scoring system.

Results: Control group was seen to have a normal structure under light microscopy. In I/R group, it was seen that mononuclear cell infiltration developed, epithelium lining villi degenerated, villus integrity was impaired and mucosa was flattened. In naringin group, impaired villus integrity was seen to continue, no improvement was seen to develop in epithelial degeneration. SOD activity and MDA amount were seen to elevate statistically significantly and significantly decreased due to naringin administration in I/R group compared to control group. GPx activity was seen to decrease significantly in I/R group compared to control group and elevated due to naringin administration.

Discussion: We consider that naringin does not have an improving effect on morphologic changes observed in intestinal tissue of rats due to I/R however it has positive effects on biochemical parameters.

Keywords: Naringin, ischemia/reperfusion, intestine, rat

Investigation of Decolorization of Reactive Violet 5R and Remazol Brilliant Orange 3R by *Bacillus* sp. DT16

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Abstract

Dye pollution in water and soil is increasing rapidly depending on the industrialization. Synthetic dyes are mutagenic, toxic and resistant to degradation due to their complex chemical structures so their effluents cannot be directly discharged. The biological remediation of textile effluents has recently received an increasing attention, representing an attractive, cheap, environmentally friendly, and publicly acceptable alternative to the physico-chemical methods. Microorganisms play an important role in the decolorization and removal of dyes from polluted sites.

In this study, decolorization of RV-5R and RBO-3R by *Bacillus* sp. which was isolated from textile effluent was investigated. The effect of environmental factors such as pH (5.5, 6.5, 7.5, 8.0, 9.0 and 10.0) temperature (20, 30, 37 and 42 °C), carbon (1 g/L: sucrose, glucose, starch and mannitol) and nitrogen sources (ammonium chloride, peptone and yeast extract) and initial dye concentration (10, 25, 50, 100, 200, 500 mg/L) on the microbial decolorization by *Bacillus* sp. was investigated.

The maximum dye removal was obtained at 500 mg/L initial dye concentration for RV-5R and 200 and 500 mg/L for RBO-3R by the bacterium. Bacterial decolorization of RV-5R was 54.54% (6h) in growth medium containing yeast extract (1g/L) and glucose (1g/L) at pH 10.0 and 37 °C. The same bacterium decolorized the RBO-3R dye at 96.15% (172 h) in growth medium containing peptone (1g/L) and sucrose (1g/L) at pH 10.0 and 30 °C.

Any report about the microbial decolorization of the RV-5R and RBO-3R dyes has not been seen in the literature. Therefore this study was the first report about the bacterial decolorization of these dyes.

Keywords: *Bacillus*, decolorization, optimization

Acknowledgments: This study was supported by the Scientific Research Council of Pamukkale University, Turkey (research grant 2012KRM015)

Partial purification of polyphenol oxidase (PPO) from potato (*Solanum tuberosum*)

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Abstract

Damage, interruption and mechanic injuries during harvesting that cause the oxygen transition, storage and industrial treatment, cause browning on most of fruit and vegetables. Browning reactions change the flavor and aroma of products, also cause the reduction in quality of theirs. This enzymatic browning is catalyzed polyphenol oxidase (PPO) enzyme. Synthesis of melanin pigments performed by browning is caused by catalyzing the oxidation of phenolic compounds this enzyme (EC.1.14.18.1) that is a member of oxidoreductase groups.

In this study, activities of polyphenol oxidase isolated from potato and partially purified by 3 different methods, were determined against catechol substrate. PPO was purified by application %80 (NH₄)₂SO₄ precipitation and then dialysis of samples that are homogenized in KH₂PO₄ (pH 6.8) buffer including ascorbic acid, PEG and Triton X-100, following production of acetone powder. In the second method, without producing acetone powder, after grated potato samples were homogenized in KH₂PO₄ (pH 6.8) buffer containing ascorbic acid, PEG and Triton X-100, filtrate was obtained. It was evaluated as crude enzyme extract. In the third and last method, PPO was purified by homogenization with citrate buffer (pH 4.8) of precipitate acquired from NaF and (NH₄)₂SO₄ treatment.

In the enzyme activity studies performed at 420 nm wavelength, activity of PPO purified above mentioned methods was detected as 231; 1338; 544 U/mL respectively. In total protein amount assay made by using Lowry methods, protein amounts in samples were respectively determined as 382; 433; 510 µg/mL. Molecular weight of PPO obtained from potato by partial purification processes, was detected by SDS-PAGE analysis.

Keywords: Polyphenol oxidase, enzymatic browning, *Solanum tuberosum*

Biodegradation of 2,4,6-Trinitrotoluene (TNT)

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Abstract

Nitroaromatic compounds known as xenobiotics, are produced as a result of industrial processes that focused on synthesis of polyurethane, parathion like pesticide, dinoseb and explosives. The wide fields of application this compounds as well as nitroaromatic contents of drug and dyes materials, are production of pecticides, plastics, polymers, textile paper and explosives.

The contaminant is caused to leak to soils and groundwaters by the participation in structures of other explosives of TNT that have continuous continued its production for military purposes since World War I, its storage processes and/or incorrect incorrect removal technologies. Besides its explosiveness, TNT is a important nitroaromatic pollutant due to its toxicological effects on many organisms including humans. Its toxic effects on the living can be prevented by providing a real degradation with not only the removal of polluted regions but also the prevention of its leakage to groundwaters.

TNT's persistent structure does not completely allow to remediation of the aromatic ring. In contrast, bacteria, fungi and many organisms such as yeasts can be metabolized TNT as nutrient source by their owned enzyme systems. In this study, biodegradation processes was carried out by the strain *Enterobacter cloacea* isolated from border lands with contaminated TNT. In biodegradation processes performed under agitated conditions for 96 h time period, the decline at initial concentrations of TNT against abiotic cultures was followed by HPLC analysis. In cultures including 50 and 75 mgL⁻¹ TNT, after 96 h of incubation, biodegradation efficiency were detected as %35 and %45 respectively. In biotic cultures, it was not observed nitrite and nitrate accumulation and 4-aminodinitrotoluene (4-ADNT) as degradation intermediate was identified by TLC and GC-MS.

Keywords: TNT, biodegradation, nitroaromatic pollutant

A content-based web image retrieval system for butterfly identification based on the structural similarity index: BICBIR

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Abstract

Butterflies can first be classified according to their outer morphological specialties. If that is not possible, their genital characteristics must be analyzed. The genital characteristics of a butterfly can be determined using various chemical substances and methods that are both time-consuming and expensive. In this study, a computer vision method, which is a content-based web image retrieval system, is proposed for automatically identifying butterfly species, as an alternative to conventional methods. We have developed an image retrieval system named as the (BICBIR=butterfly identification with content based image retrieval), which uses structural similarity (SSIM) as the method for retrieving butterfly images, similar to a given set of reference butterfly images in a database. A total of 380 butterfly images from 38 species, which were collected from Mount Ereğ, Van, between May, 2002 and August 2003, are used for evaluating the effectiveness of the proposed method. The BICBIR system can be designed to identify butterfly images with or without the similarity index threshold. The performance of the butterfly species identification from butterfly images in the image database, noisy butterfly images in the image database and butterfly images that are not in the image database, with and without the SSIM index threshold, were 96%, 94%, and 100% with the threshold, 89%, 90%, and 0% without threshold, respectively. The experimental results showed that the proposed method had achieved good recognition in terms of the accuracy rates for butterfly species identification.

Keywords: Image retrieval, butterfly identification, www, structural similarity index, expert system

Synthesis, characterization and antiproliferative activity of novel modified Poly(maleic anhydride-co-vinyl acetate)/Hydroxyurea conjugate

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Abstract

Copolymer, poly(maleic anhydride-*co*-vinyl acetate) (MAVA), was traditionally synthesized by free radical chain polymerization reaction, in methyl ethyl ketone (MEK) at 80°C, using benzoyl peroxide (BPO) as the radicalic initiator. The purified copolymer was then modified with a commercial chemotherapeutic agent, hydroxyurea (HX). The conjugation reaction was performed for 50 hours at 70 °C in dimethylformamide (DMF), using triethylamine (TEA or Et₃N) as the catalyst. The modified copolymer/drug couple was named as MAVA/HX. Structural characterization of the copolymer (MAVA) and the conjugate (MAVA/HX) was carried out by Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (¹H-NMR and ¹³C-NMR). Their molecular weights were also determined by size-exclusion chromatography (SEC). The FTIR, ¹H-NMR, ¹³C-NMR spectra, and SEC measurement confirmed that HX molecule was successfully covalently bound to the MAVA copolymer. A mechanism for MAVA and HX was then also suggested for the conjugation reaction. Antiproliferative activities of MAVA/HX were determined by the BrdU cell proliferation ELISA assay, using C6 and HeLa cell lines on eight concentrations (5, 10, 20, 30, 40, 50, 75, and 100 µg/ml). Two anti-cancer chemotherapy drugs, cisplatin and 5-fluorouracil, were included as the positive controls. By comparing with cisplatin and 5-fluorouracil, the antiproliferative activity results showed that MAVA/HX have significant activity against C6 cell line. Especially, MAVA/HX has higher activity than cisplatin at 5, 10 µg/ml. On the other hand, MAVA/HX have no antiproliferative activity against HeLa at compare with cisplatin and 5-fluorouracil. In addition, the antiproliferative activities of MAVA/HX was shown to increase depending to increasing concentration against HeLa.

Keywords: Copolymer modification, hydroxyurea, poly(maleic anhydride-*co*-vinyl acetate), FTIR spectroscopy, NMR spectroscopy

Synthesis and Characterization of Antigenic Peptide Epitopes of The Rabies Virus Protein

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Abstract

The rabies virus is acute and precise lethal infection of the central nervous system, which is the causative agent of rabies. Transmitted to humans from animals, is among the important diseases [1]. On the basis of new approaches and methods developed as an alternative vaccine to Pasteur vaccine method, there are obtaining antigens (peptide, lipopolysaccharides etc..) from structure of microbes and viruses that are disease agents, producing them synthetically and developing vaccines with this way [2].

In our study, rabies virus (rabies virus-RV) protein antigenic-peptide epitopes, 404-418 (WAVYTRIMMNGGRLK), and 253-275 (WPPDQLVNLHDFRSDEIEHLVVE) sequences were synthesized by us on the peptide synthesis apparatus (Liberty Microwave Assisted Solid Phase Peptide Synthesizer) with the solid phase peptide synthesis method using microwave energy. It is known that the microwave solid phase peptide synthesis have benefits compared to conventional methods. Chromatographic and spectroscopic analysis methods were applied for purification and characterization. Preparative HPLC was used to purify, LC-MS, GPC and HPLC was used for characterization [3,4,5].

Our aim in our ongoing study is ; doing covalent conjugation of synthesized peptides with synthetic polymers and forming of new generation synthetic vaccine prototype with developing of immunological polymer-peptide bioconjugates against the rabies.

Keywords: peptide, solid phase peptide synthesis, rabies, microwave

Microwave-Assisted Solid Phase Synthesis and Characterization of Antigenic Peptide Epitopes of Brucella Abortus Disease

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Abstract

Many studies have been performed in the application areas of biotechnology, medicine and pharmacy about water-soluble polyelectrolytes(PE)-metal-peptide complexes as functional biopolymer systems [1].

There is the possibility of creating stable structure ternary complexes of polyelectrolytes with peptides in the presence of transition metal ions. As well as such complexes have a high immune effect and stability against radiation, these complexes show vaccine feature that has been proven by our earlier studies [2,3].

Brucellosis is a zoonotic disease (infectious disease that can be passed from animals to humans) formed by the genus of Brucella bacterias. It is infected via the meat, milk, urine, body fluid, products of conception of sheep, goats, cattle, buffalo, pigs and some dairy products made of with infected milk [4].

In this study three antigenic peptide epitopes [(Gly-Gly-Asp-Asn-Tyr-Ser-Asp-Lys-Pro-Glu-Pro-Leu-Gly-Gly), (Leu-Ala-Glu-Ile-Lys-Gln-Arg-Ser-Leu-Met-Val-His-Gly-Gly) ve (Gly-Gly-Ala-Pro-Gly-Glu-Lys-Asp-Gly-Lys-Ile-Val-Pro-Ala-Gly) amino acid sequence] of virus protein of Brucella abortus disease that is often seen in recent years were synthesized with microwave-assisted solid-phase methods by us. Ternary complexes of these antigenic peptides, which synthesized, purified by preparative HPLC and characterized by LC-MS, with anionic polymers via Cu²⁺ will be obtained, structures and bonding mechanisms of these complexes will be examined. Also toxicity of these obtained peptide epitopes and complexes will be tested with cell assays [4-6].

Keywords: Synthetic Peptide, Brucella Abortus, PE-Metal-Peptide Complexes

Effect of Putrescine on Paraquat Induced Genomic Instability in *Phaseolus vulgaris*

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Abstract

In this study, we aimed to investigate DNA damage levels in *Phaseolus vulgaris* subjected to paraquat and whether putrescine has any protective effect on these changes using RAPDs (Randomly Amplified Polymorphic DNA) technique. The results showed that paraquat (10^{-6} , 10^{-5} , 10^{-4} and 10^{-3} mol/l) caused RAPDs profile changes (DNA damage) increasing, genomic template stability (GTS) decreasing. However, these effects of paraquat seen at higher levels decreased after treatment with different three concentrations (10^{-8} , 10^{-9} and 10^{-10} M) of putrescine. The results of this experiment have clearly shown that putrescine could be used effectively to protect bean seedlings from genotoxic effects of paraquat.

Keywords: Paraquat, Putrescine, Genomic instability, RAPD

Protective Role of β -estrodinol against DNA Hypomethylation of Trifluralin in *Zea mays*

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Abstract

Trifluralin (2,6-dinitro-N,N-dipropyl-4-trifluoro-methylaniline) is a widely used dinitroaniline herbicide in agriculture. The use of herbicides in agriculture may represent a potential toxic risks to some crops. Thus, the present study aimed to evaluate protective role of β -estrodinol against epigenetic effects of trifluralin by using CRED-RA (Coupled Restriction Enzyme Digestion-Random Amplification) assay in *Zea Mays* seedlings.

The results showed that trifluralin (0.5, 1, 2 and 4 ppm) caused DNA hypomethylation. However, these effects of trifluralin seen at higher levels decreased after treatment different three concentrations (10^{-8} , 10^{-9} and 10^{-10} M) of β -estrodinol. The results of this experiment have clearly shown that increasing of DNA hypomethylation under trifluralin stress may be a part of the defense system against the stress and β -estrodinol could be used effectively to protect corn seedlings from the destructive effects of trifluralin.

Keywords: Trifluralin, β -estrodinol, DNA hypomethylation, CRED-RA

The Mutagenic and Antimutagenic Activity of *Cynara syriaca*

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Abstract

Cynara syriaca BOISS. (*Asteraceae*) is a perennial plant distributed in the Palestine, Lebanon, Northern Iraq, Syria and Turkey. The potential mutagenic and antimutagenic properties of leaf extracts of *Cynara syriaca* was investigated with Ames Salmonella/microsome mutagenicity assay in (by using) *Salmonella typhimurium* TA98 and TA100 strains. The leaf of *C. syriaca* were collected from southeastern Turkey (Diyarbakır), dried, powdered and macerated with methanol. The plate incorporation method was performed to identify reverse mutations from histidine dependence to histidine independence by using *S. typhimurium* test strains TA98 and TA100 with and without S9 fraction that was the mammalian liver homogenate. In a series of experiments preceding the mutagenicity studies, it was ascertained that the different amounts of leaf extract (0.1, 0.5, 1, 5, 10, 25, 50, 100, 250, 500 µg/plate) added to the indicator bacteria should not have a toxic effect on the TA98 and TA100 test strains and mutation frequencies do not change significantly when compared with spontaneous mutation frequencies. The antimutagenic experiments showed that leaf extract has strong antimutagenicity (inhibition level > 40%) at 250 and 500 µg/plate concentration (42.63% and 53.98%) on TA98 strain and moderate antimutagenicity (inhibition level < 40-25%) at 5 and 500 µg/plate concentration (35.73% and 29.69%) on TA98 and TA100 strains respectively.

Antimicrobial, Antioxidant Activities and DNA Interactions of *Salvia siirtica* (Lamiaceae)

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Abstract

There are several biological activity studies about *Salvia* L. genus which is the largest genus of the family Lamiaceae. Several species of this genus were used for folk medicine. There is no study about *Salvia siirtica* Kahraman, Celep & Doğan which was described by Kahraman et al. in 2001 from Siirt Çatılı province. In this study antimicrobial activity on some gram positive and negative bacteria and yeast, antioxidant activity and DNA interactions of methanolic extracts of *Salvia siirtica* are studied. The extract was shown antimicrobial activity against *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* ve *Staphylococcus aureus* (inhibition zone diameter 14 ± 0 , 12 ± 1 , 12 ± 1 , 11 ± 1 and 9 ± 1 mm respectively). The extract was studied for DPPH radical scavenging and ferrous ion chelating activity and IC₅₀ values were determined as 31.50 and 12.11 µg/ml, respectively. The amounts of total phenolic contents, β carotene and lycopene in the extract were determined as 225.75 ± 4.24 mg/g; 1.943 ± 0.016 µg/g and 0.263 ± 0.019 µg/g, respectively. Interactions between extract with different concentrations and pUC18 plasmid DNA were studied by using agarose gel electrophoresis. The extract was induced conformational changes in the DNA helix. However there is low antimicrobial activity of *Salvia siirtica* methanolic extract; antioxidant activity of the extract is quite high. The advanced studies should make to rate this extract as anticancer agent. This is the first study about biological activity of *Salvia siirtica* species.

Keywords: *Salvia siirtica*, antimicrobial activity, antioxidant activity, DNA interaction

Newly Isolated thermophilic *Anoxybacillus flavithermus* SO-10, its α -amylase production, characterization and usage in fruit juice industry

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Abstract

The present study was concerned with isolation of thermophilic bacteria from hot spring in Afyonkarahisar (Omer) and production and characterization of extracellular α -amylase from *Anoxybacillus flavithermus* SO-10 (Accession number:KJ094999). To identify the thermophilic isolate morphology, biochemistry, sequencing of 16S rRNA gene, G-C content and DNA-DNA hybridisation were analysed. The influence of different fermentation conditions such as incubation time, temperature and pH, different carbon and nitrogen sources on amylase production were investigated. In addition to these, enzyme partially purified and characterised. Various parameters such as temperature and temperature stability, pH and pH stability, detergents, different starches and metal ions on effect of enzyme characterization were studied. About 71%, 43% and 23% of the activity retained after heating the partially purified enzyme solution at 50°C, 60°C and 70°C for 720 min, respectively. Enzyme was strongly inhibited by Hg²⁺(95%) and Cd²⁺(82%). The enzyme was significantly activated by Co²⁺ (198%) and Mg²⁺ (142%). Enzyme degraded the %82 of starch content in raw apple at 70°C in 30 min.

Keywords: Thermophilic bacteria, α -amylase, thermostable, 16S rRNA, starch

Determining of *Gibberella fujikuroi* as new L-glutaminase Producer by Solid State Fermentation

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Abstract

L-glutaminase (E.C. 3.5.1.2) is the enzyme that catalyzes the deamidation of L-glutamine to L-glutamic acid and ammonia. The action of L-glutaminase acts a major role in the nitrogen metabolism of both prokaryotes and eukaryotes. In recent years, L-glutaminase has received attention as a therapeutic against cancer and HIV, as a biosensing in monitoring glutamine levels, as a flavor and aroma enhancing agent in food. In addition, L-glutaminase is used anti-leukemic agent. The enzyme causes selective death of glutamine-dependent tumor cells by depriving these cells of glutamine. L-glutaminase has therapeutic and uses in industrial areas, is produced mainly from *Aspergillus* and *Trichoderma*. Although genetic manipulations by classical mutation techniques and recombinant DNA technology are frequently used to increase the expression levels of a large number of microbial enzymes in well known microorganisms, traditional screening procedures make it possible to find new attractive wild microorganisms which are able to produce useful enzymes. In our study, fungi species were supplied by the fungi collection of Arda Vocational College, Trakya University, Turkey. In the experiment the data which can help the future industrial and scientific world was obtained by Solid State Fermentation (SSF) using wheat bran as substrate and which is fairly inexpensive. 28 fungus species were screened for the L-glutaminase production by SSF (% 65 moisture, 2 mL inoculum and 6 day incubation) and *Gibberella fujikuroi* was found out to have the maximum L-glutaminase activity (85.90 ± 6.96 U/gds). This study has shown the potential of *G. fujikuroi* as a new source of L-glutaminase.

Keywords: L-glutaminase, *Gibberella fujikuroi*, solid state fermentation

Screening for Antimicrobial Activities of *Actinomycetes sp.* isolated from Afyonkarahisar, Turkey

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Abstract

We aimed to determine the characterization of antimicrobial compounds of Actinomycete isolates (A32, A33) which were determined to have antimicrobial activities in a previous study of ours. Different fermentation media were used to determine effective antimicrobial compounds production of AA32 and AA33 by fermentation. Fermentation procedures were performed in three major steps: sporulation, inoculation, and fermentation. Effective antimicrobial molecules of AA32 and AA33 were determined as test microorganisms *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used during fermentation. Raw material extraction of AA32 and AA33 were performed by solvent extraction and active compound determination was performed by using TLC, biotography, column chromatography and UV spectrophotometer methods. Also to describe these two isolates, fatty acid profiles were determined using gas chromatography. As a conclusion, the isolate AA32 was identified as *Streptomyces-halstedii-olivaceus* at a similarity index of 0.154 and the isolate AA33 was identified as *Streptomyces californicus* at a similarity index of 0.540 by the MIDI system. The antimicrobial compound isolated has O-H and/or N-H rich, having amine groups, has an R_f value of 0.527, its λ_{\max} is at 254 nm, and a polar compound. Its UV spectrum peak and, its λ_{\max} bring to mind the cephalosporin or cephalosporin like aromatic or polyene compounds.

Keywords: Actinomycete, antimicrobial compound, fatty acid profile

Investigation of Decolorization of Reactive Violet 5R and Remazol Brilliant Orange 3R by *Bacillus* sp. DT16

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Abstract

Dye pollution in water and soil is increasing rapidly depending on the industrialization. Synthetic dyes are mutagenic, toxic and resistant to degradation due to their complex chemical structures so their effluents cannot be directly discharged. The biological remediation of textile effluents has recently received an increasing attention, representing an attractive, cheap, environmentally friendly, and publicly acceptable alternative to the physico-chemical methods. Microorganisms play an important role in the decolorization and removal of dyes from polluted sites.

In this study, decolorization of RV-5R and RBO-3R by *Bacillus* sp. which was isolated from textile effluent was investigated. The effect of environmental factors such as pH (5.5, 6.5, 7.5, 8.0, 9.0 and 10.0) temperature (20, 30, 37 and 42 °C), carbon (1 g/L: sucrose, glucose, starch and mannitol) and nitrogen sources (ammonium chloride, peptone and yeast extract) and initial dye concentration (10, 25, 50, 100, 200, 500 mg/L) on the microbial decolorization by *Bacillus* sp. was investigated.

The maximum dye removal was obtained at 500 mg/L initial dye concentration for RV-5R and 200 and 500 mg/L for RBO-3R by the bacterium. Bacterial decolorization of RV-5R was 54.54% (6h) in growth medium containing yeast extract (1g/L) and glucose (1g/L) at pH 10.0 and 37 °C. The same bacterium decolorized the RBO-3R dye at 96.15% (172 h) in growth medium containing peptone (1g/L) and sucrose (1g/L) at pH 10.0 and 30 °C.

Any report about the microbial decolorization of the RV-5R and RBO-3R dyes has not been seen in the literature. Therefore this study was the first report about the bacterial decolorization of these dyes.

Keywords: *Bacillus*, decolorization, optimization

Acknowledgments: This study was supported by the Scientific Research Council of Pamukkale University, Turkey (research grant 2012KRM015)

Single-step purification of human serum albumin using agmatine functionalized affinity beads

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Abstract

Serum albumin (SA) and immunoglobulins (Igs) represent over 78% of all in human serum proteins. Several methods have been developed that attempt to separate the high-abundance serum proteins such as albumin and IgG [1]. Albumin can act as a carrier of many substances (i.e., fatty acids, bilirubin, hormones, vitamins, proteins). Such ability of albumin to bind very different ligands could favor its nonspecific binding to many immobilized ligands used for separation and depletion of serum proteins by affinity chromatography [2]. The ligand molecules have different functional groups such as $-\text{SO}_3\text{H}$, $-\text{NH}_2$, NH , $-\text{COOH}$, $-\text{OH}$ and aromatic rings for ionic, polar and hydrophobic interactions. Agmatine is also a small molecular weight affinity ligand, and it is decarboxylated form of arginine. It offers several advantages over other ligands in terms of economy, stability and ease of immobilization [3]. In this paper, novel affinity beads based on fibrous-grafting and functionalization with a salt resistance affinity ligand were developed to separate and deplete serum albumin from human serum. The binding capacity of the affinity beads to serum albumin (SA) was determined using aqueous solution of SA in a batch system. Batch adsorption studies showed that the amount of adsorbed SA was found to be 156.7 mg/g at 25 °C. The maximum adsorption capacity for affinity beads was observed at around pH 5.5. Adsorption of SA onto affinity beads significantly increased with increasing temperature, and reached a value 177.8 mg/g beads at 45 °C. The equilibrium data were found to be well described by Langmuir model, while the kinetic data were well-fitted to the pseudo second order kinetic. The degree of the purity of SA was determined by using HPLC. Before and after adsorption, the peak areas of SA was used in the calculation of separated SA.

Keywords: Affinity beads, Agmatine, Adsorption, Separation, Serum albumin, Kinetics

Preparations and characterization of biomedical materials for controlled release of doxorubicin

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Abstract

Doxorubicin is a chemotherapeutic agent commonly used in the treatment of a wide range of different cancer types. The use of doxorubicin and its derivatives causes dilated cardiomyopathy and congestive heart failure due to the accumulation of the drug and results in doxorubicin-induced cardiotoxicity. In addition, doxorubicin has been used as a model drug in many drug release studies, and different hydrogel formulations have been used for developing these drug release systems. In the previously reported work, chitosan-gold particles or chitosan-polyvinyl alcohol conjugates were reported as drug delivery systems for controlled doxorubicin release with low toxicity to healthy tissues. Selective and targeted delivery of drugs is a major challenge for effective therapy of many diseases. An ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving a high drug loading for the required blood levels, immune to accidental release, simple to apply, and easy to fabricate [1-5]. The objective of the present study was to develop 2-hydroxypropyl methacrylate-co-polyethylene methacrylate hydrogels (with different formulations) that are able to efficiently entrap doxorubicin for the application of loco-regional control of the cancer disease. Systemic chemotherapy provides low clinical benefit while localised chemotherapy might provide a therapeutic advantage. These hydrogel networks with different compositions and doxorubicin loading were prepared for the construction of a controlled drug-release system. The hydrogel films were characterized by FTIR, SEM, swelling ratios, permeability, and contact angle studies. The effects of the hydrophilic polymer chain length of the macro-monomer, cross-linker density and the amount of loaded drug on the doxorubicin release were studied. The biocompatibility of the hydrogel formulations has been investigated using two measures: i) cytotoxicity test (using lactate dehydrogenase assay) and major serum proteins adsorption studies. Anti-tumor activity of the released doxorubicin was assessed using a human SNU398 human hepatocellular carcinoma cell line. It was observed that doxorubicin released from all of the hydrogel formulations remained biologically active and had the capability to kill the tested cancer cells.

Keywords: Controlled release, Biocompatible materials, Doxorubicin

Molecular characterisation of *Phytomonospora* sp. KT1403 isolate

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Abstract

Objectives: The family *Micromonosporaceae* was first described by Krasil'nikov (1938). The family is phylogenetically distinct, but encompasses a chemotaxonomically and morphologically diverse group of filamentous organisms. The genus *Phytomonospora* which belongs to this family described in 2011, and an aerobic and Gram-reaction-positive actinomycetes that forms branched substrate mycelium. In this study, it was made characterisation of a novel actinomycetes from compost soil sample from Turkish Republic of Northern Cyprus, using by molecular methods.

Material and Methods: The strain KT1403, isolated from SM3 medium (Gauze's medium), supplemented with cycloheximide (50 µg/ml), nalidixic acid (10 µg/ml), neomycin sulphate (50 µg/ml), novobiocin (10 µg/ml), and nystatin (50 µg/ml) incubated at 28°C for 30 days. Genomic DNA extraction and PCR-mediated amplification of the 16S rRNA gene were performed as described by Chun & Goodfellow (1995), using 27f and 1525r universal primers. Phylogenetic analysis was carried out by using three different algorithms. DNA-DNA hybridisation, whole cell sugars, polar lipids and isoprenoid quinones were determined using the described procedures by De Ley *et al.* (1970), Hasegawa *et al.* (1983), Minnikin *et al.* (1984) and Kroppenstedt (1982), respectively.

Results: The strain KT1403 shared highest 16S rRNA gene sequences similarity (99.73 %, 4 nucleotides differences) with *Phytomonospora endophytica* YIM 65646^T. DNA-DNA hybridisation value with strain KT1403 and related type species of *Phytomonospora endophytica* YIM 65646^T was 33.4 %. On the basis of a combination of phylogenetic, chemotaxonomic and morphological characteristics, strain KT1403 represents a novel species in the genus *Phytomonospora*.

Keywords: *Phytomonospora*, Molecular characterisation, 16S rRNA gene

Changes in Gene Expression Levels of Antioxidant Enzymes in Brain Tissues of Diabetic Rats: Effects of Resveratrol

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Abstract

Diabetes mellitus is a disease characterized by persistent hyperglycemia, which may lead to brain tissue damage due to oxidative stress and also contributes to neuronal death and changes in synaptic transmission. This study evaluated the effect of diabetes and the use of resveratrol supplementation on gene expression levels of antioxidant enzymes in the brains of chronic diabetic rats induced by streptozotocin. After induction of diabetes and resveratrol supplementation, gene expression levels of antioxidant enzymes were determined by Real Time PCR technology in the brain tissues. Accordingly, it was found that chronic effect of the diabetes led to the upregulation of superoxide dismutase (SOD1), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) gene expression in diabetes and after resveratrol supplementation, diabetic rats restored most of the the expression levels toward the control levels. Our results suggest that resveratrol supplementation reduces oxidative stress in the brain tissues of diabetic rats and also regulates the the gene expression levels of antioxidant enzymes which should be beneficial to individuals with diabetes/chronic hyperglycemia.

Keywords: Diabetes, Resveratrol, Brain Tissues, Gene Expression, Antioxidant Enzymes

Biological Evaluation of Grape Seeds from Wine Processing Industry Wastes and Chemical Characterization

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Abstract

Vitis L. is a genus of Vitaceae with about 60 species of agricultural and economical importance. Wine industry causes annually more than 5 M tons of waste including grape seeds. The waste contains many natural products including polyphenols and fatty acids. The purpose of this study was to evaluate the antibacterial, antioxidant and lipoxygenase of fixed oil acquired from grape seed as a result of industrial production process of wine industry from Nevşehir, Turkey. The air dried seeds were subjected to *n*-hexane extraction using Soxhlet apparatus. The resulting extract was methylated with BF₃/MeOH, which was analysed by GC and GC/MS, simultaneously. As a result, 11 fatty acids were detected representing 99.9% of the analysed material. The major constituents were identified as linoleic acid (66.5%), oleic acid (18%) and palmitic acid (9.1%), respectively. In addition, the extract was subjected to *in vitro* antioxidant activity evaluation by the β -caroten bleaching and ABTS scavenging methods, spectrophotometric *in vitro* enzyme lipoxygenase inhibition and antibacterial activities using the CLSI microdilution method. Antibacterial activity against was evaluated against the pathogens *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhimurium*, *Bacillus cereus*, *Agrobacterium tumefaciens*, *Pseudomonas citronellosis*, *Bacillus velezensis*. The biological evaluation results of *Vitis* sp. L. semen fixed oil were that the lipoxygenase inhibition and antioxidant activity was not observed at the tested concentration (20 mg/mL) when compared to standard compounds. The most prominent antibacterial inhibition was observed against *S. typhimurium* at MIC: <160 μ g/mL. As a conclusion the fixed oil can be used as sustainable antibacterial source.

Keywords: Waste Grape Seeds, Soxhlet Extraction, Fatty Acid Composition, GC and GC/MS, Antibacterial Activity

Molecular detection of gastrointestinal viruses in patients with acute gastroenteritis

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Abstract

Introduction and Aim: Viral gastroenteritis is a common cause of morbidity and mortality in humans worldwide, affecting all age groups. Viral pathogens causing acute gastroenteritis include rotavirus (RV), norovirus (NoV), sapovirus (SaV), adenovirus (HAdV), astrovirus (HAstV) and bocavirus (BoV). We aimed gastrointestinal viruses in patients with acute gastroenteritis using polymerase chain reaction (PCR) assays.

Material and Methods; During the period January 2013 to December 2013, a total of 275 patients who were admitted to the Medical Microbiology Laboratory of Aydın Adnan Menderes University Medicine Faculty and Kocaeli Derince Training and Research Hospital and were included in the study. The stool samples from patients with acute gastroenteritis were examined for the presence of rotavirus, norovirus, astrovirus, and adenovirus using multiplex PCR assay. Bocavirus is studied by RT-PCR.

Results: Gastrointestinal viral agents were detected in 69 (25.1 %) patients of the 275 analyzed. Of these, 62 (22%) patients had rotavirus 6 (2%) in patients with norovirus , 2 (0.7%) patients were positive for bocavirus. Adenovirus was not detected. One patient was positive for rotavirus and norovirus at the same time.

Conclusion: In all cases with acute gastroenteritis, rotavirus positivity was found to be more frequent than other gastroenteritis viruses.

Keywords: gastroenteritis, rotavirus, norovirus, bocavirus

A Study On Bacteriologic, Physicochemical And Melissopallinologic Analysis Of Commercial Honeys From Turkey

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Abstract

In this study, bacteriological, physicochemical and melissopallinological contents of commercial honey in Turkey was investigated.

Total mesophilic, coliform, *Bacillus* screening has been done to determine the bacteriological contents of 18 commercial honeys. PCA for total mesophilic bacteria, VRB agar for coliform bacteria and J-agar has been used for *Bacillus* screening. The physicochemical tests; HMF, humidity, ash, total protein, pH, diastase, total acidity, electrical conductivity, briks, color, invert sugar. Melissopallinological analysis was conducted with 24x24 mm coverslip.

As a results total mesophilic bacteria count $2,54 \times 10^5$ - $1,14 \times 10^2$ cfu/g, bacteria count of *Bacillus* genus $8,25 \times 10^3$ - 1×10^2 cfu/g was found in the range. Coliform weren't detected in any of samples. Physicochemical analysis results are as follows; HMF 3,13-88,84; humidity %14,6-17,7; ash %0,06-0,74; total protein %0,13-0,19; pH 3,38-4,68; diastase 29,29-433,33; total acidity 22,67-49,22 meq/kg; electrical conductivity 0,24-1,43 mS/cm; briks %80,57-83,57; color L 6,85-21,61, a 2,01-13,43, b 2,88-13,83; invert sugar %53,59-71,52. Pollen diversity 13-25, pollen count 1223-7832 was found with melissopallinological analysis. The dominant pollens are in *Centaurea*, *Leguminosae*, *Compositae*, *Cruciferae*, *Labiatae*, *Astragalus* ve *Ericaceae* family.

The results obtained in this study is a important record for differences in types of bacteria in Turkish honey. In addition, contrary to the expectations of our country honeys compared to the world honeys are higher bacterial load. According to the physicochemical properties of processing and storage conditions should be improved. Honey isn't rich in terms of pollen.

Keywords: Honey, Bacteriological, Physicochemical and Melissopallinological analysis

Acknowledgment: The authors would like to thank to the scientific research council of Pamukkale University, Turkey, for the research grant 2012FBE025.

Effects of Potassium Humate and Different Concentration of MS Medium in *Brassica napus* L. cv. Licosmos on Shoot Regeneration and Some Physiological Parameters

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Abstract

In this study, rapeseed (*Brassica napus* L.) cultivars Licosmos plant used. Doses of various MS medium, MS ½ and MS ¼ and 25, 50 and 100 ppm potassium humate concentrations were used. Germination increased in MS 0 medium with increasing doses of potassium humate. 100 ppm potassium humate dose decreased the root length showing adverse effects in the MS ¼ medium. 25 ppm potassium humate increased in the stem length in MS ¼ medium. With increase of concentration of the fresh weight increase was observed in MS 0 medium. 100 ppm had a negative impact on MS ½ medium. 25 ppm potassium humate increased and positive impact in the MS ¼ medium fresh weight but 50 and 100 ppm potassium humate was observed a significant decrease in fresh weight. 25 ppm potassium humate decrease in the total chlorophyll content and chlorophyll a an amount in MS 0 medium. Chlorophyll a has not been a significant change in the MS ½ medium. MS ¼ medium in a concentration of 25 ppm potassium humate increased in the amount of chlorophyll and total chlorophyll. 25 ppm also negative effects caused on in MS 0 medium and have decreased in amount of chlorophyll b. 25 ppm potassium humate also positive impact on MS ¼ and MS ½ medium dose of increased the amount of chlorophyll b. 25 ppm potassium humate decreased the amount of protein in the MS 0 medium. 25 and 50 ppm potassium humate has increased the amount of protein in MS ¼ medium. SOD activity increased with raising doses of potassium humate.

Keywords: Antioxidant enzyme, *Brassica napus*, potassium humate, photosynthetic pigments

Determination of Phyteremediation Capacity of Mercury in Linum (*Linum usitatissimum* L. cv. VERNE) via *In Vitro* Culture

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Abstract

In this study that it was determined that phyteremediation capacity of mercury 15, 30, 45 and 60 ppm doses on linum (*Linum usitatissimum* L. cv. Verne) via in vitro culture. Seeds were sowed in holder what dimension was 13 × 19 × 4,5 in 22-23 °C temperature. Sowing had been monitored seeding rates as two week. At the end of the two week, stem and root lengths of seedlings was measured by millimeter scale, dry/fresh gravity were measured, Spectrophotometer analysis of photosynthetic pigments were also measured. As a result the plant germination showed a significant decrease in 30 ppm .All the mercury concentration on the root and stem lengths was caused a significant decrease in (P<0,05). 45 ppm was effective in fresh and dry weights of plant and has been extremely important in weight reduction.

Chlorophyll a, total chlorophyll, total carotenoids amounts to 15 ppm, 30 ppm, 45 ppm, no adverse effects were observed at concentrations. In addition, 60 ppm in the disappearance of the yield strength of the plant significantly reduced amounts of photosynthetic pigments. Chlorophyll b was not significant changes in all concentrations. Phytoremediation of mercury capacity of flax was determined till 30 ppm.

Keywords: Germiation , Linum, Mercury , Phyteremediation

Investigation of *In Vitro* and *In Vivo* Antiproliferative Activity of A New Coordination Compound Containing $\text{Ag}^{\text{I}}(\text{CN})_2$ On Some Cancer Cell Lines

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Abstract

Background: Definitive treatment for cancer is not possible, however, the discovery of novel antiproliferative agents is needed. Coordination compounds have provides exciting the development of metal-based therapeutics. We have been exploring the antiproliferative and apoptotic effect of newly synthesized cyano-bridged $\{\text{Ag}^{\text{I}}(\text{CN})_2\}$ coordination compound, coded as AN12 ($\text{NiC}_{17}\text{H}_{32}\text{N}_9\text{O}_4\text{Ag}_3$), against on HeLa, C6 and HT29 cancer cell lines.

Materials and Methods: The new coordination compound containing $\text{Ag}^{\text{I}}(\text{CN})_2$ was synthesized using "brick-mortar" method [1]. *In vivo* cytotoxicity of AN12 was evaluated by lactate dehydrogenase assay (LDH assay) against on cancer cell lines. The antiproliferative activity of AN12 was assessed against cancer cell lines using BrdU Cell Proliferation Assay (BCPA), 5-fluorouracil (5-FU) was used as a reference standard. DNA laddering assay and topoisomerase I assay were used to determine whether this compound induce cell apoptosis.

Results: According to BCPA and LDH test results, this coordination compound was inhibited the cell viability of cancer cells compared to positive control anticancer drug, 5-fluorouracil (5-FU). Remarkably, the LDH test results disclosed that AN12 was significantly cytotoxic than 5-FU, suggesting that this compound may affect by lose membrane integrity of cell as a result of [apoptosis](#). Furthermore, the compound AN12 caused laddering of genomic DNA, indicating that it may act through inducing apoptosis on the cells.

Conclusion: The results of the study suggest that the AN12 may have potential to be used as promising antiproliferative agent.

Keywords: Coordination Complexes, Anticancer Activity, AN12

A Novel Coordination Compound Containing Au^I(CN)₂ Displays Anticancer Ability On HT29, HeLa and C6 Cancer Cells Lines *In Vitro* and *In Vivo*

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Abstract

Objectives: We have been exploring the antiproliferative and apoptotic effect of newly synthesized cyano-bridged {Au^I(CN)₂} coordination compound, coded as AK9b (NiC₁₄H₃₂N₆O₄Au), against on HeLa, C6 and HT29 cancer cell lines.

Methods: The new coordination compound containing Au^I(CN)₂ was synthesized using "brick-mortar" method [1]. *In vivo* cytotoxicity of AK9b was evaluated by lactate dehydrogenase assay (LDH assay) against on cancer cell lines. The antiproliferative activity of AK9b was assessed against cancer cell lines using BrdU Cell Proliferation Assay (BCPA), 5-fluorouracil (5-FU) was used as a reference standard. DNA laddering assay and topoisomerase I assay were used to determine whether this compound induce cell apoptosis.

Results: According to BCPA and LDH test results, this coordination compound was inhibited the cell viability of cancer cells compared to positive control anticancer drug, 5-fluorouracil (5-FU). Remarkably, the LDH test results disclosed that AK9b was significantly cytotoxic than 5-FU, suggesting that this compound may affect by lose membrane integrity of cell as a result of apoptosis. Furthermore, the compound AK9b caused laddering of genomic DNA, indicating that it may act through inducing apoptosis on the cells.

Conclusion: These results demonstrate that the AK9b is a potential therapeutic agent for cancer cell lines.

Keywords: Coordination Complexes, Anticancer Activity, AK9b

Antimicrobial Activity of Various Extracts of *Ocimum basilicum* L. and by Using of Scanning Electron Microscopy, Observation of the Inhibitory Effect on Bacterial Cells

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Abstract

The antimicrobial activities of chloroform, acetone and two different concentrations of methanol extracts of *Ocimum basilicum* L. were studied. These extracts were tested *in vitro* against 10 bacteria and 4 yeasts strains by the disc diffusion method. The results indicated that the methanol extracts of *O. basilicum* exhibited the antimicrobial activity against tested microorganisms. While the chloroform and acetone extracts had no effect, the methanol extracts showed inhibition zones against strains of *Pseudomonas aeruginosa*, *Shigella sp.*, *Listeria monocytogenes*, *Staphylococcus aureus* and two different strains of *Escherichia coli*. The cells of microorganisms, which were treated and untreated with plant extracts, were observed by using the scanning electron microscope. It was observed that the treated cells were damaged.

Keywords: Antimicrobial activity, Disc diffusion method, *Ocimum basilicum*, Plant extract, SEM, Spices

Carbonic Anhydrase Isoenzymes I and II Inhibitory Effects of Bromophenol Derivatives Including Cyclopropane

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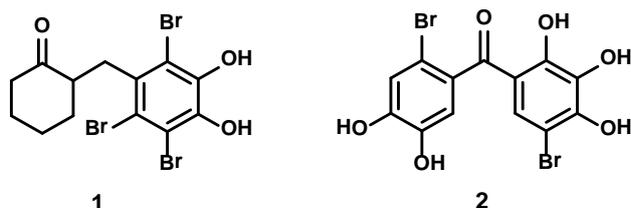
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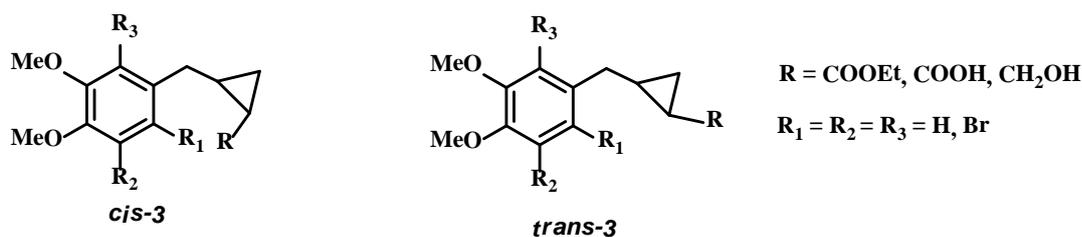
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Abstract

Carbonic anhydrases (CAs, E.C.4.2.1.1) are metalloenzymes that is a member of the family, present in most living organisms. It classically participates in the maintenance of pH homeostasis in human body, catalysing the reversible hydration of CO₂ in a two-step reaction to yield bicarbonate and protons.^{1,2} Many bromophenol compounds are natural products, and they have important biological activities. Molecules **1** and **2** shown below exhibit carbonic anhydrase inhibition effects.^{3,4}



As far as we know carbonic anhydrase inhibition effects of bromophenol compounds including cyclopropane ring or their derivatives have not investigated. To investigate such molecules, phenol and bromophenol derivatives with cyclopropane as *cis-3* and *trans-3* were synthesized. Carbonic anhydrase inhibitory effects of all the synthesized products have been investigated.



Keywords: Bromophenol; Cyclopropane; carbonic anhydrase; inhibitor; inhibition

Optimization of *Aspergillus terreus* Xylanase Production by Using Response Surface Methodology

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Abstract

Fossil fuel sources are limited and nowadays, they have almost come to an end. Therefore, These findings resulted in an increase on researches to find new and sustainable energy resources. One of resources belonging to renewable energy sources is waste of agricultural residues that they have been considered as environmental pollution previously. Within the scope of biorafinery concept, the lignocellulosic structure of agricultural residues used in bioethanol production at one hand, while the other hand, it can be used to produce high value-added materials. For this purpose, firstly cellulase to hydrolyse the cellulose after that for the degradation of hemicellulose xylanase take roles to breakdown the lignocellulosic material for its complete usage. *Aspergillus terreus* is one of the molds used in industry for production of high value-added product, itaconic acid. In our laboratory, previous studies showed that it has a capacity for xylanase production. In this research, by using response surface methodology its xylanase production increased almost 60%.

Keywords: Xylanase; Response Surface Methodology; Biorafinery

Effect of Leaf Rolling on Gene Expression in Plants under Drought Stress

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Abstract

In order to determine the effects of leaf rolling on expression of genes in plants under drought stress, maize (*Zea mays* L.) seedlings were exposed to 10% PEG-6000 for one day. Leaf rolling was prevented by hanging transparent wires to some of drought stressed seedlings. Total RNA was isolated from control leaves, rolled leaves and rolling prevented leaves then, microarray analysis was performed. According to the microarray results, some of genes were upregulated by leaf rolling while some were found to downregulate. For example, in the leaf rolling group expression level of *dehydrin1* gene was 284 times, *galactinol synthase1* gene was 34 times, *beta-amylase* was 33 times, *heat shock factor protein1* was 29 times, *dehydrin13* was 22 times and *metallothionein-like protein type2* was 20 times higher than the control group. Interestingly, expression levels of same genes were downregulated by prevention of leaf rolling. In addition, down regulation of some gene expression was also determined in the rolled leaves as compared to the control. For instance, *benzoxazine6* was 30 times, *sucrose phosphate synthase1* was 30 times, *chlorophyll a-b binding protein2* was 19 times, and *12-oxo-phytodienoic acid reductase6* was 16 times lower than the control. Expressions of the same genes increased by prevention of leaf rolling. Consequently, We concluded that the genes may play a role in the regulation of the leaf rolling.

Keywords: Drought, Maize, Microarray, Leaf rolling

Effect of Hydrogen Peroxide on Osmoregulation in Plants under Drought Stress

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Abstract

Maize (*Zea mays* L.) seedlings grown to four-leaf stage were treated with 10 mM hydrogen peroxide (H₂O₂) for 6 hours then exposed to 3% PEG-6000 for 12 hours to determine effect of H₂O₂ on osmoregulation in the seedlings under drought stress. Immediately after the application, measurements of leaf water potential, stomatal conductance, lipid peroxidation and osmolyte content (proline, total soluble sugars and polyamine) were done. Total RNA was isolated for microarray analysis. As compared to untreated seedlings, maintained leaf water content, increased stomatal conductance and reduced lipid peroxidation were determined in H₂O₂-treated seedlings under drought stress. In addition, contents of proline, total soluble sugars and polyamines (putrescine, spermine and spermidine) were found to increase by H₂O₂ treatment in the seedlings under drought stress. H₂O₂ pretreatment also upregulated some genes related to osmolyte synthesis under drought stress compared to untreated group. For example, by H₂O₂ treatment, *sucrose synthase 2.1* fold, *sucrose synthase 2.5* fold, the *UDP-glucose-6-dehydrogenase* 1.4 fold, *hexokinase 2*, 1.7 fold, *beta amylase 5* 2.6 fold and *spermidine synthase 1* 2.2 fold increased under drought stress as compared to untreated group. Our results showed that H₂O₂ stimulated osmolyte accumulation by upregulating genes in osmolyte synthesis thus, osmoregulation was provided then, membrane damage was reduced, stomatal closure was ensured and leaves retained their water contents under drought stress.

Keywords: Drought, Hydrogen peroxide, Maize, Microarray, Osmoregulation

Investigation of the Role of MTHFR A1298C Gene Polymorphism in Colorectal Cancer

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Abstract

Objective: Colorectal cancer (CRC) defined as the cancerous growths in the colon or rectum. CRC is one of the major causes of mortality and morbidity that occurs by genetic and/or nutritional factors. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism. It irreversibly converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. 5-methyltetrahydrofolate provides the methyl group for methylation of homocysteine to methionine. Imbalanced DNA methylation has been implicated in colorectal carcinogenesis. A1298C gene polymorphism in exon 7 of MTHFR, resulting in a substitution of glutamate with alanine at codon 429, reduces the activity of MTHFR enzyme and so may contribute to CRC disease.

Material and Methods: The study population included colorectal cancer patients group (71 patient), and control group (82 control). DNA was isolated from peripheral blood, containing EDTA, by Invitrogen blood DNA kits. DNA purity and quantity were assessed in spectrophotometer and checked by 0.8% agarose gel electrophoresis. To determine the MTHFR A1298C gene polymorphism polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods were used.

Results: The MTHFR A1298C genotype distribution in patient group with colorectal cancer (AA=%47,89, AC=%42,25, CC=%9,86) did not differ from those in control group (AA=%39,02, AC=%52,44, CC=%8,54).

Conclusion: This study shows that MTHFR A1298C gene polymorphism was not genetic risk factors for colorectal cancer.

Keywords: CRC, MTHFR A1298C gene polymorphism, PCR, RFLP

Computational Modelling of Tyrosine 444 and Tyrosine 407 Mutation for Human MAO A

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Abstract

Monomine oxidases are flavoenzymes that catalyze the oxidation of various amine neurotransmitters, such as serotonin, dopamin and norepinephrine. There are two isozymic forms, MAO A and MAO B. The compounds that selectively inhibit MAO A exhibit antidepressant activity, whereas the ones that selectively inhibit MAO B are used in the treatment of Parkinson's disease [1, 2]. In the active site of MAO A and MAO B, there are two tyrosyl residues (Tyr407, Tyr444 and Tyr398, Tyr435, respectively) forming an "aromatic cage". Li et al., reported that Tyr435 MAO B mutants exhibit lower activities in comparison to the wild type enzyme [3]. The similar behavior was also observed with MAO A. Our previous ONIOM calculations on MAO B and Tyr435 mutants revealed that aromatic cage play a role in enhancing the nucleophilicity of the amine, and hence increasing the reactivity of amine towards flavin [4]. The purpose of the present study is to extend these calculations to MAO A to further investigate the role of the aromatic cage in catalysis. ONIOM (HF/6-31G*:PM3:UFF) calculations were performed on the wild type human MAO A and six mutant enzymes, Tyr444Phe, Tyr444His, Tyr444Leu, Tyr444Tryp, Tyr407Phe and Tyr407Leu. Alterations in the energies, structural and electronic properties of the parallel and perpendicular benzylamine conformations have been investigated as they travel in the active site along the path towards flavin. The results indicated that the aromatic cage in MAO A enhances the amine nucleophilicity of the substrate such that Tyr407 exhibits a greater effect than Tyr444.

Keywords: MAO, ONIOM calculations, oxidation mechanisms, enzyme modelling, flavo enzymes

The Effect of Tissue Water and Growth Regulators Contents on *In Vitro* Shoot Regeneration Capacity

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Abstract

The water content of an actively growing plant can be as much as 95% of its live weight. A plant requires water as an essential ingredient of photolysis, the photochemical stage of photosynthesis where water is split using light energy. Neither carbon dioxide nor oxygen required for photosynthesis is usable by plant unless it is in solution in water. Therefore, water is the key to plant's survival and growth. Water is also an excellent solvent. The substances (solutes) that become dissolved in water in plants include mineral ions such as potassium (K⁺), sugars (glucose and sucrose), and amino acids, main components of proteins. The reduction in growth, yield and quality by water stress has been well recognized in field conditions. This study showed that enriching tissue water and growth regulators contents provides cells with a high regeneration capacity and consequently increasing explant's culture response.

Keywords: Water, growth regulators, regeneration

Investigation of Antibacterial Property of Pennyroyal (*Mentha pulegium* L.) Plant

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Abstract

In this research, the effect of extracts derived from leaves of pennyroyal (*Mentha pulegium* L.) plant under *in vitro* conditions on bacteria strains '*Eschericia coli* ATTC 25922', '*Entereococcus faecalis* ATCC 29212', '*Staphylococcus aureus* ATCC 19213' and '*Staphylococcus epidermidis* ATCC 12228'. Plant dry leaves were grinded in a mortar and were kept in methanol and acetone over night. Then, extracts were harvested by evaporating methanol and acetone in evaporator. The effects of extract on bacteria were observed by using extract-absorbed-discs via disc diffusion method. Empty antibiotic discs were used as negative control. Amoksisilin – Klavunat, Klindamisin and Tetrasiklin antibiotic discs were evaluated as positive control. It was determined that acetone-derived extract was more effective than the one of methanol. Extract was observed to be more effective on '*Staphylococcus epidermidis*'.

Keywords: Pennyroyal, plant extract, antibacterial effect

Anti-cancer and anticholinesterase activities on different parts of *Hypericum amblysepalum*

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Abstract

Cancer is a grand health problem in both developed and developing countries. Since many years, plants were known to have anticancer activities against different cancer cell lines. In this study, we have reported a study based on anticancer and anticholinesterase properties of flower, fruit and seed parts of *Hypericum amblysepalum*. The parts of shade dried plant were extracted with methanol. Anticancer activities were determined with standard MTT colorimetric procedure against HeLa and NRK-52E cell lines. From the analysis it was found that fruits of *H. amblysepalum* showed 4.12% and 4.39% inhibition activities against to HeLa and NRK-52E cell at the concentration of 90 mg/ml respectively. The same values for seeds were 11.67% and 2.86% at 100 mg/ml. On the other hand flowers of *H. amblysepalum* did not display anticancer activities against HeLa and NRK-52E cancer cell line. *H. amblysepalum* flower, fruit and seeds methanol extracts were screened for their acetyl and butyryl-cholinesterase inhibitory activity by using *in vitro* Ellman method at 200 µg/ml concentration. Galanthamine (87.08%) was used as standard inhibitor (positive control). Only seed of *H. amblysepalum* showed 20.39% inhibition against acetylcholinesterase (AChE). However flower, fruit and seed extracts exhibited 53.67, 38.63 and 76.89% inhibition against butyrylcholinesterase (BChE) respectively.

Keywords: *Hypericum amblysepalum*, MTT assay, HeLa, NRK-52E, anticholinesterase

The Impact of Resveratrol on Fructose-Induced Vascular Insulin Resistance in Female Rats

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Abstract

Objective: The present study aims to evaluate the levels of endothelial function, insulin receptor substrate (IRS-2) and endothelial nitric oxide synthase (eNOS) gene expression and to determine the effects of resveratrol supplementation on these parameters.

Methods: Four-week-old female Wistar rats were allocated into four groups: Control, resveratrol, fructose and fructose combined with resveratrol. The fructose group received 10% fructose in drinking water for four months while the resveratrol group was fed with standard chow pellet that was fortified with resveratrol at a dose of 0.05 g per kilogram. The metabolic and vascular functions of the recruited rats were assessed by the examination of aortic tissue and arterial blood specimens. Endothelial functions were evaluated by the utilization of force-displacement transducer on aortic tissues. Real Time-PCR was used to measure the aortic concentrations of IRS-2 and eNOS gene expression.

Results: Body weight, plasma insulin concentration and serum triglyceride level increased significantly while the levels of IRS-2, eNOS gene expression within the aortic tissues decreased significantly in the fructose group. Moreover, the endothelium-dependent relaxation response of acetylcholine (10^{-9} - 10^{-4} M) was significantly decreased in the fructose group. When resveratrol supplementation was added, the levels of IRS-2, eNOS gene expression and the endothelium-dependent relaxation response of acetylcholine (10^{-9} - 10^{-4} M) within the aortic tissues were increased significantly.

Conclusion: Long-term fructose consumption induced endothelial dysfunction in female Wistar rats by means of attenuating IRS-2/eNOS mRNA expression. Resveratrol supplementation improves the endothelial functions and the triggers the up-regulation of associated genes in rats fed with fructose. The dramatic recovery of the fructose-induced metabolic and vascular dysfunction after resveratrol supplementation may be of importance for the individuals who are exposed to hazardous effects of sweetened beverages.

Keywords: Fructose, endothelial dysfunction, eNOS, IRS-2, resveratrol

The Impact of Resveratrol on Fructose-Induced Metabolic and Hepatic Dysfunction in Female Rats

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Abstract

Objective: The present study aims to investigate the hepatic insulin signaling pathway including insulin receptor (IR), insulin receptor substrate-1 (IRS-1), insulin receptor substrate-2 (IRS-2) in a model of metabolic syndrome induced by high-fructose fed female rats. In accordance, plasma insulin and hepatic triglyceride levels, as well liver IR, IRS-1 and IRS-2 mRNA expressions were determined. We also examined the impact of resveratrol on the alterations induced by fructose consumption.

Methods: Four-week-old female Wistar rats were divided into four groups: Control, resveratrol, fructose and fructose combined with resveratrol. The fructose was administrated to rats in drinking water, as 10% (w/v) solution, for four? six months. Rats on a resveratrol supplemented diet were fed with standard chow pellet that was fortified with resveratrol at a dose of 0.05 g per kilogram. The metabolic functions of the recruited rats were assessed by the examination of hepatic tissue and blood specimens. Real Time-PCR was utilized to specify the levels of IR, IRS-1 and IRS-2 gene expression.

Results: Increased body weight, plasma insulin and hepatic triglyceride were associated with decreased IR and IRS-2 mRNA levels in the liver of fructose fed rats. Resveratrol supplementation significantly restored the abnormalities in IR and IRS-2 gene expressions. However, the level of IRS-1 gene expression was not altered by either fructose or resveratrol treatment.

Conclusion: Here, hepatic triglyceride accumulation induced by high fructose consumption is positively correlated with the attenuation of hepatic IR, and IRS-2 expression. Resveratrol improves these irregularities, that may attribute to IR and IRS-2 up-regulation. Resveratrol supplementation could offer an alternative way to prevention of metabolic syndrome.

Keywords: Fructose, liver, IR, IRS-1, IRS-2, resveratrol

Apoptotic and Anti-Angiogenic Effects of Acorus Calamus Root Extract on Prostat Cancer Cell Line

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Abstract

Background / Aim: Acorus calamus have long been considered to have anti-carcinogenic and medicinal properties especially in Asia. We examined whether ethanolic extract of acorus calamus root affects the survival of prostate cancer LNCaP cells and induces apoptosis and angiogenesis of these cells in vitro.

Materials and Methods: Cells were incubated during 24 and 48 hours with different concentrations of extract to determine the effect of the extract on the cells proliferation. Three extract doses selected and cleaved poly-(ADP-ribose) polymerase (PARP), vascular endothelial growth factor-A (VEGF-A) protein and gene expression amounts determined.

Results: Extract with these concentrations reduced the number of LNCaP living cells up to 44 % as compared to the control at dose and time dependent manner at 24 and 48 hours. Meanwhile, significantly increased by 180% had been observed in cleaved PARP after 24 hours at 750 µg/ml extract concentration, and after 48 hours cleaved PARP levels at 500 and 750 µg/ml extract concentrations were found %198 and %244, respectively. After 48 hours VEGF-A protein and gene expression amounts at 750 µg/ml extract concentrations were found 142 pg/ml and 6%, respectively.

Conclusion: The present study reveals the possibility that ethanolic extract of acorus calamus root possesses a dose and time dependent anticancer, apoptotic and anti-angiogenic properties.

How To Access Forest Fires On Time To Minimize Environmental Damages?: A Case of Erbil, Iraq

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Abstract

In effectively fighting against forest fires, initiation of fire extinguishing activities on time and especially arrival of ground team into the fire zone in the shortest time period is very important. Computer assisted methods has been widely used in solving the transportation problems requiring the determination of the shortest path. In this study, network analysis based Geographic Information Systems (GIS) techniques were used to determine the optimum path providing the transportation to the fire zone at the shortest time. This system was applied for 10 different potential fire zones by considering 9 firefighting teams located in the city of Erbil, Iraq. The results indicated that it is necessary to establish new fire headquarters as an addition to current headquarters. Besides, extending road network in the study area by building new roads and increasing transportation speed by improving standards of existing roads can provide solution to the problem.

Keywords: Forest Fires, Fire Fighting Teams, GIS, Network Analysis, Shortest Path, Safest Route

mRNA Expressions of FOXO1, FOXO3*1, FOXO3*2 and FOXO4*1 Genes in multiple sclerosis

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Abstract

Introduction And Aim:

Multiple sclerosis (MS), is a chronic disease characterized by demyelination and axonal damage. There have been many studies on multiple sclerosis disease. However, in spite of all these studies, the exact cause of MS is unknown. In our study, we investigated the relationship between FOXO1, FOXO3*1, FOXO3*2 and FOXO4*1 a member of FOXO (Homo sapiens forkhead box O) gene family which has effect on growth and differentiation of myogenic that belongs to Forkhead Family and MS disease. We analyzed FOXO gene expression levels using real-time PCR.

Material And Methods:

mRNA was measured in peripheral blood samples of 95 patients with MS and 95 healthy individuals without a known neurodegenerative disease and compared with expression of FOXO genes in both groups by Fluidigm quantitative RT-PCR Array. The data obtained were analyzed with Mann–Whitney U test, after GAPDH and Beta – Actin normalization and calculation of the ratios of the results of patients and healthy controls.

Finding And Discussion:

According to the results of analysis there is no difference between FOXO1, FOXO3*1, FOXO3*2 and FOXO4*1 rates in patients with multiple sclerosis and healthy control group ($p>0,05$). These findings suggest that it would be important to investigate the necessity of the continuation of the treatment and diagnosis of MS further molecular studies and other genes in charge of the suppressing demyelination and axonal damage.

Keywords: multiple sclerosis, RT-PCR, FOXO Genes

The Determination of Genotoxicity Using RAPD Analysis in *Pseudevernia furfuracea* (L.) Zopf Species Exposed to Heavy Metal Pollution in Kırıkkale City

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Abstract

Biological systems, especially plants and lichens are also used effectively and successfully to detect the atmospheric pollution in beside instrumental methods. In depth analysis by addition of molecular biological techniques to environmental pollution studies with lichen, provide not only information about the degree of pollution in the subject area but also provide the identification of the extent of pollution levels directly from the genetic structure of living organisms.

Effects of environmental pollution on DNA was investigated by RAPD analysis in lichen species *Pseudevernia furfuracea* (L.) Zopf thalli collected from Kırıkkale City. Lichen samples were transplanted to the different pollution sources in province of Kırıkkale for two time periods of the year. Ten decamer primers were used, and polymorphism was calculated in relation to the appearance of new bands and disappearance of normal bands considering the control's band patterns. RAPD polymorphism rates obtained from two periods have shown significant differences.

Keywords: Lichen, RAPD, heavy metal pollution, genotoxic effect, *Pseudevernia furfuracea*

The Determination of Genotoxicity Using RAPD Analysis in *Pseudevernia furfuracea* (L.) Zopf Species Exposed to Heavy Metal Pollution in Ankara City

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Abstract

Heavy metals have a great impact on environmental pollution because of their long term and destructive effects on organisms. Lichens are used effectively and successfully to detect the atmospheric pollution in beside instrumental methods. Analysis by addition of molecular biological techniques to environmental pollution studies with lichen, provide the identification of the extent of pollution levels directly from the genetic structure of living organisms.

In this study, we aimed to describe the DNA changes in *Pseudevernia furfuracea* (L.) Zopf samples exposed to pollution in Ankara City by RAPD (random amplified polymorphic DNA) analysis in order to reveal the pattern of genetic variation influenced by the environmental pollution.

Lichen samples were transplanted to the different pollution sources in province of Ankara for two time periods of the year. Ten decamer primers were used, and polymorphism was calculated in relation to the appearance of new bands and disappearance of normal bands considering the control's band patterns. RAPD polymorphism rates obtained from two periods have shown significant differences.

Keywords: Lichen, RAPD, heavy metal pollution, genotoxic effect, *Pseudevernia furfuracea*

The Determination of Genotoxicity Using RAPD Analysis in *Pseudevernia furfuracea* (L.) Zopf Species Exposed to Heavy Metal Pollution in Çorum City

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Abstract

Heavy metals are classified among the most dangerous groups of environmental pollutants due to their toxicity and persistence in the environment. Lichens have effectively been used as biomonitors of metal contamination in the atmosphere. Analysis by addition of molecular biological techniques to environmental pollution studies with lichen, provide the identification of the extent of pollution levels directly from the genetic structure of living organisms.

Effects of environmental pollution on DNA was investigated by RAPD analysis in lichen species *Pseudevernia furfuracea* (L.) Zopf thalli collected from around the Çorum City. Lichen samples were transplanted to the different pollution sources in province of Çorum for two time periods of the year. Specimens were analysed using RAPD to detect the probable pollution effects and changes on DNA molecules. Polymorphism was calculated in relation to the appearance of new bands and disappearance of normal bands considering the band patterns of the control samples. RAPD polymorphism rates obtained from two periods have shown significant differences.

Keywords: Lichen, RAPD, heavy metal pollution, genotoxic effect, *Pseudevernia furfuracea*

The *in vitro* Genotoxic Effects of 1-Naphthaleneacetamide in Human Peripheral Blood Lymphocytes

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Abstract

1-Naphthaleneacetamide (NAAm) is a synthetic plant growth regulator in the auxin family that has been used for several decades in agriculture to promote the growth of numerous fruits, for root cuttings and as a fruit thinning agent.

The potential genotoxic effects of NAAm on human peripheral blood lymphocytes were investigated *in vitro* by the chromosome aberrations (CAs), and cytokinesis-block micronucleus (MN) assays. The human lymphocytes were treated with 20, 40, 80, and 160 µg/mL of NAAm for 24 and 48 hr. NAAm increased the structural CAs and the percentage of micronucleated binuclear cells (MNBN %) significantly for all concentrations (20, 40, 80 and 160 µg/mL) and treatment periods (24 and 48 hr) when compared with the negative and the solvent control. In the both 24 and 48 hr treated cultures, NAAm was also found to significantly induce the nuclear bud formation in the binuclear cells when compared with the only negative control at 80 µg/mL, and compared to the negative and the solvent control at the highest concentration, 160 µg/mL. At all the tested concentrations, NAAm caused a statistically significant reduction in the mitotic index (MI) only for 48 hr treatment period and also in the nuclear division index (NDI) for both 24 and 48 hr treatment periods as compared to the control groups. The MI and NDI were reduced by NAAm in a concentration-dependent manner during both treatment times.

In conclusion, results of both genotoxicity assays indicate that NAAm was genotoxic and cytotoxic/cytostatic on human peripheral blood lymphocytes *in vitro*.

This study was funded by Mustafa Kemal University Research Fund (project code: 281).

Keywords: 1-Naphthaleneacetamide; human peripheral blood lymphocytes; chromosome aberration; micronucleus

Cloning, Expression and Zymogram Analysis of a β -class Carbonic Anhydrase from *Enterobacter asburiae*

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Abstract

Carbonic anhydrases (CAs) [EC4.2.1.1] are zinc containing metalloenzymes that catalyse the reversible hydration of CO₂ to HCO₃⁻ and H⁺ and have an essential role in the regulation of CaCO₃ mineralization. Bacterial mineralization of calcium carbonates has found applications in the remediation (fixation) of metal contaminated soil and groundwater, atmospheric CO₂ sequestration, strengthening and consolidation of sand, limestone monument repairs, reduction of permeability of cement mortar, pores and cracks filling in concrete and enhancing the strength of ash bricks. A β -class CA encoded by *can* gene (also known as CA 2) of *Enterobacter asburiae* (*canEas*), which was isolated by our laboratory was cloned, sequenced and overexpressed. CA activity was determined in Native-PAGE and zymography staining of the esterase activity was performed on SDS-PAGE (15 %) with α -naphthyl acetate (α -NAC) after renaturation. *canEas* was found to be 663 bp long and encodes a protein of 220 amino acids in length. Based on the DELTA-BLAST results, it is 99 % identical with *Enterobacter cloacae* WSU1 carbonic anhydrase 2 from β -class clade A and was 95 % identical with *E. coli* K12 *can* gene, which was essential for growth at atmospheric pCO₂. The CA activity was observed after a few second in the CO₂-saturated ddH₂O as a yellow band on the Native-PAGE. The zymogram analysis of *canEas* was demonstrated a characteristic esterase activity band via fast red staining. To date, most of the CA study has been conducted on pathogenic bacteria and thermophilic archaea, however there have been no comprehensive characterization of a recombinant CA from *Enterobacteraceae*. On this basis our studies on the purification and comprehensive characterization of the CAEas is still in progress.

Keywords: CO₂ sequestration, microbial mineralization of CaCO₃, carbonic anhydrase 2

***In silico* Analysis and Comparative Modelling of *Camellia sinensis* Heat Shock Protein 70 (Hsp70)**

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Abstract

The 70-kD heat shock proteins (Hsp70s) are molecular chaperones involved in a variety of cellular processes including protein folding, protein transport across membranes, modulation of protein activity, regulation of protein degradation, prevention of irreversible protein aggregation and for the differentiation of germinating seeds. All Hsp70s are composed of a nucleotide-binding domain (NBD) and a protein substrate-binding domain (SBD), with the latter subdivided into β -sandwich (SBD β) and α -helical (SBD α) subdomains. Although widescale experimental studies have been carried out in many eukaryotes on determination of 3D structure of this protein, however there have been no study on plants. In this study, we predicted the 3D structure of *Camellia sinensis* Hsp70 by using different modelling tools and some structural and functional properties via *in silico* analysis. The protein sequence of CsHsp70 was retrieved from NCBI. Physicochemical analysis, secondary structure prediction, domain and motif analysis and subcellular localization were predicted by several major bioinformatic tools. Templates were selected using PSI-BLAST against PDB and with PRC against HMM database. Comparative modelling was carried out by LOMETS and MODELLER v.9.12. Selected models were verified through ProQ, ProSA and QMEAN based on a number of structural features to predict the model quality. The stereochemical quality of the selected models was evaluated with PROCHECK by Ramachandran plot analysis. The 3D structure of protein was visualized by PyMOL v1.5.0.3. The model generated by LOMETS was chosen with a 469.200 Zscore as best. Ramchandran Map calculation revealed following results; 95.3 % residues in most favoured regions, 4.7 % residues in additional allowed regions, 0.0 % residues in generously allowed regions and 0.0 % residues in disallowed regions. Ribbon diagrams and stick representations of binding properties were determined.

Keywords: *Camellia sinensis*, Hsp70, comparative modelling

Chemically Modified Superoxide Dismutase Prevents Lipid Peroxidation and Improve Antioxidant Status in Streptozotocin-Induced Diabetes

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Abstract

Antioxidant enzyme superoxide dismutase, Cu,Zn type [EC 1.15.1.1] (SOD) catalyzes the dismutation of highly reactive superoxide anion radical ($O_2^{\cdot -}$), to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). This enzyme prevents tissue damage from cytotoxic oxygen radicals produced in pathological states such as inflammatory diseases, diabetes mellitus, cancer, ischemic/reperfusion injury in transplantation. However, SOD has short plasma half-life in vivo (less than 5 minutes) currently limits clinical applications of this enzyme. In addition, SOD is rapidly inactivated by its own reactive products H_2O_2 , yielding highly reactive oxidant species. In the present study SOD enzyme was chemically modified with poly methyl vinyl ether-co-maleic anhydride (PMVE/MA). Physicochemical properties of the conjugates, such as temperature stability and stability to oxidation with H_2O_2 were studied and compared to native SOD. For in vivo biological evaluation of these conjugates 30 Sprague Dawley rats were divided into four groups: Group I, non-diabetic (Normal Control) rats; Group II, Diabetic Control rats; Group III, native SOD treated diabetic rats; Group IV, SOD-PMVE/MA conjugate treated diabetic rats. The brain, kidney and liver tissues were excised after 5 weeks of chemically modified SOD treatment, the levels of malondialdehyde (MDA) and the activities of SOD and Glutathione (GSH) of all groups were analyzed. The results demonstrated that SOD-PMVE/MA conjugate potent protective effect comparable with native SOD in STZ induced diabetic rats.

Keywords: Superoxide Dismutase, Chemical modification, Lipid Peroxidation, Antioxidant Status

Circulating Levels of Cytokines (IL-1 β , TNF- α ,IL-33), the NF-kB1 ATTG ins/del Polymorphism and Risk of Atherosclerosis in a Turkish Population

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Abstract

Introduction: Atherosclerosis, a common and complex disease, is a chronic immuno-inflammatory disease characterized by inflammation and immune activation in arterial wall. Nuclear Factor-kB (NF-kB) is responsible a major transcription regulator inflammation and immune responses control genes and associated with atherosclerosis .

Objectives: To evaluate IL-1 β , TNF- α and IL-33 blood levels depending on ATTG ins/del polymorphism of NFKB1 gene in atherosclerosis. The present study was to investigate the roles of cytokines the polymorphism during development and progression of atherosclerosis.

Methods: 76 analyzed patients with atherosclerosis (coronary artery, carotid artery and peripheral artery disease) and 58 patients lower extremity venous insufficiency as control group were operated due to disease participated in this study. We analyzed the distribution of NFKB1 -94 ins/del ATTG polymorphism using PCR-RFLP method. Plasma levels of IL-1 β , TNF- α and IL-33 using ELISA method in Turkish population. GraphPad static's software were used for the analyses of the patients and control values.

Results: There was no statistically significant difference in distribution of the genotypes and alleles of NFKB1 -94 ins/del ATTG polymorphism in patients and control ($p > 0.05$). Although, no significant differences in levels of IL-33 and TNF- α were found between patients and control group (15.94 \pm 5.2 and 16.18 \pm 6.05 pg/ml; 12.76 \pm 3.18 and 12.15 \pm 2.64 pg/ml , respectively), IL- 1 β levels of patient group were significantly higher than the control group (9.61 \pm 1.71 and 8.83 \pm 2.26 pg/ml ,respectively) ($p=0.0008$). Furthermore, when we compared plasma levels of IL-1 β according to the genotypes of NFKB1 -94 ins/del ATTG polymorphism in atherosclerosis patients, there was a significant difference among the genotypes ($p=0.0028$). We observed that the patients with del/ins genotype had higher IL-1 β concentration than the control with del/ins while patients with del/del genotype had lower than the control with del/del.

Conclusion: Our findings suggest that the functional promoter NFKB1 -94 ins/del ATTG polymorphism was significantly associated with population atherosclerosis disease through acting by directly modulating IL-1 β plasma levels while no significant differences in levels of IL-33 and TNF- α .

Keywords: NF-kB1 ATTG ins/del, polymorphism, cytokine levels

Characterization of Non-Replicative DNA polymerase III (DNA pol E) from Thermophilic *Geobacillus kaustophilus* HTA

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Abstract

Geobacillus kaustophilus HTA (*Gkau*) has two copies of DNA polymerase III gene, *pol C* and *pol E*, which encode two DNA polymerases with presumptive functions in DNA replication and repair, respectively. We cloned a 3273 bp long DNA *pol E* gene from *Gkau*, overexpressed and purified the recombinant DNA pol E, with a molecular mass of 124 kDa, to near homogeneity. *Gkau* pol E exhibited an optimum DNA polymerase activity at 50-55°C and it is active at this temperature up to 3 hours. The enzyme preferred Mn²⁺ over Mg²⁺ for polymerase activity with 2 and 5 mM being optimal concentrations respectively. *Gkau* pol E did not display 3'→5' exonuclease activity. The DNA binding affinity (*K_D.DNA*) and apparent dNTP binding affinity (*K_m.dNTP*) for this enzyme were 24 nM and 0.15 μM, respectively. Contrary to the expectation, *Gkau* pol E showed relatively high fidelity. However, it extended mismatched termini, a characteristic reported for some Y-family polymerases. *Gkau* pol E efficiently carried out strand displacement synthesis of DNA (SDSD). Three residues in putative fingers subdomain, namely, F581, R674 and R675 were identified as essential for this activity. Structural comparison of the model built pol E and the ternary complex structure of *Gkau* pol C revealed that subtle differences near the active site may be responsible for the accommodation of mismatched template-primers by pol E.

Keywords: DNA polymerase III, *Geobacillus kaustophilus*, Site-directed mutagenesis

Evaluation of the Genotoxicity and Antigenotoxicity of Cynarin in Human Lymphocytes by Comet Assay

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Abstract

Artichoke is an edible vegetable, originating from the Mediterranean region and, grown all over the world at present. Its flower head and leaves are widely consumed as a vegetable or tea. Its leaves have also been used in traditional medicine for therapeutic properties since ancient times. There is growing evidence that artichoke extracts exhibit major biological roles such as hepatoprotection, anti-oxidative, anti-inflammatory, anti-microbial, anti-mutagenic, and anti-proliferative potential. It has been determined to be rich source of phytochemicals such as mono- and dicaffeoylquinic acids and flavonoids. Cynarin (1,5-dicaffeoylquinic acid) is a phenolic compound that is derivative of caffeic acid in artichoke head and leaves and it possesses the most antioxidant activity. In this study, we investigated genotoxic and antigenotoxic effect of cynarin against H₂O₂ induced DNA damage on human lymphocyte by comet assay. For this purpose, peripheral blood was taken from two healthy donors (1 male and 1 female) and lymphocytes were isolated using Biocoll separating solution. Isolated lymphocytes were incubated with different concentration of cynarin (6.25, 12.5, 25, 50 and 100 µg/ml) alone and also combination with H₂O₂ (100 mM) at 37°C for 1 hour. A negative (distilled water), a solvent (%50 metanol) and a positive control (H₂O₂) were also maintained. The tail length (µm), tail intensity (%) and tail moment of 100 cells for each experiment (total of 200 comets per concentration) were examined, using specialized Image Analysis System (Comet Assay IV, Perceptive Instruments Ltd., UK). Our findings showed that cynarin alone did not significantly increased the comet tail length, tail intensity and tail moment at all concentrations compared to control groups. However, cynarin+H₂O₂ treatment significantly decreased DNA damage at all concentration compared to positive control. Our data demonstrated that cynarin showed a strong chemopreventive effect in isolated human lymphocytes against H₂O₂ induced DNA damage.

Keywords: Antigenotoxicity, cynarin, human lymphocytes, comet assay

Use of TLC in Plant Lipidomics Analysis

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Abstract

“Lipidomics” refers to detailed, comprehensive profiling of lipid classes, often in relation to genotype. Comparison of the lipid profiles of wild-type plants with those of plants that have been subjected to forward- or reverse-genetic manipulation, in parallel with developmental and physiological phenotyping, can aid in characterization of the roles of the manipulated genes and enzymes. For example, analysis of changes in individual lipid classes can provide clues as to the physiological substrates and products of genetically-altered enzymes.

In plant lipidomic analysis, quantitative information on numerous individual lipid species is typically acquired directly from organic extracts of plant material without chemical modification. Complex lipids, and especially membrane lipids, are particularly amenable to these direct, comprehensive analyses.

Between the many chromatographic techniques, thin-layer chromatography (TLC) has its special place for large-scale and rapid “lipidomic” analysis, being one of the first separation methods introduced in the lipid analysis. TLC is a simple, flexible and cost efficient separation technique for both qualitative and quantitative analysis, enabling analysis of many lipid classes with minimal time requirement. TLC is easy to perform, versatile, and does not require expensive instrumentation. TLC also allows direct quantification of lipid classes by using scanning densitometry or photo-scanner with the requirement to calibrate the measurements against a reference sample, a requirement that is valid also for all other chromatographic techniques.

Keywords: Lipidomics, plant, TLC

Isolation and Characterization of a Novel Acidophilic, Halotolerant and Cold-adaptive Cellulase(CMCCase) from a native isolate *Bacillus* sp.

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Abstract

A cold-adaptive acidic cellulase producing *Bacillus* sp. TG-1 strain was isolated from soil samples. In SDS-PAGE analysis the enzyme showed single bond of 52.4 kDa. The Cellulase TG-1 had a pH optimum of 5.0 and displayed maximum activity at 45°C. The enzyme was stable from 20 to 40°C with 100 % activity and more than 44% of its original activity was observed after preheating at 60°C for 60 min. Also, it was stable from pH 4.0 to 9.0 with more than 85% of original activity after pre-incubation at 30°C for 24 h. It conserved its activity in the presence of 5mM EDTA, ZnCl₂, MgCl₂, CaCl₂, 34mM SDS, 16mM Triton-X-100, 0.8mM Tween-20 and Tween-80 up to 79, 72, 76, 82, 69, 75, 76 and 76 %, respectively after pre-incubation at 60°C for 60 min. The Cellulase TG-1 was strongly inhibited in the presence of 8M Urea, 29mM H₂O₂, 3mM PMSF and 1, 10-Phenanthroline up to 60, 77, 71, and 67%, respectively. However, the activity was increased in the presence of 127mM β-mercaptoethanol and 5mM MnCl₂ up to 53, 94 %, respectively. The enzyme showed more than 63 % of original activity in different NaCl concentrations from 51mM to 5.0 M after pre-incubating at 60°C for 60 min. TLC analyses of the hydrolysate of CMC revealed the presence of maltose and etc. The results of this study showed that the enzyme TG-1 is an acidophilic, halotolerant, cold-adaptive, detergent resistant and surfactant resistant enzyme. To days Cold-active detergents are in great demand in order to energy saving, so owing to the mentioned characteristics the isolated TG-1 cellulase enzyme can be useful in detergent industries.

Keywords: *Bacillus* sp, Cold-adaptive cellulase, detergent, energy saving

Investigation of Antidepressant Drug Desipramine Genotoxicity In Human Peripheral Lymphocytes Using Micronucleus and Comet Assays

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Abstract

Desipramine is a widely used tricyclic antidepressant for treatment of depression. Its mechanism of action is mainly the inhibition of the neuronal recapture of monoamines such as noradrenalin and serotonin. The present study was planned to determine the genotoxic effects of desipramine in human peripheral blood lymphocytes using micronucleus (MN) and comet assays. For this purpose, peripheral blood obtained from two healthy young donors, a man and a woman, was treated with six different concentrations (1,50; 3,00; 6,00; 12,00; 18,00 and 24,00 µg/ml) of desipramine in culture conditions. In MN test, a negative and a positive control (mitomycin-C) were also applied for each treatment. In comet assay isolated lymphocytes were treated with the same concentrations of desipramine. Hydrogen peroxide (100 µM) and distilled water were used as positive and negative control, respectively. According to the results, desipramine significantly increased the frequency of MN in all concentrations (except 1,50 µg/ml), compared to negative control, in a dose-dependent manner ($r=0,98$). However, it did not affect cytokinesis-block proliferation index (CBPI). Besides, desipramine increased the comet tail intensity, tail length and tail moment at all concentrations but not in a dose-dependent manner (weak correlation for all comet parameters). The results suggest that desipramine may have genotoxic potential on human lymphocytes *in vitro*.

Keywords: Antidepressant drug, desipramine, micronucleus, comet assay

Phisto: A Web Platform for Studying Infection Mechanisms through Pathogen-Human Interactions

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Abstract

Infectious diseases are one of the leading causes of death and disability worldwide. The molecular interactions between the infectious microorganisms, pathogens and their human hosts allow the microorganisms to manipulate human cellular mechanisms to their own advantage, resulting in infection. A thorough understanding of these pathogen-host interactions (PHIs) will elucidate the mechanisms involved in infections, and allow new-age therapeutic solutions to be devised. In the post-genomic era, following the advances in genomics, proteomics, and then interactomics, PHI data can be produced in large-scale within the last decade. We have developed a Web platform, PHISTO (Pathogen Host Interaction Search Tool) to make PHI data available from one single source for systems pharmacology research. It enables access to the most up-to-date PHI data for all pathogen types whose experimentally-verified protein interactions with human are available, via a user-friendly and functional Web interface at www.phisto.org. Our goal is to facilitate the efforts focusing on enlightening infection mechanisms through PHIs. The platform also offers integrated tools for visualization of PHI networks, graph theoretical analysis of human proteins targeted by pathogens and BLAST search. Recently, we have implemented additional bioinformatics tools into PHISTO to enable users to analyze the functional properties of human proteins targeted by pathogens during infections. This type of analysis may give crucial insights on infection strategies used by pathogens, in terms of their attacking behavior. Such bioinformatics tools increase the potential of PHISTO to serve as a network analysis platform to investigate the interspecies protein interaction networks between pathogens and human to elaborate on infection mechanisms.

Antioxidant Effects of Methanolic and Aqueous Extract from *Lentinus tigrinus*

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Abstract

Antioxidant activities methanolic and aqueous extracts of *Lentinus tigrinus* were assayed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, reducing power and iron chelating. Furthermore, total phenol, flavonoid, β -carotene and lycopene contents of these mushroom extracts were also determined. All extracts exhibited potent and concentration dependent free radical scavenging activity in all the assays tested. IC₅₀ values for methanol and water extract were 7.451 and 22.638 mg/ml, respectively. Reductive abilities of these extracts were also found to increase with increase at concentration. At maximum concentration (10 mg/ml), while absorbance value for reducing power of water extract had 0.27, methanol extract had absorbance value of 0.45. Total phenol flavonoid, β -carotene and lycopene content determination showed that all the extracts are rich in antioxidant compounds. The highest quantity was determined in total phenol content (46.76 μ g/mg) for water extract. IC₅₀ values for chelating on ferrous ions were 14.119 (for methanol extract) and 9.513 (for water extract) mg/ml. The present study showed that methanolic and aqueous extracts of *L. tigrinus* might be beneficial to protect human body against oxidative damage.

Keywords: antioxidant activity, *Lentinus tigrinus*, macrofungi

Determination of Cytotoxic Properties of *Schizophyllum commune*

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Abstract

The main objective of this study was to evaluate the cytotoxicity capacity of methanolic and aqueous extracts from *Schizophyllum commune* against liver hepatocellular carcinoma (HepG2) cell lines. After 48 and 72 h, cytotoxicity effects of two extracts were investigated. It was determined that cell viability decreased depending on passing of time. When compared the IC₅₀ values, the highest cytotoxicity capacities were determined after 72 h. While 72 h IC₅₀ value of methanol extract of *S. commune* was 42.632 mg/ml, 72 h IC₅₀ value of water extract was 3.465 mg/ml. At the same time, IC₅₀ values of two extracts after 48 and 72 h were statistically ($p < 0.05$) different from each other.

Keywords: cytotoxicity, HepG2, macrofungi, *Schizophyllum commune*

Preconcentration of Ni(II) and Co(II) ions from real samples by using immobilized thermophilic *Geobacillus stearothermophilus* SO-20

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Abstract

The thermophilic *Geobacillus stearothermophilus* SO-20 (Accession number: KJ095002) immobilized on XAD-4 was used as a new biosorbent for the preconcentrations and recoveries of cobalt and nickel ions. The effects of some analytical parameters such as pH, flow rate, amount of biomass and resin, sample volume, eluent volume and type on the quantitative recoveries of analytes were experimented. The retained cobalt and nickel ions on the biosorbent were eluted by using various concentration of (0.5-1M) HCl and HNO₃ and analysed by ICP-OES. It was observed that 5.0 mL of 1M HCl was sufficient to recover cobalt and nickel ions. The maximum biosorption capacity was found 16.8 and 21.6 mg g⁻¹ for Ni (II) and Co (II) ions, respectively. In order to validate the accuracy of new method for determination of cobalt and nickel ions, certified reference material of tea sample (NCS ZC-73014) was used. The new preconcentration method was experimented to river water sample, soil and some vegetables.

Keywords: *Geobacillus stearothermophilus*, Preconcentration; Co; Ni; Sensitivity improvement

Carbonic Anhydrase I and II Isoenzymes Inhibition Effects of Some of the Compounds Synthesized from Eugenol

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Abstract

The aroma of clove bud components, eugenol and eugenil acetate. Eugenol, clove and other spices in the contents of certain fields and clove delicious is a component having the characteristic odor.^{1,2}

Carbonic anhydrase (CA, Carbonate hydrolyase, EC 4.2.1.1) is a well-characterized pH regulatory enzyme in most tissues including erythrocytes and catalyzes reversible hydration of CO₂ to HCO₃⁻ and H⁺. In this study, the CA I, and II isoenzymes from human blood were purified in a single step by using Sepharose-4B-L-tyrosine-sulfanilamide affinity. Purity of isoenzymes was checked by SDS-PAGE and a single band was observed.

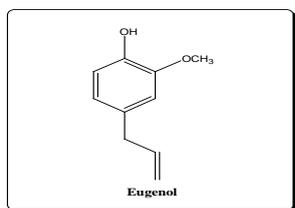


Figure 1. The chemical structure of eugenol

CA isoenzymes in biomedical applications for the design of inhibitors or activators have been the target of interest. Accordingly, some of the compounds synthesized from eugenol on hCA I and II isoenzymes were investigated. According to the results obtained inhibition was observed in the micromolar range. Therefore at the present study leads to various areas, such as drug design is thought to be.

Keywords: Eugenol, carbonic anhydrase, inhibitor

The Effects of MESNA (2-Mercaptoethane Sulfonate) on Nitric Oxide Levels and Arginase Activity in the Ischemia-Reperfusion Injury of Small Intestine

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Abstract

Ischemia-reperfusion (I/R) injury of small intestine is a serious and common condition, a result of blockage of the superior mesenteric artery (SMA) due to significant clinical problems. In this study, it has been aimed to evaluate the effects of Mesna, which is an antioxidant, on the intestinal nitric oxide (NO) levels and arginase activity in the I/R injury at biochemical, immunohistochemical and light microscopy level and to determine the tissue malondialdehyde (MDA) level as a marker of oxidative damage. In the study, 10-12 weeks old male Wistar albino rats weighing 200-250g were used. The animals were grouped as Group A-Sham-control group, Group B-Intestinal I / R group and Group C-Mesna (n = 7). I / R was performed by binding and opening of SMA after 120 min. In Mesna group, Mesna (150 mg / kg, ip) was given immediately after opening of SMA. Following surgical procedures, intestinal tissue samples were obtained for biochemical parameters, light microscopy and immunohistochemical studies. The inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) have been shown in the tissue. The levels of NO and MDA and the activity of arginase were measured. Histopathological findings in the morphological level were evaluated with microscopy and stated by histological scoring. It has been observed in I/R group that, mononuclear cell infiltration in the connective tissue degeneration and deteriorated of integrity of epithelium and smoothing in mucosa of small intestine. The levels of MDA, and NO, arginase activity, immunoreactivities of iNOS and eNOS were compared with the control group, it has been observed that, the values were significantly increased ($p < 0.05$) in I/R group and decreased in the MESNA group ($p < 0.05$).

As a result, it has been observed that, MESNA was helpful against the intestinal I/R injury due to its antioxidant properties considering biochemical parameters, however, an improvement in intestinal tissue has not been identified in light microscopic examination.

Keywords: Ischemia-reperfusion, Small intestine, Mesna, Nitric oxide, Arginase, iNOS, eNOS

Antimicrobial activity on Gram negative and Gram positive microorganisms by TiO₂ nanocomposites

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Abstract

Antimicrobial materials are natural or synthetic chemicals that kill microorganisms or prevent their to reproduction. In recent studies, it has been determined that some of the photocatalyst materials also have antimicrobial activity on microorganisms. Various studies have been carried out through photocatalyst materials such as water purification, air cleaning, putrefaction of prevention and purification from bad odors and volatile organic materials. In addition, photocatalyst materials have some effects on pathogenic microorganisms as well.

In this study, *Escherichia coli* which is Gram negative and *Staphylococcus aureus* which is Gram positive, were used. Nanocomposites, which contain 3%, 5% and 10% of TiO₂, were cut into equal sizes. A similar study as antibiogram was conducted by using cultures with different McFarland turbidity standards. During the study, the effect of UV on photocatalyst nanocomposites has also been researched. At the end of the study, was of non-breeding areas which shows the antimicrobial effect, was calculated. As a result the inhibition is in a descending order as Gram positive bacteria > Gram negative bacteria.

Keywords: Antimicrobial, nanocomposite, TiO₂

Construction and Replication of Two Protein Kinases Deficient Recombinant *Amsacta moorei* Entomopoxvirus (AMEV)

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Abstract

Entomopoxviruses are a group of important viruses infecting insects. *Amsacta moorei* Entomopoxvirus (AMEV) is a typical Entomopoxvirus that can easily grow in cell culture. The AMEV genome has been completely sequenced and functional analysis of a few genes has been done. Bioinformatics analyses of AMV197 and AMV153 open reading frames (ORFs) suggested that they are encoding Serin/Threonine (Ser/Thr) protein kinases. It is known that Ser/Thr protein kinases of Poxviruses have roles in virus replication, morphogenesis, regulation of host cell cycle, and apoptosis. However, there is little information about characterization and functional analysis of the genes coding these proteins in Entomopoxviruses.

In this study, AMV197 and AMV153 ORFs have been deleted from AMEV genome by homolog recombination. Because of their marker genes, first recombinant virus that lacking AMV197 named as Am Δ PK/*gfp* and second recombinant virus that lacking AMV153 named as Am Δ 153/*Cherry*. Also, double recombinant virus (Am Δ PK-153/*gfp-Cherry*) that lacking both AMV197 and AMV153 was done by same technics. After construction and purification of recombinant viruses, we tested them for the progeny virus production in cell culture. Our results showed that three recombinant viruses and wild type virus are able replicate in *Lymantria dispar* cell culture (Ld cells). Further experiments are needed to identify the exact role of these genes in AMEV genome.

Keywords: Am Δ PK/*gfp*, Am Δ 153/*Cherry*, Am Δ PK-153/*gfp-Cherry*, *Amsacta moorei* Entomopoxvirus (AMEV), protein kinases

Isolation and Characterisation of Petroleum-Degrading Bacteria from Petroleum Contaminated Soils

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Abstract

Two bacterial strains belong to *Acinetobacter* and *Stenotrophomonas* genus were isolated from petroleum contaminated soils in Diyarbakir and Batman petroleum fields. Morphological, physiological, biochemical and kemotaxonomical characterisation of bacterial strains were carried out. According to 16S rRNA gene sequencing analysis, two strains designated as GC2 and 2TP1A were found to be closely related to *Acinetobacter iwafii* and *Stenotrophomonas maltophilia* species by phylogeny, respectively. Both bacterial strains were found to use crude petroleum as carbon sources in order to grow, moreover the strain close to *Acinetobacter iwafii* species was found to degrade hexadecane analysed by GC-MS.

Keywords: Bacterial Isolation and Characterisation, Petroleum degrading bacteria, 16S rRNA sequencing, GC-MS

Antimicrobial Activities of Chroococcus dispersus and C. Minutus

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Abstract

In this study, antimicrobial activity of *Chroococcus dispersus* and *C. minutus* which were grown in proper culture condition was searched with their extracts. The antimicrobial activity of algal extracts were tested on *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O 157:H7, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, *Candida albicans* ATCC 10239 by using disc diffusion method. *Chroococcus dispersus* extracts was observed that all test microorganisms and highly effective to varying degrees affect the antimicrobial effect.

However, *C.minutus* extracts shown especially the high antimicrobial activity against *Salmonella typhimurium*.

According to estimates by analysis of variance, the test results were significant differences between the groups in terms.

In Duncan test, the algal species, was found to be far superior to the control group. The differences between the results was regarded as statistically significant ($p<0.01$).

Keywords: Antimicrobial activity, *Chroococcus dispersus*, *Chroococcus minutus*, Disc diffusion methods

*This study Gazi University Scientific Research Projects (BAP) by Unit (05/2011-62 coded projects) are supported.

Effects of Nicotine on Drug Resistance of Breast Cancer Cells

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Abstract

Breast cancer is the most common cancer in women, and the second most common cause of cancer death after lung cancer. Cancer stem cells represents a novel approach to target the cancer and reduce cancer recurrence and metastasis. It is well known that many breast cancer patients continue to smoke after diagnosed with cancer. Smoking cessation is usually very little therapeutic effect in cancer. There are studies to suggest reduction of chemotherapeutic drug effects if patient continue to smoking. Nicotine, the major component in cigarette smoke originally thought to be only responsible for tobacco addiction, also alters some cellular functions, such as activation of mitogenic pathways, angiogenesis, and cell growth in many cell types. In the present study, we investigated the effects of nicotine on the population of MCF-7 and cancer stem cells in MCF-7 human breast cancer cells. Nicotine stimulated a dose-dependent migration and CD44⁺/CD24⁻ cancer stem cell numbers of MCF-7 cells was determined by SEM (scanning electron microscope), and flow cytometry analysis. Also we investigated that nicotine effects on chemoresistance in these cells. These findings underscore the importance of smoking cessation following a metastases of cancer, and chemoresistance of continued smoking that may be detrimental to treatment.

Keywords: Cancer stem cells, nicotine, chemoresistance, flow cytometry

The Removal of Textile Dyes Reactive Orange 14 and Reactive Blue 2 with White Rot Fungus *Lentinus concinnus*

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Abstract

Fungal biosorption is an economic, simple and effective method for removal of organic pollutants from aqueous media. In this study, a white rot fungus has been used as biosorbent, due to, it can be produced using inexpensive materials and it provides high biosorption capacity for many pollutants. *Lentinus concinnus* has been used for removal of two reactive textile dyes from aqueous media. Reactive Orange 14 and Reactive Blue 2 textile dyes, have been chosen as model. The effect of pre-treatment on biosorption capacity has been searched through realizing biosorption experiments using native and heat treated inactive fungal preparations. The effect of pH and initial dye concentration on biosorption of textile dyes from aqueous media has been examined. Optimum pH value was searched in the pH range of 1,0 -10,0 and the optimum value has been found as pH 2,0 for the two textile dyes and two fungal preparations. Maximum biosorption capacity of native and heat treated fungal biomass has been found as 201 mg/g and 158 mg/g for Reactive Orange 14, and; 194 mg/g and 116 mg/g for Reactive Blue 2, respectively.

Keywords: Biological removal, *Lentinus concinnus*, Reactive Orange 14, Reactive Blue 2

The Using Of *Lentinus concinnus* The White Rot Fungus For Removal Of Chromium (VI) From Aqueous Solutions

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Abstract

In this study, biosorption of Cr (VI) metal ions from aqueous solutions onto *Lentinus concinnus*, the white-rot fungus biomass were studied. The effects of pH, biosorbent dose, initial concentration of metal ions, temperature and contact time parameters on Cr (VI) removal were investigated in a batch system. Inactivation of fungal biomass was carried out by three different ways: acid, base and heat treatments. Active fungal biosorbent has lower Cr (VI) adsorption capacity (36,00 mg/g), than all of the inactivated biosorbents. Cr (VI) adsorption capacities of inactive biosorbents, are ranked from higher to lower as, acid treated (54,25 mg/g), base treated (62,38 mg/g) and heat treated biomass (74,75 mg/g). The heat treatment was evaluated as the most effective inactivation method for Cr (VI) removal. Maximum removal ratio for Cr (VI) was determined as 80,5% for 200 mg of heat treated biomass in 100 ml total volume. This obtained result was significantly higher than the results reported in literature. The maximum biosorption of Cr (VI) ions on the fungal biosorbents were obtained at pH 1.0. There is no any reported study in the literature about *Lentinus concinnus*. According the results obtained from these experiments, *Lentinus concinnus* biomass has the high potential to be used for the removal Cr (VI) ions from industrial wastewater.

Keywords: White rot fungi, *Lentinus concinnus*, Cr (VI), Biosorption

Direct Quantitative Analysis of 2D-TLC Plates

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Abstract

Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. TLC consists of a stationary phase immobilized on a glass, aluminum, or plastic plate, and an organic solvent (mobile phase). The sample, either liquid or dissolved in a solvent, is applied as a spot on the stationary phase. The different components in the mixture move up the plate at different rates due to differences in their partitioning properties between the mobile liquid phase and the stationary phase and constituents of a sample can be identified by simultaneously running standards.

2D-TLC uses the TLC method twice to separate spots that are unresolved by only one solvent. After running a sample in one solvent, the TLC plate is removed, dried, rotated 90⁰, and run in another solvent. This method gives higher resolution than in a single run. The finished chromatogram is a two-dimensional array of spots. The conventional quantitative analysis of 2D-TLC plate is comprised visual detection of the spots corresponding to a specific standard, excise the spots from the plate, and then transfer to vials quantitative analysis. But 2-D Gel analysis software offers direct, comprehensive, flexible and fast 2D-TLC plate analysis. Powerful auto-matching algorithms of programme quickly and accurately match spots on plates and flexible annotation features establish a centralized information source, which allows virtually any type of characterizing data to be linked to each spot on a master plate image. Furthermore, it is easy to view and share information associated with identified spots.

Keywords: 2D-TLC, quantitative analysis, 2D-Gel analysis software

Relationships between university students' self regulation strategies (managing time and study environment, cognitive and metacognitive strategies) in biochemistry and gender

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Abstract

Self-regulated learning is defined as the strategies that students use to regulate their cognition (use of various cognitive and metacognitive strategies) as well as the use of resource management strategies which students use to manage and control their environment and also their learning. Therefore, this study is based on case study method with a purpose of describing low achievement students' self-regulated strategies and exploring relationships between male and female students' self-regulated strategies in biochemistry. The study was conducted with third class 25 university students. The Motivated Strategies for Learning Questionnaire (MSLQ), developed by Pintrich, Smith, Garcia and McKeachie (1991) was used to collect the data. The Turkish version of the Motivated Strategies for Learning Questionnaire was translated and adapted into Turkish by Sungur (2004). In accordance with the purpose of the study, only three subscales of learning strategies section (cognitive and metacognitive strategies, managing time and study environment) were used to assess students' learning strategies. T-test was carried out to explore the relationships that might exist among students' self-regulated learning strategies and gender. Results revealed that there was significant difference between male and female students' cognitive strategies; however, there was no significant difference between male and female students' metacognitive strategies and time/study environmental management strategies.

Keywords: self-regulated learning strategies, biochemistry

Isolation of Thermophilic *Geobacillus stearothermophilus* and its α -amylase production and characterisation

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Abstract

In this study, a novel thermophilic bacteria was isolated from hot spring of Afyokarahisar (Gecek). The isolate was characterized by morphology, biochemistry and sequencing of its 16S rRNA gene. The sequence of 16S rRNA gene showed maximum identity 96% similarity with *Geobacillus stearothermophilus* (Accession number: KJ095002). The influences of different carbon and nitrogen sources and surfactants on α -amylase production by newly isolated thermophilic *G. stearothermophilus* were tested. The maximum α -amylase production (3587 U/mg) was obtained with SDS. Various parameters such as temperature and temperature stability, pH and pH stability, detergents and metal ions on partially purified enzyme characterization were studied. The optimum temperature and pH on enzyme activity were found to be 75 °C and 6.0, respectively. In addition to these, starch content in raw apple and its degradation by partially purified enzyme were experimented. After 30 minute incubation, enzyme degraded the %71 of starch content in apple juice at 75 °C.

Keywords: *Geobacillus stearothermophilus* α -amylase production and characterization, detergents, Apple juice

Antiproliferative activity of quinoline compound DIE-17 against various cancer cell lines

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Abstract

In the designs of many synthetic compounds, quinoline derivatives have received great attention due to their diverse pharmacological properties. The aim of this study was to investigate the anticancer properties and mechanism of action of the novel quinolin derivatives, DIE-17, synthesized by substitution reactions of any quinoline molecule. Antiproliferative and cytotoxic activities of DIE-17 was tested on HeLa, HT29 and C6 cell lines *in vitro* using BrdU Cell Proliferation ELISA, LDH assay. DIE-17 was more antiproliferative against HT29 and C6 tumor cells than 5-FU but not in HeLa. The mechanism of anticancer activity of this compound was determined using DNA laddering assay. Although the results showed that the DIE-17 may be a potent anticancer drug candidate with high antiproliferative activity, further studies are needed to elucidate its mechanism of action.

Keywords: Quinoline, anti-cancer, cytotoxicity

*This study is supported by a grant (TBAG 112T394) from TUBITAK.

Polyphasic Taxonomy Of A Novel *Streptomyces* sp. Z1R7, Isolated from the Burgazada

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Abstract

Object of the research: Members of the genus *Streptomyces* are aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes which form extensively branched substrate and aerial mycelia. The present polyphasic taxonomic study was designed to determine the taxonomic position of the novel *Streptomyces* sp. Z1R7.

Materials and Methods: Z1R7 isolate, was picked after 3 weeks of incubation at 28°C on Bennett's Agar supplemented with cycloheximide (50 µg/ml), rifampicin (0.5 µg/ml) and nalidixic acid (10 µg/ml). Genomic DNA isolation was performed by DNA isolation kit (Invitrogen, USA). The 16S rRNA gene was amplified by PCR by using universal primers 27f and 1525r. Phylogenetic analyses were performed by using three tree-making algorithms, namely the neighbour-joining, maximum-likelihood and maximum-parsimony methods. Sugars in cell wall hydrolysates were analysed by using the methods established by Hasegawa *et al.* (1983). Polar lipids were extracted, examined by using two-dimensional TLC and identified on the basis of procedures described by Minnikin *et al.* (1984). Isoprenoid quinones were analysed by HPLC as described by Minnikin *et al.* (1984) and Kroppenstedt (1982).

Results: Z1R7 shared highest 16S rRNA gene sequence similarity with *Streptomyces specialis* GW 41-1564^T (95.76 %). The *meso*-diaminopimelic acid was the diagnostic amino acid in the cell wall.

Conclusions: Z1R7 isolate is a novel species of *Streptomyces* genus according to result the 16S rRNA gene sequence, phenotypic and chemical analyses.

Keywords: Polyphasic Taxonomy, *Streptomyces*, Burgazada, 16S rRNA

Acknowledgements: This study was supported by Ondokuz Mayıs University (PYO. FEN. 1904.13.004). We would thank Prof. Dr. Kiyem Guven for her helps to study fatty acids analyses.

Bioaccumulation factors of some heavy metals in *Cucumis sativus*

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Abstract

Phytoremediation is soils contaminated by heavy metals is an important application in environmental problems. *Cucumis sativus* (cucumber) was tested to assess a bioaccumulation in soils contaminated by the heavy metals cadmium (Cd), lead (Pb), copper (Cu), and nickel (Ni) respectively in glasshouse experiment. In three separate pot culture experiments, four levels of soil cadmium (Cd) and copper (Cu) concentrations as 5, 10, 15, and 20 mg/L, three levels of lead (Pb) and nickel (Ni) including 75, 150, and 300 mg/L were tested. Bioaccumulation factors of heavy metals in *C. sativus* were determined. Bioaccumulation factor of Cd was >1 , which shows *C. sativus* could be accumulator of Cd in contrast to Pb, Cu and Ni. Our results also showed leaves of *C. sativus*, when exposed to the higher Cd in soil, such as the other plants indicates a great performance of this plant for Cd might be introduced as Cd- hyperaccumulator plant.

Keywords: Bioaccumulation, *Cucumis sativus*, Heavy metal, Phytoremediation

Synthesis And Biological Evaluation Of Superoxide Dismutase Enzyme Modified By Carboxymethylcellulose

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Abstract

Diabetes is considered to be one of the most common chronic diseases worldwide. There is a growing scientific and public interest in connecting free radicals, reactive oxygen species (ROS) mediated oxidative stress with a variety of pathological conditions including diabetes mellitus (DM). Superoxide dismutase (SOD) enzyme has been proposed as potential enzyme drug for several diseases caused by overproduction of ROS. However, the pharmacological activity of this scavenger enzyme is limited by its rapid clearance through kidney and inactivation by H₂O₂. Due to its poor pharmacokinetic profile and short half-life in biological system, new controlled delivery strategies have been developed to increase the intravascular half-life of SOD. In this study SOD enzyme was chemically modified with carboxymethylcellulose (CMC) and physicochemical properties of these conjugates clearly analyzed. The stability of SOD-CMC conjugates was examined against temperature and externally added H₂O₂. In addition, we investigate the effect of chemical modification of enzyme on lipid peroxidation and antioxidant status in Streptozotocin (STZ)-induced diabetic rats. Antioxidant status in diabetic groups was significantly increased by treatment of CMC-SOD conjugates. The protective effect on degenerative changes in diabetic rats was also further confirmed by histopathological examination. This study provides the preventative activity of SOD-CMC against complication of oxidative stress in experimentally induced diabetic rats. In conclusion, our results suggest that chemically modified SOD is effective on the oxidative stress-associated disease and offer a therapeutic advantage in clinical use.

Keywords: Superoxide Dismutase, Chemical modification, Lipid Peroxidation, Antioxidant Status

Molecular characterization of *Klebsiella* spp: comparison of multi-locus variable-number of tandem repeat analysis (MLVA) and pulsed-field gel electrophoresis.

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Abstract

Klebsiella species especially *Klebsiella pneumoniae* is an important pathogen and may cause society and hospital based infections. In this study, it was aimed to fulfill the characterization of 51 *Klebsiella* spp. (2 *K. oxytoca*, 49 *K. pneumoniae*) strain with multiple-locus variable number tandem-repeat assay (MLVA) and pulsed-field gel electrophoresis (PFGE). As a result of the characterization performed with two different methods; 7 locus was aimed at and while 29 patterns were acquired, 6 different patterns were acquired with PFGE method. As a result of typing *Klebsiella spp* strains, MLVA method was found out to have a high separation power when compared with PFGE method.

Keywords: *Klebsiella*, characterization, comparison

The Anti-cancer Effect of the Copolymer-drug Conjugate Containing Cytarabine on MCF-7 Cell Lines and Its Toxic Effect on L929 Cell Lines

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Abstract

Objective: The drug product Cytarabine, which is used for treatment of cancer, has a limited use due to the fact that it has too many side effects depending on its toxic effect on tissues. The objectives of this study are to bind the drug product Cytarabine to maleic anhydride vinyl acetate copolymer (MAVA) to increase its solubility in body fluids; to decrease its toxic effects; and to increase its anticancer activity compared to crude drug. Furthermore, antimicrobial activity of the synthesized copolymer was investigated.

Material and Methods: Synthesized MAVA copolymer's antibacterial and antifungal effects were determined through disc diffusion method. Structural characterization of the MAVA-Cytarabine copolymer-drug conjugate was performed by Fourier Transform Infrared Spectroscopy (FTIR) and Proton Nuclear Magnetic Resonance Spectroscopy (¹HNMR). Its solubility in water and the behavior of copolymer-drug conjugate in PBS (Phosphate Buffer Saline) at hour 1, 24 and 48 were investigated. Anticancer activity of copolymer-drug conjugate on MCF-7 cells was determined by XTT assay in comparison with anticancer activity of the crude drug, while its toxic effects on L929 cells were determined by XTT Assay again in comparison with the crude drug. The results were analyzed statistically with the Mann-Whitney U Test.

Results: It was found that MAVA copolymer doesn't have any antimicrobial activity according to our study. The synthesized copolymer-drug conjugate which is structurally characterized and whose solubility in water is studied was determined to be bound to each other with a quite successful reaction by means of amidation mechanism; to be water-soluble; and to have a long time effect in PBS. While the highest concentration of Cytarabine has a killing effect at a rate of 60,61% on MCF-7 cells, MAVA-Cytarabine conjugate has a rate of 60,64%. When the toxic effect of copolymer-drug conjugate on L929 cell lines was investigated, it was observed that its vitality rate (91,89%) was greater than the drug product (76,01%); that is to say, its toxic effect on cells was considerably lesser ($p < 0,05$).

Conclusion: Since the conjugation reaction of the synthesized MAVA-Cytarabine copolymer-drug conjugate was successful, the conjugate was determined to be biocompatible; to have good water-soluble characteristics; and to show compatible behaviors in PBS buffer solution as well, unlike the copolymer it contains. It was observed that although drug's active ingredient had some effects on breast cancer cell line through the formation of copolymer-drug conjugate, anticancer activity didn't increase. Besides it was found that with binding drug to copolymer, its toxic effect was almost eradicated. It can be suggested to test MAVA-Cytarabine conjugate in animal experiments and to develop it into chemotherapy drug, directing it to clinical research.

Keywords: Cytarabine, anticancer, antimicrobial, MAVA

Functional interactome of Aquaporin 2 sub-family reveals new physiological functions in *Arabidopsis Thaliana*

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Abstract

Aquaporins plasma membrane proteins are intercellular channel proteins found in different cellular compartments, facilitate the water flow and control the movement of gases and solutes across the cellular plasma membranes. This in silico analysis is focused on the subfamily plasma membrane intrinsic protein 2 (AtPIP2), predicting their interactome homolog's and 3-D models. AtPIP2 homologs interact with many proteins with different plant physiological roles in *Arabidopsis thaliana* including PIP1 subfamily, involved in the transport of water, controlling the cell turgor and cell expansion and involved in root water uptake respectively. The results revealed that AtPIP2-5, AtPIP2-7 and AtPIP2-8 interact with Syntaxin 132 protein (SYP132), functioning in vesicle trafficking in the secretory pathway. In *Medicago tarancula* SYP132 protein is shown to function in infection thread development or growth and the early stages of symbiosome formation. Another important interaction of PIP2-5 and PIP2-7 proteins is with NAC transcription factor-like 9 proteins (NTL9), which is shown to control the floral development. In addition, AtPIP2-2 and AtPIP2-3 proteins, are shown to interact with Nodulin-26-like major intrinsic protein subfamily (NIPs) in *Arabidopsis thaliana*. NIPs are having minimal water and glycerol transport, Instead, AtNIP2;1 displays transport of lactic acid and therefore may play a role in adaptation to lactic fermentation under anaerobic conditions. According this in silico analysis we can conclude that AtPIP2 subfamily, besides the water transport control is also involved in other physiological functions such as symbiosome formation, abiotic stress control and possibly in lactic acid transport.

In vivo Genotoxic Effects of Calcium Propionate

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Abstract

Calcium propionate is an antimicrobial food additive that is especially used in long life breads. The aim of this study was to assess the effect of calcium propionate on bone marrow cells of Swiss Albino mice using chromosomal aberration test. Animals treated with four i.p. calcium propionate doses for 24h. 75, 150, 300 and 600 mg/kg doses of calcium propionate were injected to animals by i.p. Our results indicates that calcium propionate significantly increased the abnormal cell frequency and cells with chromosomal aberrations compared with negative control.

The Effects of Irrigation Pumps on The Zooplankton Composition in Lake Eğirdir

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Abstract

The aim of this study was to found the effects of irrigation pumps on the distribution zooplankton in the Lake Eğirdir. The abundance of zooplankton in the Lake were determined in different zones (pelagic and pump outlet) between May -September 2010 with 5 monthly periods. Zooplankton samples were taken from pelagic and pump outlet zones by using plankton net a mesh size of 55 µm. A total of 49 taxa were recorded as 31 Rotifera, 16 Cladocera and 2 Copepoda. Sørensen similarity index and indicator species analysis were used. *Testudinella patina*, *Euchlanis dilatata*, *Lecane luna*, *Lecane bulla*, *Disparalona rostrata*, *Nitocra hibernica*, *Trichotria pocillum* and *Mesocyclops leuckarti bodanicola*, which were found in the lake, are indicator species of pelagic and pump outlet zones.

Keywords: Zooplankton, pelagic zone, pump outlet zone, Lake Eğirdir

Efficacy of different MS concentrations on in vitro shoot regeneration of Water Hyssop (*Bacopa monnieri* L. Pennel)

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Abstract

Water hyssop (*Bacopa monnieri* L.) has great importance in traditional medicine system of Pakistan and India due to containing active compounds like alkaloids, saponins, flavonoids, betulinic acid, stigmasterol, beta-sitosterol and saponins. In Turkey, water hyssop is very popular aquarium plant due to its appearance and adaptability under wide range of water environment. In the present study, shoot tip explant and leaf explants were cultured on different concentrations of MS (Murashige and Skoog, 1962) solidified basal mediums containing 1.0 mg/l BA. Different concentrations of MS concentrations; full MS (4.4 mg/l), ½ MS (2.2 mg/l), ¼ MS (1.1 mg/l) and 1/8 MS (0.55 mg/l) were tested for shoot regeneration. Shoot regeneration from shoot tip explant started earlier than leaf explant on all MS concentrations. Results further showed that MS concentrations had insignificant effects on shoots per explant but significantly affected the shoot length of both explants. Regenerated shoots were rooted successfully (100 %) on MS rooting medium containing different concentrations of 0.25-1.0 mg/l IBA. Rooted plantlets were acclimatized successfully in aquariums and continued their growth without showing any sign of necrosis or death.

Keywords: Aquatic plant, Acclimatization, Basal medium, Medicinal plant, Shoot regeneration, Water hyssop

Axillary shoot regeneration from different nodal segments of *Rotala rotundifolia*

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Abstract

Roundleaf toothcup-*Rotala rotundifolia* [(Buch-Ham. ex Roxb) Koehne] is an aquatic tropical and sub-tropical medicinal plant native to south and southeast Asia extending from Eastern India to Japan. *Rotala rotundifolia* is used as anti pyretic, detoxication, antismelling and diuresis properties and for treating cirrhosis ascetic fluids, gonorrhea, menstrual cramps and piles in the south of China. In Turkey, the plant is solely used as aquarium plants due to attractive leaf color from green to pinkish red. The present study was designed to obtain axillary shoot regeneration from different nodal (shoot tip, 1st and 2nd nodal segment) explant. They were cultured on agar solidified MS medium supplemented with 0.05, 0.10, 0.20, 0.40 and 0.80 mg/l BAP with 0.10 mg/l NAA. All explants showed similar response to BA-NAA concentrations and maximum shoots per explant were recorded on MS medium containing 0.40 mg/l BAP+0.10 mg/l NAA. Results further revealed that shoot length decreased with increase of BA concentration and longer shoots were recorded on MS medium containing 0.05 mg/l BAP+0.10 mg/l NAA. Regenerated shoots rooted directly in the regeneration medium and were transferred to aquariums for acclimatization. 100 % survival rate of plants without having negative effects on plant growth and development. was recorded after 4 weeks of culture in aquariums.

Keywords: Aquatic plant, Acclimatization, Axillary shoot regeneration, Medicinal plant, *Rotala rotundifolia*

In vitro Multiple Shoot regeneration from different nodal segments of aquatic *Shinnersia rivularis*

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Abstract

Mexican oak leaf (*Shinnersia rivularis*) is freshwater aquarium plant of Asteraceae family. The plant is native to Mexico and Texax (USA) and spread all over the world as aquarium plant. The present study was designed to develop successful and repeatable protocol of *Shinnersia rivularis* under in vitro conditions. Twigs with 3-4 nodes were surface sterilized with 20 % H₂O₂ (v/v) for 10 min. followed by rinsing thrice with sterilized distilled water. Thereafter, different nodal segments explants (shoot tip, 1st and 2nd nodal segments) were cultured on MS medium containing different concentrations of BAP (0.05, 0.10, 0.20 0.40 and 0.80 mg/l BAP) and 0.20 mg/l NAA. Shoot regeneration started from shoot tip explant followed by 1st and 2 nd nodal explant. After 6 weeks of culture, explants were transferred to MS medium without any growth variants for 6 weeks. All explants gave maximum number of shoots per explant on MS medium 0.20 mg/l BAP+0.20 mg/l NAA. Whereas, longer shoots were obtained from MS medium containing 0.05 mg/l BAP+0.20 mg/l NAA. Regenerated shoots were rooted on MS medium supplemented with 0.25-1.0 mg/l IBA followed by successful acclimatization in the aquariums.

Keywords: Aquatic plant, Acclimatization, Axillary shoot regeneration, *Shinnersia rivularis*

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