

Modeling and Optimization of Biohydrogen Production from De-oiled Jatropha Using the Response Surface Method

- Gopalakrishnan Kumar , Periyasamy Sivagurunathan , Sang-Hyoun Kim , Peter Bakonyi , Chiu-Yue Lin

Abstract:

In this study, optimization of hydrogen fermentation process from de-oiled Jatropha waste using a central composite design was modeled. A series of batch assays was performed at various substrate concentrations (32–368 g/L), temperatures (38–72°C) and pHs (4.8–8.2) according to the experimental design. The optimal conditions for cumulative hydrogen production (CHP) were determined by response surface methodology. The optimal substrate concentration, pH and temperature values were 211g/L, 6.5 and 55.4°C, respectively. Under these circumstances, the highest achievable CHP of 296 mL H₂ was predicted. To validate the model, verification experiments were conducted. The results were satisfactorily matched with the statistically estimated values with peak CHP value of 307.4 ± 4.5mL H₂. Microbial community profiling (PCR-DGGE) has revealed that the dominant bacterial community present belonged to *Clostridium thermopalmarium*, *Clostridium buytricum*, *Bacillus ginsengihumi* and *Bacillus coagulans*.

A Dual Role of Marine Microalga *Chlorella* sp. (PSDK01) in Aquaculture Effluent with Emphasis on Initial Population Density

- S. Dinesh Kumar , P. Santhanam , F. Lewis-Oscar , N. Thajuddin

Abstract:

The marine microalga *Chlorella* sp. (PSDK01) was cultured in shrimp-cultured effluent under different initial population densities (IPDs) ranging from 0.25 to 12.0 g L⁻¹ for dual purpose (excess nutrient consumption and biomass productivity). It was found that the IPD had affected the nutrient consumption and biomass significantly ($P < 0.001$). A higher biomass productivity compared to that of other IPDs was achieved in 0.5 g L⁻¹ IPD, and the concentration had reached 0.78 g L⁻¹ d⁻¹. In the same time, higher IPD derived higher biomass concentration (up to 5.5 g L⁻¹) in 6 days growth, but at the end of the experiment (9th day), the biomass was slightly decreased (2 %). In the current observation, while starting IPDs 0.25–0.5 g L⁻¹, with the increase in IPD, the biomass productivity also increased, when IPD exceed the 0.5 g L⁻¹, the biomass productivity reversely decreased. The maximum nutrients consumption was recorded in 0.5 g L⁻¹ IPD at the end of the experiment (9th day) as 96, 69, and 67 % for phosphate, nitrate, and nitrite, respectively. However, the highest NH₃ consumption (63 %) was observed in 0.25 g L⁻¹ on 9th day. Maximum ammonia consumption in other IPDs was resulted at 6th day, after that ammonia concentration was slightly increased from the previous concentration due to the decay of microalgae. Based on these results, to obtain the maximum nutrient consumption and biomass productivity of *Chlorella* sp. (PSDK01) in diverse wastewater on large-scale level, it is necessary to select a suitable IPD at around 0.5 g L⁻¹.

Isolation and Optimization of Growth Condition of *Bacillus* sp. from Fermented Shrimp Paste for High Fibrinolytic Enzyme Production

- Dinh Bui Quynh Anh , Nguyen Thi Tieu Mi , Do Ngoc Anh Huy , Pham Van Hung

Abstract:

The fibrinolytic enzyme has been found in the traditional fermented foods including the traditional shrimp paste product. In this study, six Vietnamese traditional shrimp paste products collected in three regions (north, south and middle of Vietnam) were screened for fibrinolytic enzyme and the related bacteria were isolated and identified. The fibrinolytic enzyme was found in all Vietnamese shrimp paste products, in which the products in the middle of Vietnam had higher fibrinolytic enzyme activity (2.43–2.95 FU/ml) than those in other regions. The isolated bacterium which produced the highest fibrinolytic enzyme was identified as a strain of *Bacillus* sp., closely related to the species of *Bacillus weihenstephanensis* with 99 % identities. The optimal fermentation conditions were also investigated using response surface methodology based on Box–Behnken design for high fibrinolytic enzyme production by the isolated strain. As a result, the fibrinolytic enzyme activity reached to 6.85 FU/ml at the optimal fermentation condition of 1.50 % of shrimp shell powder, 1.44 % of NaCl, at 33 °C and 32-h fermentation. Thus, the fibrinolytic enzyme produced by the strain of *Bacillus* sp. in this study might be used as a thrombolytic agent in pharmaceutical industries.

Single-Gene Versus Double-Gene Tree Analyses in Molecular Classification of Saudi Venomous Snakes

- Ahmed M. Alshammari, Eman El-Abd , Massimo Ciccozzi , Alessandra Lo Presti , Marta Giovanetti , Eleonora Cella

Abstract:

Aiming to use DNA sequence for proper identification of venomous snakes in Saudi Arabia, mitochondrial 12S rRNA gene sequences of *Bitis arietans*, *Cerastes cerastes*, *C. gasperettii*, and *Echis coloratus* were investigated. Concatenated analysis of 16S and 12S rRNA gene sequences (as mosaic) was also performed to validate the usefulness of using more than one gene marker in molecular taxonomy of venomous snakes. DNA extracted from whole blood samples and 12S rRNA was amplified using PCR. Sequences submitted to GenBank were analyzed for similarity using BLAST and aligned using Clustal X followed by manual editing with BioEdit software. Phylogenetic analysis was generated using PAUP version 4, and the evolutionary distance among different groups was calculated using MEGA5 software for both 12S and 16S rRNA genes. Contrary to the morphological classification, phylogenetic analysis revealed that the collected isolates formed three different statistically supported clusters (*B. arietans*, *C. cerastes*, and *E. coloratus*). Although the combined analysis of 16S and 12S rRNA sequences improved the signal, the *C. cerastes* and *C. gasperettii* intermixing have not been resolved. Both single and combined genetic distance analyses showed that *Echis* is more distant from *Cerastes* than from *Bitis*. Further studies are mandatory to evaluate other gene markers in molecular classification.

Effect of Daily Chewing Soft Buds and Leaves of *Catha edulis* (Khat) on the Antioxidant Defense System and Oxidative Stress Markers in Blood

- Khalid Mohammed Naji , Maher Ali Al-Maqtari , Adnan Ali Al-Asbahi , Qais Yusuf M. Abdullah , R. Nagesh Babu , Varadahally Rangaiah Devaraj

Abstract:

Catha edulis (Khat) is one of the major economic, social and health problems in Yemen. This paper aimed to study the effect of Khat on the oxidative status of Khat chewers by measuring the levels of enzymatic and non-enzymatic antioxidant as well as lipid peroxidation. The results exhibited significant reduction in erythrocytes superoxide dismutase (SOD, EC: 1.15.1.1), and catalase (CAT, EC: 1.11.1.6) in Khat chewers, in addition to elevation of serum glutathione-S-transferase (GST, EC: 2.5.1.18). Furthermore, non-enzymatic antioxidants glutathione (GSH) and vitamin C were significantly reduced ($p < 0.001$; $p < 0.015$), whereas malondialdehyde (MDA) was significantly elevated ($p < 0.001$). The depletion of GSH and vitamin C along with MDA elevation in Khat chewers compared with control reflects the obvious oxidative status, a result of enormous reactive oxygen species (ROS) formation, leading to membrane damage. ROS possibly induced by active components of Khat or by pesticides added to the Khat tree. In addition, the reduction of SOD and CAT is indicative to cellular proteins damage which occurred by ROS. As well, the elevation of GST may due to a leakage of cellular GST to blood stream; this implies that GST active site was not affected. This study concludes that daily chewing Khat for long period certainly induce ROS production, leading to oxidative toxicity. Both enzymatic and non-enzymatic antioxidants are involved in the protection against this toxicity. People who habitually chew Khat for long term will be susceptible to the oxidative toxicity; therefore, they recommended giving up of Khat chewing.

Detection of *Citrus psorosis virus* Using an Improved One-Step RT-PCR

- Asmae Achachi , Mohamed Haïssam Jijakli , Elmostafa El Fahime , Abdelmajid Soulaymani , Mohammed Ibriz

Abstract:

We report an improvement of a one-step reverse transcription polymerase chain reaction (RT-PCR) assay for the detection of *Citrus psorosis virus* (CPsV; genus *Ophiovirus*) in citrus trees. Two different sample preparation procedures were compared. The data showed that when using total RNA extracted by the Qiagen procedure more virus isolates could be detected compared with the Trizol procedure. Three pairs of primers reported in literature and selected within the coat protein (CP) region of the virus were used for a reliable detection of CPsV. Only one primer pair has been able to detect all CPsV isolates collected from different citrus regions of Morocco. In addition, this report showed that one-step RT-PCR was more sensitive and more consistent than ELISA test. Notably, 15 out of 22 samples tested positive by one-step RT-PCR could not be detected by Mab Ps29, thus providing a degree of differentiation among isolates. The detection protocol described in this study could be used in citrus certification programs and to test trees in nurseries and commercial orchards for CPsV infection.

Exploration of Microbial Diversity of Taptapani (India) Hot Spring Through Molecular Phylogenetic Analysis

- Sudip Kumar Sen , Smita Raut , Soumya Satpathy , Bidyut Bandyopadhyay , Pradeep Kumar Das Mohapatra , Sangeeta Raut

Abstract:

The nature of microbial communities and their relation to enzyme activities in the Taptapani hot spring Odisha, India, is a neglected area of investigation. To address this, the microbial diversity, enzyme activities and the physicochemical factors of the hot spring water were studied. Organisms were identified using the 16S rDNA, 28S rDNA and 18S region of rDNA for bacteria, fungi and algae, respectively, after amplification by the polymerase chain reaction. Phylogenetic analysis of the sequenced gel electrophoresis bands revealed a great diversity of microorganisms. Based on morphology, nine groups of bacteria, three groups of fungi and four groups of algae were dominant during all seasons. The growth behavior of the selected isolates was studied at different elevated temperatures which revealed that all ten isolates (six bacteria, three fungi and one alga) were able to grow in the temperature range of 50–80°C and at pH 4–9. Maximum temperature and pH for the amylase activity were found to be 80 and 9°C, respectively, while an optimum amylase activity was observed at 60°C and pH 9. Considerable activity was observed at both acidic and alkaline pH (4.0–10.0), suggesting wide technical applications for these enzymes and indicating that these enzymes are novel.

Monitoring the Effect of pH on Bacterial Cellulose Production and *Acetobacter xylinum* 0416 Growth in a Rotary Discs Reactor

- Khairul Azly Zahan , Norhayati Pa'e , Ida Idayu Muhamad

Abstract:

Bacterial cellulose demonstrates unique properties including high mechanical strength, crystallinity and water retention ability that are suitable for industrial applications, such as food, paper manufacturing and pharmaceutical. In this study, *Acetobacter xylinum* 0416 was cultured in a designed 10-L Rotary Discs Reactor (RDR) to produce bacterial cellulose. The effects of different pH in the range of 3.5–7.5 on the bacterial cellulose production and the bacterial growth were investigated. The highest yield was obtained at pH 5.0 with a total dried weight of 28.3 g, while the lowest yield was obtained at pH 3.5 with a total dried weight of 4.7 g. Results also showed that the dried weight of bacterial cellulose was 60% higher when the pH of the medium was controlled during the experiments compared with uncontrolled pH. In addition, *A. xylinum* 0416 growth decreased to around 30% when pH value dropped from 5.05 to 3.56. The results also proved that the formation of acetic acid as a by-product caused the pH to drop during fermentation process in 10-L RDR.

Optimization of Critical Medium Components for Protein Production by *Nostoc ellipsosporum* Using Response Surface Methodology

- Deepika Mittal , Anshuman Srivatsava , Sharmila Govindasamy ,
Muthukumaran Chandrasekaran

Abstract:

Nostoc ellipsosporum is a pharmaceutically important cyanobacteria known for the production of anti-HIV drug called cyanovirin. A five-level four factor central composite design of response surface methodology (RSM) was performed to identify the optimal level for maximum protein production. Four medium variables, PHA extract, glucose, Fe-EDTA and micronutrients, were chosen for RSM optimization study. Experimental data were analyzed by regression, and model equation was constructed. Maximum protein production was expected at the predicted optimal level of PHA extract, 2.50 % (v/v), glucose, 0.05 % (w/v), Fe-EDTA, 0.125 % (v/v) and micronutrients, 0.125 % (v/v). Validation experiment results were in good agreement with the results predicted by RSM. Results of this study showed that optimization by RSM approach improves the protein production, and also PHA extract was found to be a significant medium component for enhancing the protein synthesis.

Influence of Extraction Duration on the Chemical Composition and Biological Activities of Essential Oil of *Thymus pallezens* de Noé

- Otmane Benchabane, Mohamed Hazzit , Fazia Mouhouche, Aoumeur Baaliouamer

Abstract:

Thymus pallezens de Noé essential oils were isolated from the plant's dried leaves, by hydrodistillation, with different distillation times (30 min, 1, 2 and 3 h). The essential oils compositions and their antioxidant, antimicrobial and insecticidal activities were examined. The chemical compositions of the oils were determined by GC and GC-MS. The antioxidant capacity was tested by four complementary methods: thiobarbituric acid reactive substances, ferric reducing power and scavenging of radicals DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) and ABTS^{•+} [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)]. The antimicrobial activity was tested against three bacteria, two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and one gram-negative (*Escherichia coli*) and one yeast (*Candida albicans*). The insecticidal activity against confused flour beetle *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) was evaluated using direct contact application and fumigant method. The predominant components in the oils were carvacrol (63.3–68.2 %), γ -terpinene (6.9–9.1 %), *p*-cymene (6.2–10.3 %) and thymol (6.5–7.5 %). The antioxidant activities were dependent on the time of hydrodistillation and the method used. The essential oils extracted for 30 min and 1 h were the most efficient against all the bacteria used. Toxicity tests against *T. confusum* showed that the oils isolated for 3 h and 30 min caused highest mortality with contact method and fumigant test, respectively. The results suggest that distillation time can determine the value of the oil designed to be applied to any biological material.

Biosorption and Bioaccumulation of Copper and Lead by Heavy Metal-Resistant Fungal Isolates

- Shazia Iram, Rabia Shabbir , Hunnia Zafar , Mehwish Javaid

Abstract:

Microorganisms play an important role in the bioremediation of heavy metal-contaminated wastewater and soil. In this research, isolation of heavy metal-resistant fungi was carried out from wastewater-treated soil samples of Hudiara drain, Lahore. The purpose of the present investigation was to observe fungal absorption behavior toward heavy metal. The optimum pH and temperature conditions for heavy metal removal were determined for highly tolerant isolates of *Aspergillus* spp. along with the initial metal concentration and contact time. Biosorption capacity of *A. flavus* and *A. niger* was checked against Cu(II) and Pb(II), respectively. The optimal pH was 8–9 for *A. flavus* and 4–5.4 for *A. niger*, whereas optimal temperature was 26 and 37 °C, respectively. Moreover, the biosorption capacity of *A. flavus* was 20.75–93.65 mg g⁻¹ for Cu(II) with initial concentration 200–1400 ppm. On the other hand, biosorption capacity of *A. niger* for Pb(II) ranged from 3.25 to 172.25 mg g⁻¹ with the same range of initial metal concentration. It was also found that equilibrium was maintained after maximum adsorption. The adsorption data were then fitted to Langmuir model with a coefficient of determination >0.90. The knowledge of the present study will be helpful for further research on the bioremediation of polluted soil.

Evaluation of Emulsification Index in Marine Bacteria *Pseudomonas* sp. and *Bacillus* sp.

- Fatemeh Shahaliyan , Alireza Safahieh , Hajar Abyar

Abstract:

In the present study, two bacteria *Pseudomonas* sp. and *Bacillus* sp. resistant to the crude oil were isolated from the Khor Musa sediments, Persian Gulf, Iran. Hemolysis, oil spreading and drop collapse methods were applied to measure the ability of bacteria in producing the biosurfactant. Eventually, the bacterial emulsification activity was reviewed in the presence of crude oil and kerosene. *Pseudomonas* sp. showed a clear zone around a colony on blood agar medium and made a 2.7-cm-diameter halo. The results of the emulsification test for crude oil and kerosene indicated that *Pseudomonas* sp. emulsified approximately a half of the kerosene within 2 h, whereas the rate of the kerosene emulsification was lower in *Bacillus* sp. as emulsification percentage reached to 41.2 % within 48 h. Moreover, the crude oil emulsion was only observed in *Pseudomonas* sp. The results of this article suggested using the ability of *Pseudomonas* sp. in producing surfactant to accelerate the hydrocarbons bioremediation.

Micropollutants in Sewage Sludge: Elemental Composition and Heavy Metals Uptake by *Phaseolus vulgaris* and *Vicia faba* Seedlings

- M. Smiri , S. Elarbaoui , T. Missaoui , A. Ben Dekhil

Abstract:

Sludge is a major economic and environmental concern, and it can be reused for agricultural purposes. The germination success of seed and early growth regulation of plants treated with sludge requires more attention. The study evaluated the influence of municipal sewage sludge (0 and 5%) containing Cd, Co, Cu, Ni, Pb, and Zn, obtained from several sites and sampling dates, on germination and growth of bean (*Phaseolus vulgaris* L.) and Faba bean (*Vicia faba* L.) incubated at 25°C in the dark. Water uptake, reserve mobilization, and mineral nutrition in germinating *P. vulgaris* and *V. faba* seedlings were analyzed. Among studied heavy metals, zinc concentration was highest in sewage sludge ($450 \pm 5 \text{ mg kg}^{-1} \text{ DW}$), while cadmium concentration was lowest ($0.54 \pm 0.1 \text{ mg kg}^{-1} \text{ DW}$). The contents of Co, Cu, Ni, and Pb in the sewage sludge were about 5 ± 0.5 , 104 ± 4 , 17 ± 0.4 , and $35 \pm 0.5 \text{ mg kg}^{-1} \text{ DW}$, respectively. Heavy metal concentrations were relatively similar independent of sewage sludge site (S1; S2; S3). Concentration of Zn is 85 ± 2 and $124 \pm 2 \text{ } \mu\text{g g}^{-1}$ dry weight in *P. vulgaris* and *V. faba* controls, respectively, to about 127 ± 2 and $148 \pm 2 \text{ } \mu\text{g g}^{-1}$ in both plants treated with sewage sludge. Metal amounts in this sewage sludge application rate (5%) are not toxic for germination process and growth of two tested leguminous plants. In this context, we shall try to use the 5% sewage sludge for seed germination. It appears that treatment of seed with the 5% sewage sludge was beneficial to germination and early plant development.

Role of *Bacillus subtilis* and *Pseudomonas aeruginosa* on Corrosion Behaviour of Stainless Steel

- Hafiz Zeshan Wadood , Aruliah Rajasekar , Yen-Peng Ting , Anjum Nasim Sabari

Abstract:

Corrosion behavior of SS304 in minimal salt medium with 1.5 % NaCl as a corrosive agent in presence of *Bacillus subtilis* strain S1X and *Pseudomonas aeruginosa* strain ZK has been investigated. Electrochemical techniques such as Tafel polarization and electrochemical impedance spectroscopy with surface analytical techniques like atomic force microscopy, scanning electron microscopy–energy dispersive spectrum analysis and Fourier transform infrared spectroscopy showed that both bacteria inhibit corrosion of SS304 due to the development of a protective biofilm on metal surface. The pH values of bacterial-inoculated systems decreased with increasing incubation time showing the production of some acidic metabolites by bacterial isolates.

Dietary Toxicity of Lead and Hyper-Accumulation in *Petroselinum crispum*

- Jamshaid Hussain , Wajeeha Saeed , Tatheer Alam Naqvi , Mohammad Maroof Shah , Raza Ahmad , Amjad Hassan , Qaisar Mahmood

Abstract:

The current study investigated the bioaccumulation potential of *Petroselinum crispum* (Parsley) for lead (Pb) contaminated soils. Different concentrations of lead nitrate (0, 200, 600, 1,000 and 1,200 mg kg⁻¹ soil) were applied to the soil, and Parsley plants were grown in contaminated soil for a period of 3 months. One set of treatments was supplemented with EDTA as chelating agent to enhance the Pb uptake. The growth parameters, phytotoxic effects and Pb accumulation in different parts of experimental plants were recorded. Increasing Pb concentrations in soil caused delay in germination rate, decrease in plant height, root length and fresh and dry weight. The Pb accumulation in roots, shoots and seeds of treated plants linearly increased with Pb concentration in soil. For the highest treatment, Pb accumulation in roots and leaves was recorded to be 641 and 439.5 mg kg⁻¹ dry weight (DW) over 20.5 and 17.25 mg kg⁻¹ DW of control plants, respectively. At higher Pb treatment, the addition of 10 mmol EDTA had a significant effect on Pb accumulation in plants. Although Parsley seems to be a promising candidate to reclaim Pb-contaminated sites, being edible plant dietary toxicity of the plant needs to be seriously considered.

Response Surface Methodology Mediated Modulation of Laccase Production by *Polyporus arcularius*

- M. Jegatheesan, M. Eyini

Abstract:

Screening of thirteen mushroom fungal isolates collected from Alagar Hill and Thandikudi Hill of Tamil Nadu, India, for laccase production revealed the high laccase production potential of the isolate MI 51. The mushroom isolate MI 51 was identified as *Polyporus* sp., based on sporophore (fruiting body) morphology and spore characteristics. Molecular identification of the fungal isolate MI 51 using primer ITS1 and ITS4 showed that isolate MI 51 shared 98 % sequence similarity with *Polyporus arcularius*, a basidiomycete fungus. Initial screening of production parameters using Plackett–Burman design helped in identifying the system variables/parameters which directly influenced laccase production. Laccase production by *P. arcularius* was optimized using central composite design (CCD) of experiments and response surface methodology (RSM) by studying influence of the growth supplements and media additives on modulating laccase production by *P. arcularius*. High laccase production by *P. arcularius* growing in basal salt medium, (9.30 IU/ml), was observed in 21 days of incubation. Results from experiments designed by CCD indicated that nearly threefold increase in laccase production (28.30 IU/ml) over the control experiments in basal salt medium could be obtained earlier at 15 days of incubation by increasing the content of the nitrogen source, yeast extract to 0.5 g/l, and by adding 250 μ M CuSO₄ to the basal salt medium. RSM plots showed high interaction between the variables, incubation time, yeast extract concentration, and CuSO₄ concentration in the medium.

Statistical Analysis of Cultural Parameters Influencing Delta-Endotoxins and Proteases Productions by *Bacillus thuringiensis kurstaki*

- Karim Ennouri, Hanen Ben Hassen, Nabil Zouari

Abstract

Bacillus thuringiensis is a spore-forming bacterium that produces delta-endotoxins, which are toxic to lepidopterans, dipterans and coleopterans. Concomitantly with production of delta-endotoxins, *B. thuringiensis* produces proteolytic enzymes during growth and sporulation, affecting yields of toxins production. In this work, an experimental factorial design was employed for the dual evaluation of effects of delta-endotoxins and proteolytic enzyme on activity of a *B. thuringiensis* subsp. *kurstaki* strain. Main effects and interactions of three factors (soybean meal, starch and aeration) under two conditions (0 and 7 g/l NaCl) were analysed using statistical techniques. Regression models, known as useful tool to build relationships between variables and subsequently to make predictions, were suggested to fit the experimental data. Yields ranged between $6.10\text{--}15.76 \times 10^{-7}$ μg and $18.18\text{--}30.30 \times 10^{-7}$ IU for delta-endotoxin and proteolytic enzymes per cell, respectively. When NaCl (7 g/l) was added to the medium, yields ranged from 15.51 to 38.06 for delta-endotoxins and from 9.56 to 48.73 for proteases. Starch, soybean meal and aeration improved the delta-endotoxins production. Interactions (starch \times air) and (soybean meal \times air) showed a negative effect on delta-endotoxin yield, while (starch \times soybean meal) had no effect. The active function of aeration was enhanced when NaCl (7 g/l) was added, and effects of all interactions on delta-endotoxins yield had been nullified. Soybean meal and aeration had negative effects on proteolytic activity, in contrast to starch, (starch \times air) and (soybean \times air), which improved proteolytic activity yields. The inhibitory role of soybean meal shifted to positive effect according to the presence of NaCl (7 g/l) in the culture medium.

Purification and Characterization of Extracellular Protease and Amylase Produced by the Bacterial Strain, *Corynebacterium alkanolyticum* ATH3 Isolated from Fish Gut

- Goutam Banerjee, Sandip Mukherjee, Shelley Bhattacharya, Arun K. Ray

Abstract:

The use of enzymes in different industrial sectors increased significantly due to huge industrialization. Different types of protease and amylase are randomly used in industries including food, textile, and paper. For this purpose, purification of extracellular protease and amylase produced by the bacterium, *Corynebacterium alkanolyticum* ATH3 (Acc. No. JX656749) isolated from the distal intestine of a freshwater fish, *Anabas testudineus*, was carried out using column chromatography. The specific activity of protease and amylase significantly increased with each step of purification and finally became 93.73 and 88.1 U/mg protein with a purification fold 26.03 and 44.94, respectively. The sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis showed molecular weight of purified protease and amylase was ~17 and ~28 kDa, respectively. To the authors' knowledge, this is the first report of ~17 kDa protease and ~28 kDa amylase from the bacterial strain *C. alkanolyticum* ATH3. Further, enzyme activity was also evidenced by zymography analysis. The enzymes acted optimally at pH 7.5–8.0 and temperature 35–45 °C, respectively. So, due to cheapest source, these two enzymes are very important for various purposes in industrial sectors.

Biochemical Biomarkers in the Halophilic Nanophytoplankton: *Dunaliella salina* Isolated from the Saline of Sfax (Tunisia)

- Taheni Belghith, Khaled Athmouni, Jannet Elloumi, Wassim Guermazi, Thorsten Stoeck, Habib Ayadi

Abstract

The purpose of the present work was to study the potential biotechnological use of *Dunaliella* species isolated from the solar saltern of Sfax (Tunisia). *D. salina* was grown in artificial seawater (3.4M NaCl) under a constant temperature (25°C) and light ($265\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The maximal cellular density was about 4.66×10^6 cells/ml, and the growth rate ranged between 0.13 and 0.16 doublings/day. Chlorophyll *a* reached $52.32 \pm 0.12 \text{ mg/l}$ in the exponential phase. Protein and carbohydrates content reached maximum values in the lag phase (52.4 ± 0.2 and $5.7 \pm 0.34 \text{ mg/l}$, respectively). The maximal values of total lipids content were obtained at the decline phase ($92 \pm 0.87 \text{ mg/l}$). In addition, during this study, the analyses of the fatty acids profile have shown the presence of essential fatty acids that accumulate in *D. salina* during the stress phase.

Diversity of Silver Nanoparticle Synthesizing Actinobacteria Isolated from Marine Soil, Tamil Nadu, India

- A. Ranjani, P. M. Gopinath, K. Rajesh, D. Dhanasekaran, P. Priyadharsini

Abstract

Marine actinobacteria have been considered as a potential source of bioactive compounds holding a prominent position as targets in screening programs due to their diversity and their proven ability to produce novel metabolites and other molecules of pharmaceutical importance. In this regard, an attempt was made to study the diversity of actinobacteria and its ability to synthesize silver nanoparticles in Ramanathapuram and Thoothukudi districts of Tamil Nadu. A total of 1143 actinobacteria were isolated, out of which 49 were morphologically distinct based on the spore color, mycelia formation and pigment production. The morphologically distinct isolates were characterized and identified using light microscope as *Streptomyces* sp. (16), *Nocardiopsis* sp. (8), *Kitasatosporia* sp. (7), *Actinopolyspora* sp. (2), *Thermoactinomyces* sp. (5), *Actinomadura* sp. (4), *Kibdelosporangium* sp. (3), *Saccharopolyspora* sp. (3) and *Thermomonospora* sp. (1). The correlation coefficient analysis between the isolates and the physicochemical parameters of the soil revealed positive correlation with nitrogen. Silver nanoparticles production by the green chemistry approach was investigated using the isolated marine actinobacteria which showed that 25 isolates out of 49, synthesized silver nanoparticles.

Ultrasound-Assisted Rapid Extraction of Bacterial Intracellular Medium-Chain-Length Poly(3-Hydroxyalkanoates) (mcl-PHAs) in Medium Mixture of Solvent/Marginal Non-solvent

- K. A. Ishak, M. S. M. Annuar, T. Heidelberg, A. M. Gumel

Abstract

Intracellular medium-chain-length poly(3-hydroxyalkanoates) (PHAs) produced by *Pseudomonasputida* Bet001 were extracted in an ultrasound-assisted process. A mixture of acetone (solvent) and heptane (marginal non-solvent) was used as the extraction medium. The effects of volumetric energy dissipation, extraction medium ratio and irradiation time on the extraction process were investigated. Sonication frequency of 37 kHz and heptane as marginal non-solvent facilitated the process. Following optimization, high PHA extraction rate of $74 \times 10^{-3} \text{ g PHA g}^{-1} \text{ g}^{-1} \text{ dried biomass min}^{-1} \text{ min}^{-1}$ was observed at ultrasonic energy output of $1151 \pm 3 \text{ Jml}^{-1}$ with 50:50 solvent/marginal non-solvent ratio for irradiation time of 5 min. PHA showed good stability under the rapid extraction process.

Transcriptome Sequencing and Analysis of Wild Pear (*Pyrus hopeiensis*) Using the Illumina Platform

- Ting Ting Liang, Yan Ma, Jing Guo, De Kui Zang

Abstract

Pears are cultivated worldwide as an economically valuable fruit of the Rosaceae. Because of the lack of genomic resources, few molecular biology studies have focused on wild pear, which are less abundant than partially cultivated pear species. However, high-throughput transcriptome sequencing technologies enabled the advent of genomic studies requiring shorter time and minimal costs, allowing efficient wild pear research. *Pyrus hopeiensis* is an endangered and valuable horticultural plant species, and its transcriptome was sequenced in this study. Overall, 25,877,477 high-quality reads and 48,278 unigenes were generated with the Illumina platform from four different tissue samples. A total of 27,005 (55.94 %) unigenes were successfully annotated. Of these, 26,941 and 19,248 unigenes were annotated in the National Center for Biotechnology Information non-redundant (Nr) protein database and the Swiss-Prot protein database, respectively. In addition, 18,590 and 7505 unigenes were assigned to Gene Ontology and Cluster of Orthologous Groups classifications, respectively, and a total of 5104 unigenes were mapped to 123 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Furthermore, 4301 simple sequence repeat markers were identified in *P. hopeiensis*, which will be used in future research of the genetic diversity of *Pyrus*. Finally, the results indicated that lignin biosynthesis, secondary metabolism and suberin/cutin/wax biosynthesis were involved in the russet pericarp formation process of *P. hopeiensis*. Detailed examination of these pathways revealed 54 candidate genes were predicted based on the KEGG pathways, which encoded 10 key enzymes involved in lignin biosynthesis; moreover, 33 additional regulatory genes for pigmentation of wild pear russet pericarp were also summarized. In summary, this effective RNA-Seq study provides comprehensive genetic and genomic insight into the endangered *P. hopeiensis* species, which will improve future studies on the molecular mechanism of the appearance and quality of this fruit.

Environmental Epidemiology of Cancer in South Asian Population: Risk Assessment Against Exposure to Polycyclic Aromatic Hydrocarbons and Volatile Organic Compounds

- Muhammad Manzoor, Aqib Hassan Ali Khan, Rahim Ullah, Muhammad Zuhaib Khan, Irshad Ahmad

Abstract

Environmental epidemiology is a scientific approach used in risk assessment of a disease in a population. Despite of its merits, the proper aetiological explanation for a root cause is still not possible or very difficult to assess, but it offers an attractive strategy for the identification of root causes to health issues. Polycyclic aromatic hydrocarbons and volatile organic compounds are strong risk factors responsible for increasing cancer in South Asian population. Levels of these polycyclic aromatic hydrocarbons and volatile organic compounds are relatively far higher in South Asian region as compared to other regions of the globe. An additional major problem arises due to genetic susceptibility to cancer, because of gene polymorphisms in a large proportion of population. This review focuses on establishing a broad concept regarding an advance method of epidemiology and some key factors discussed regarded as important in evaluation of cancer risk with special reference to South Asian population.

Improved Lactic Acid Production by In Situ Removal of Lactic Acid During Fermentation and a Proposed Scheme for Its Recovery

- Mallika Boonmee, Onanong Cotano, Sittipong Amnuaypanich, Nurak Grisadanurak

Abstract

Extractive fermentation with 155.8 g/L initial glucose and 364 g of anion exchange resin yielded a 1.2-fold increase in total lactate produced and a 5.9-fold increase in productivity compared with standard batch fermentation at the same glucose concentration. The addition of resin also served as a pH control strategy. Elution of the resin-bound lactate using 1 M HCl at 0.1 bed volume/min resulted in complete recovery of lactate. Further extraction of the eluted lactate and lactate in fermentation broth was conducted with reactive extraction using trioctylamine in octanol. The extraction also provided partial purification of the product, as protein from fermentation broth did not migrate to the extracted solution with the lactate. The recovery of lactate was up to 70 % of the total lactate produced.

Biodegradation of Petrochemical Hydrocarbons Using an Efficient Bacterial Consortium: A2457

- Inam Ali Larik, Muneer Ahmed Qazi, Asif Raza Kanhar, Shahida Mangi, Safia Ahmed, Muhammad Rahib Jamali, Nisar Ahmed Kanhar

Abstract

Petrochemical hydrocarbons are considered to be the most significant environmental pollutants and need to be removed. In present study, the biodegradation of used engine oil and diesel oil was achieved under shake flask conditions using an efficient bacterial consortium A2457: comprising bacterial strains of *Stenotrophomonas maltophilia*, *Bacillus cereus* and *Bacillus pumilus*. The strains were isolated and screened by enrichment technique. The bacterial strains were identified on the basis of 16S rRNA gene sequence homology. The percent removal and biodegradation of the petrochemicals was evaluated through UV–Vis spectrophotometer and FTIR spectrometry. The bacterial consortium resulted in significant degradation of diesel oil (94.13 %) and used engine oil (99.77 %) under experimental conditions. FTIR spectra of the hydrocarbons, before and after biodegradation experiments, also revealed significant changes in the characteristic peaks in the wavenumber range of 4000–600 cm^{-1} . The consortium displayed significant biosurfactant production and lipase activity during 28 days of incubation and reduced the surface tension of culture medium to $27.95 \pm 0.3 \text{ mN m}^{-1}$. The petrochemical hydrocarbon degradation by the bacterial consortium: A2457 of present study could be attributed with the production of biosurfactants and lipases to utilize diesel oil and used engine oil as sole source of carbon and energy.

Bactericidal Property of Macro-, Micro- and Nanocurcumin: An Assessment

- Judy Gopal, Manikandan Muthu, Sechul Chun

Abstract

The bactericidal properties of curcumin are well established. In the current communication, we have studied the effect of macro-, micro- and nanocurcumin on their respective bactericidal properties. The bactericidal property was evaluated through interacting curcumin with four predominant pathogenic bacteria. The results demonstrated a fivefold enhancement in bactericidal activity by nanocurcumin. CLSM was instrumental in understanding the mechanism behind the enhanced activity of the nanocurcumin. The higher penetration as shown by the CLSM imaging and the enhanced solubility of the nanocurcumin are speculated to be reasons for the observed superior bactericidal property of nanocurcumin.

16S rDNA Phylogenetic and RAPD–PCR Analyses of Petroleum Polycyclic Aromatic Hydrocarbons-Degrading Bacteria Enriched from Oil-Polluted Soils

- Abd El-Latif Hesham, Sulaiman A. Alrumman, Jawaher A. Al-Amari

Abstract

Petroleum polycyclic aromatic hydrocarbons (PAHs) are health risks to human, as they can be toxic, mutagenic and carcinogenic. As the Kingdom of Saudi Arabia is one of the major petroleum-producing countries, they inevitably suffer from worsening this environmental problem. Therefore, removal of these compounds from the environment is a necessity for ensuring human health. In this study, 54 bacterial isolates were obtained by enrichment techniques from oil-contaminated soil samples. Out of them, seven gram-negative bacterial strains, KKU-J1, KKU-J2, KKU-J4, KKU-J7, KKU-J9, KKU-J14 and KKU-J17, exhibiting ability for petroleum PAHs degradation were selected. The isolates showing the highest growth during screening as demonstrated by the increase in their optical densities (OD_{600}) and a concentration-dependent growth in all examined PAH compounds that grew in it, with strains KKU-J2, KKU-J7 and KKU-J17 were the best. The highest optimum growth rate of 0.333 ± 0.0 , 0.364 ± 0.016 , 0.333 ± 0.0 , 0.364 ± 0.016 and 0.357 ± 0.004 (OD_{600}) 0.357 ± 0.004 (OD_{600}) was recorded for the strains KKU-J2, KKU-J7 and KKU-J17, respectively, when the level of phenanthrene was 100 mg/l. On the other hand, strain KKU-J2 was found to be the best one (0.413 ± 0.0 , 0.413 ± 0.0) when the level of naphthalene was 100 mg/l. Molecular identification of the selected isolates was detected based on 16S rRNA gene amplification and partial sequence determination. Alignment results and the comparison of 16S rRNA gene sequences of the isolates to the 16S rRNA gene sequences available in GenBank database, as well as the phylogenetic analysis, confirmed the accurate position of the isolates as *Sphingomonas paucimobilis* KKU-J1, *Pseudomonas alcaligenes* KKU-J2, *Micrococcus antarcticus* KKU-J4, *Arthrobacter oxydans* KKU-J7, *Stenotrophomonas rhizophila* KKU-J9, *Kocuria rhizophila* KKU-J14 and *Shinella zoogloeoides* KKU-J17. RAPD–PCR fingerprinting was carried out for the seven isolates, and the DNA patterns revealed that there is no correlation between the RAPD profile and geographic origin sites where these isolates were collected from. This study indicates that the contaminated soil samples contain a diverse population of PAH-degrading bacteria, and the use of soil-associated microorganisms could be recommended for PAHs bioremediation in the environment.

Purification and Characterization of a Membrane-Unbound Highly Thermostable Metalloprotease from *Aeromonas Caviae*

- Santhalembi Laishram, Gautam Pennathur

Abstract

The main objective of the current study was to purify and characterize an alkaline thermostable metalloprotease from *Aeromonas caviae*. The enzyme was subjected to a two-step purification scheme using ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ fractionation and ion-exchange chromatography on a DEAE-sepharose fast flow column. The fraction which precipitated with 40–60 % (w/v) of $[(\text{NH}_4)_2\text{SO}_4]$ exhibited the highest enzyme activity. Anion-exchange chromatography resulted in approximately 51-fold purification, with a yield of 64.7 %. The enzyme was successfully purified to homogeneity, and this was confirmed on sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). A single band with an approximate molecular mass of ~42 kDa was observed. The maximum enzymatic activity was observed at pH 8 with azocasein as the substrate, and the enzyme was stable in the pH range of 6–10 after 5 h of incubation. The purified enzyme was active in the temperature range between 50 and 60 °C and showed 80 % loss in enzymatic activity upon heating at 70 °C. The K_m and V_{max} values for the purified protease, with azocasein as substrate under optimal reaction conditions (pH 8 and 50 °C) were 0.74 and 122.4 mg/ml, respectively. The protease was investigated for its application for the degradation of chicken feathers. The high enzymatic activity, pH, and thermal stability of AU04 protease make it an industrially important enzyme.

Assessment of competence of the *Pseudomonas aeruginosa* to solubilize insoluble form of zinc under various cultural parameters

- S. N. Padma Devi, K Sunitha kumari, S Vasandha

Abstract

Efficiency of *Pseudomonas aeruginosa* to solubilize insoluble form of zinc to soluble form was studied under various cultural parameters such as carbon (glucose, fructose, sucrose, maltose and lactose), nitrogen (ammonium sulphate, sodium nitrate, potassium nitrate and urea), pH (5.0, 7.0 and 9.0) and temperature (20, 30 and 40°C). The experimental study was carried out by both qualitative (plate assay) and quantitative (broth assay) methods. The Zn-solubilizing efficiency of the isolate was found more when glucose was added as C-source in both plate (150 %) and broth (16.62 mg/l) assays, and the efficiency was less in the medium amended with sucrose as carbon source in both qualitative (105.8 %) and quantitative (2.56 mg/l) estimation. Among the four nitrogen sources, *P. aeruginosa* showed best solubilizing efficiency (262.5 %) and solubilization of Zn (29.91 mg/l) in the presence of ammonium sulphate and recorded least solubilization efficiency in the presence of sodium nitrate as nitrogen source in both plate (181.82 %) and broth (21.92 mg/l) assays. It showed highest solubilization of zinc from ZnO at the incubation temperature of 30°C, and the activity was less at the temperature of 40°C. pH 7 was the most favourable pH for solubilization, and the efficacy was least when the pH reached its alkalinity. Thus the study confirmed the efficiency of *P. aeruginosa* to solubilize insoluble form of zinc and can be used as a source of bioinoculants to eradicate zinc deficiency in plants.

Phytochemical and Antimicrobial Properties of *Tamarix aphylla* L. Leaves Growing Naturally in the Abha Region, Saudi Arabia

- Sulaiman Abdullah Alrumman

Abstract

In this study, antimicrobial activities of some solvent extracts of fresh and dry leaves of *Tamarix aphylla* against eight clinical isolates of bacteria and *Candida* and the chemical compounds present in the hexane extract were determined. The results showed that cold-water extract has no antimicrobial effect against any of the microbes tested, while hot-water extract showed 75 % antimicrobial activities against the same microbes. Extracts involving methanol, chloroform, petroleum ether, acetone and diethyl ether of dry and fresh *T. aphylla* leaves showed a 100 % lethal effect against all the tested pathogenic microbes. Additionally, superior performance has been observed in the cases of methanol extract against *Proteus mirabilis*, chloroform and diethyl ether extracts against *Klebsiella oxytoca*, petroleum ether extract against *Candida* sp. compared with the standard antibiotic drug. The GC–MS analysis revealed that the leaves contain ten different chemical compounds; propenoic acid (28.99 %) and beta-d-mannofuranose (23.04 %) are found to be dominant. In conclusion, extraction methods using polar and nonpolar solvents of *T. aphylla* leaves showed great antimicrobial effect on pathogenic tested microbes. This could be used in the treatment of infectious diseases caused by multidrug-resistant bacterial strains “that are resistant to multidrug treatment” and *Candida* sp. From the pharmaceutical viewpoint, the identified phytochemical in the *T. aphylla* is reported to be biologically important and needs further investigation with regard to isolations to develop the safe and cheap drugs.

Isolation of Phosphorus-Solubilizing Fungus from Soil to Supplement Biofertilizer

- Rubina Nelofer, Quratulain Syed, Muhammad Nadeem, Farzana Bashir, Sania Mazhar, Ammara Hassan

Abstract

A number of bacterial and fungal isolates from soil were screened for phosphorus solubilization. One fungal strain was found to be efficient phosphorus solubilizer in Pikovskaya medium. The selected strain was identified as *Aspergillus niger* based on morphological and microscopic studies. Some important cultural conditions were optimized, and optimal conditions for temperature, glucose, ammonium sulfate and pH were found 30 °C, 20, 2 and 5 g/L, respectively, in 48 h of incubation. The fungal strain produces organic acids as well as phosphatases and phytases that make the medium clear within 48 h by solubilizing the added tricalcium phosphate. The soluble phosphorus concentration of medium decreased after 48 h of incubation, possibly due to the consumption of phosphorus by the fungal mycelium itself.

Decontamination of *Streptococcus pyogenes* and *Escherichia coli* from Solid Surfaces by Singlet and Triplet Atmospheric Pressure Plasma Jet Arrays

- Abasalt Hosseinzadeh Colagar, Omid Alavi, Samaneh Motallebi, Farshad Sohbatzadeh

Abstract

In this work, we studied the manipulation of bactericidal effects of non-thermal atmospheric pressure plasma jet on a gram-positive (*Streptococcus pyogenes*) and a gram-negative (*Escherichia coli*) bacteria. Decontamination of *S. pyogenes* and *E. coli* was assayed by measurement of the growth inhibition zones on solid surface. Singlet and triplet argon jets were employed to manipulate the bactericidal effect of the jet arrays. The distance effect of the contaminated plates to the plasma nozzle was analyzed, too. It was concluded that the jet distance to the sample could alter the state of downstream plasma jet and in turn changes the growth inhibition zone cross section, significantly. On the other hand, by scaling the inhibition zones, we showed that each plasma jet enabled to disinfect a surface with two orders of magnitude larger than that of the contact jet cross section in 150-s treatment. The results of this work could be used in developing atmospheric pressure plasma jet arrays for extended surface decontamination.

Phytochemical Analysis of Methanolic Extracts of *Artemisia absinthium* L. 1753 (Asteraceae), *Juniperus phoenicea* L., and *Tetraclinis articulata* (Vahl) Mast, 1892 (Cupressaceae) and evaluation of their biological activity for stored grain protection

- Yasmine Dane, Fazia Mouhouche, Ramon Canela-Garayoa, Antoni Delpino-Rius

Abstract

Research is increasingly focused on identifying alternatives to the use of chemicals for the management of pests in stored grains. In this context, we tested the insecticidal and antifungal activities of methanolic extracts of *Artemisia absinthium* L. (Asteraceae), and two Cupressaceae species, namely *Juniperus phoenicea* L. and *Tetraclinis articulata* (Vahl) Mast., collected in the north of Algeria, against *Sitophilus oryzae* L. (Coleoptera, Curculionidea)—a pest of primary infestation of stored grains—and *Fusarium* sp., mycotoxigenic fungi. The extracts were analyzed by UPLC-PDA-MS. The extraction yield was in the range of 11–21 %. UPLC chromatographic data showed the presence of several phenolic acids, flavonoid glycosides, and flavonoid aglycones. Insecticidal activity was evaluated by means of contact toxicity test, which showed that all three extracts were highly effective. Pest mortality was recorded after 24 h for *A. absinthium*. The LC50 results revealed that the most toxic extract was that of *A. absinthium* (15.53 mg/cm²), followed by that of *J. phoenicea* (22.14 mg/cm²), and *T. articulata* (24.05 mg/cm²). The antifungal activities of these extracts were evaluated using the poisonous medium technique on potato dextrose agar medium. All three extracts reduced the growth of *Fusarium culmorum* and *F. graminearum* under in vitro conditions. *T. articulata* had the greatest inhibitory effect (55.8 %) on mycelial growth of the former. Our results reveal the potential of plant methanolic extracts to control pests common to stored grains.

Cloning and In Silico Analysis of a High-Temperature Inducible Lipase from *Brevibacillus*

- A. K. Panda, S. P. S. Bisht, A. K. Panigrahi, S. De Mandal, N. Senthil Kumar

Abstract

The genus *Brevibacillus* comprises diverse collection of gram-positive/gram-variable, endospore-forming, rod-shaped, aerobic/facultative anaerobic bacteria inhabitant of varied environmental habitats contributing many industrial enzymes. A thermophilic lipolytic bacterium was previously isolated from a hot spring of Orissa, India, and identified as *Brevibacillus* sp. AK-P2 via 16S rRNA gene technology. A 750-bp lipase gene from *Brevibacillus* sp. AK-P2 was isolated and sequenced, which encoded a deduced polypeptide of 250 amino acid residues. The comparative analysis of amino acid composition among thermostable and mesostable homologue of *Brevibacillus* lipases ascertains the role of neutral, charged, and aromatic amino acid residues in thermostability. Three amino acid residues Ser 94, His 242, and Asp 213 were identified from this putative lipase of *Brevibacillus* sp. AK-P2 as catalytic triad. The consensus 'P-loop' motif (-[AG]-X4-G-K-[ST]-) previously reported for *Bacillus* thermoalkalophilic lipases is modified to (-[AT]-[GE]-X5-G-[RQ]-[S]-) in *Brevibacillus* lipases. Multiple sequence alignment (MSA) revealed that there is a methionine residue (M) in the oxyanion hole consensus sequence of thermostable *Brevibacillus* lipases. The frequency of occurrence of AXXXA motif is more for thermophilic *Brevibacillus* lipases than their mesophilic counterparts ensures strong van der Waals interaction and stabilization of proteins. Higher percentage of Alanine (A) in thermophilic *Brevibacillus* lipases attributed toward thermostabilization of lipases. Codon usage analysis revealed that there was intermediate codon usage bias in lipase-coding genes, and the same supports our hypothesis that GC mutation pressure might determine codon usage bias.