Optimization of *Brucella abortus* Fermenter Cultural Conditions and LPS Extraction Method for Antigen Production

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We modified *Brucella* fermentation medium from FAO/WHO for enhancing of *Brucella abortus* S99 biomass. The modified media composed of 15 g L^{-1} peptic digest animal tissue, 15 g L^{-1} pancreatic digest of casein, 10 g L^{-1} yeast extract and 0.10 g L^{-1} sodium bisulphate. Glucose was added during the incubation by fed-batch method (1-30 g L^{-1}). Agitation speed and air flow rates were controlled at 300-500 rpm and 4-8 L min^{-1}, respectively. Cell density was 9-10% and viable count was 3-3.3×10^{11} mL^{-1}. The modified conditions enhance the biomass production to more 2 times than the FAO/WHO method. Three methods were accomplished for LPS production: Extraction by butanol with enzymatic digestion, hot phenol extraction with enzymatic digestion, modified hot phenol with trichloroacetic acid (TCA) procedure. Yield of LPS extraction was 0.2, 0.8 and 1.3%, respectively. Method III results in a greater yield of LPS which is 6 and 1.5 times the yields of methods I and II, respectively. Protein contamination of LPS was <2, <2 and <2.9% and nucleic acid contamination of LPS was <1, <1 and <1.4%, respectively. The ketodeoxyoctonate content of LPS (in each of the three methods) was in agreement with ketodeoxyoctonate values obtained previously for highly purified LPS of *B. abortus*. According to present study, hot phenol with trichloroacetic acid (TCA) procedure is the most suitable procedure for large-scale LPS production from Brucellae, which can be employed for the production of *Brucella* biomass for vaccine and antigen preparations. *(Research Journal of Microbiology 3 (1): 1-8, 2008;* ***doi:*** *10.3923/jm.2008.1.8)*

Optimization of Pectinase Production from *Manihot utilissima* by *Aspergillus niger* NCIM 548 Using Statistical Experimental Design

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The effect of nutritional ingredients on pectinase production was studied using *Aspergillus niger* NCIM 548 in submerged fermentation. Substrate concentration (Tapioca starch), C/N ratio (Glucose/Ammonium sulphate), salt concentration (Potassium dihydrogen ortho phosphate) produced high pectinase yields and were

selected for optimization. A response surface methodology using the Box-Behnken design was used in the design of experiments and in the analysis of results. The maximum productivity of pectinase under optimum conditions was 22.87 U mL^{-1}. Tapioca starch concentration 3.71% w/v, C/N ratio 5.94 and salt concentration 0.256% w/v were found to be optimum for pectinase production. This method was efficient because only 15 experiments are necessary to assess these conditions and the model accuracy was very satisfactory, as the coefficient of determination was 0.984. *(Research Journal of Microbiology 3 (1): 9-16, 2008;* ***doi:*** *10.3923/jm.2008.9.16)*

Occupational Exposure of Buffalo Gynaecologists to Zoonotic Bacterial Diseases

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In the present study, gynaecological examinations were carried out on 916 buffaloes and samples of vaginal swabs, blood and milk were collected. Serum samples were checked for brucellosis and assayed for progesterone level. Vaginal swabs and milk samples were examined for zoonotic bacteria that may be transmitted to veterinarians during handling and examination of these animals during the different phases of the reproductive cycle. 1.09% of the serum samples were positive for brucella antibodies. Zoonotic bacteria were isolated from vaginal swabs (*E. coli*, *Y. enterocolitica*, *Klebsiella* sp., *E. faecalis*, *S. aureus* and *Bacillus* sp.) and milk samples (*E. coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Serratia marescens*, *S. aureus* and *Streptococcus agalactiae*). PCR analysis showed that *E. coli* O157 and O119 isolated from animal suffering from ovarian inactivity were positive for the toxigenic genes (*VT-II*, *stx-2* and *eae-A*). It can be concluded that risk of development of a zoonotic disease can be lessened by early recognition of infected animals, proper animal handling, basic biosecurity precautions and most importantly, personal hygiene. *(Research Journal of Microbiology 3 (1): 17-23, 2008;* ***doi:*** *10.3923/jm.2008.17.23)*

Production of β-D-Galactosidase from Whey Using *Kluyveromyces marxianus*

Parmjit S. Panesar

β-D-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23), most commonly known as lactase is widely distributed in nature and has wide scope in food industry, because of its applications in the production of lactose hydrolyzed

milk and low lactose dairy products. The present study reports the use of whey as the fermentation medium for the production of β-D-galactosidase using a culture of *Kluyveromyces marxianus* as the producer organism. The effect of different process parameters such as pH of the medium, temperature, inoculum size, age of inoculum, agitation and incubation time was monitored to enhance the production of β-D-galactosidase. The maximum enzyme activity was observed with pH 5.0, temperature 30°C, inoculum size 6% (v/v) having 20 h age, under shaking conditions 100 rpm after 28 h of incubation. *(Research Journal of Microbiology 3 (1): 24-29, 2008;* ***doi:*** *10.3923/jm.2008.24.29)*

Effect of Temperature and Storage Period on the Constituents of Milk Inoculated with *Pseudomonas aeruginosa*

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The effect of incubation temperature and storage period on inoculated milk by *Pseudomonas aeruginosa* which well known as milk-spoilage microorganism was studied. Sterile milk sample were inoculated by 10^8-10^9 cfu mL^{-1} *P. aeruginosa* and stored at 0, 7, 12, 21, 37 and 45°C. The chemical analysis was conducted daily for all stored milk samples to investigate the effect of bacteria on milk constituents (fat%, protein% and acidity) and total bacterial count. The present results showed that milk which inoculated with *P. aeruginosa* and stored at different storage periods and temperatures, showed variations for fat, protein, acidity and bacterial count. Moreover, the inoculated milk samples showed a shelf life of 1-2 days at 37 and 21°C. However, the milk samples stored at 0, 7 and 12°C showed shelf life that ranged between 4-9 days. The present study concluded that the number of psychrotrophic bacteria significantly affected by both storage period and incubation temperature. *(Research Journal of Microbiology 3 (1): 30-34, 2008;* ***doi:*** *10.3923/jm.2008.30.34)*

Fumaric Acid Production by *Rhizopus oryzae* on Corn Distillers' Grains with Solubles

Thomas P. West

Three strains of the fungus *Rhizopus oryzae* were screened for their ability to produce fumaric acid on untreated or treated corn distillers' dried grains with solubles. The treatment of the grains included autoclaving alone or with low levels of sulfuric acid. After fungal growth, the grains were processed and fumaric acid production was assayed enzymatically while biomass production was determined

gravimetrically. It was found that fumaric acid production by the three strains of *R. oryzae* after 240 h was higher on the autoclaved grains or acid-hydrolyzed grains compared to the untreated grains. Biomass production by *R. oryzae* ATCC 20344 after 240 h on the untreated and treated grains was higher than observed for *R. oryzae* ATCC 10260 and ATCC 52918. Fumaric acid productivity was higher for all three strains grown on the acid-hydrolyzed grains relative to their productivity on the untreated or autoclaved grains. *(Research Journal of Microbiology 3 (1): 35-40, 2008; **doi:** 10.3923/jm.2008.35.40)*

Microbiological and Physicochemical Characteristics of Cassava Cultivated Soils

D.J. Arotupin and F.A. Akinyosoye

Sixteen soil samples were collected from four different plots of cassava plantation and analyzed for their microbiological and physicochemical characteristics. A total of twelve microorganisms were isolated consisting four bacteria, seven fungi and one actinomyces. The bacteria were *Bacillus cereus*, *B. megaterium*, *B. polymyxa*, *B. subtilis*, while the fungi included *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. repens*, *Botrytis cinerea*, *Neurospora sitophila*, *Varicosporium elodea* and *Actionmyces reticuli*. Plot A recorded the highest microbial counts of 7.95×10^5 cfu g^{-1} and 4.18×10^3 sfu g^{-1} for bacteria and fungi respectively, while the control (uncultivated soils) had the lowest microbial counts of 1.73×10^5 cfu g^{-1} and 1.50×10^3 sfu g^{-1} for bacteria and fungi, respectively. Actinomycetes were found only in plots B and D. The colour of the soils varied from black, brownish black, yellowish brown to complete brown, while the texture ranged from very coarse, through granular to very fine. Chemical analysis revealed pH range of 5.67 to 6.70, moisture content of 10.08 to 14.70%, organic matter content of 8.48 to 13.90% oxidizable organic carbon of 0.11 to 0.41% and ash content of 8.37 to 13.40%. Mineral analysis showed the presence of N, P, K^+, Na^+, Ca^{2+}, Mg^{2+} ppm in varying proportions. Therefore, cassava cultivated soils has not suffered any significant depletion of nutrients. *(Research Journal of Microbiology 3 (1): 41-46, 2008; **doi:** 10.3923/jm.2008.41.46)*

Evaluation of Halophilic Actinomycete *Actinopolyspora* sp. for Osmolyte Production

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Halophilic actinomycetes *Actinopolyspora* sp. was evaluated for osmolyte production. Different concentrations of NaCl were used in the medium for

osmolyte synthesis and the growth started declining from 28th day onwards. Poor growth was noticed in the medium containing 15 and 25% NaCl concentrations. At 20% NaCl concentration, protein content of the strain was higher (14 μg mL^{-1}) on the 20th day and most of the halophilic proteins have been recorded to be higher in content during the late exponential period. SDS-PAGE profile showed a distinct band of protein with a molecular weight 92 kDa on 20% NaCl concentration. *(Research Journal of Microbiology 3 (1): 47-50, 2008;* ***doi:*** *10.3923/jm.2008.47.50)*

Arbuscular Mycorrhizal Status of Indigenous Tree Species Used to Restore Seasonally Dry Tropical Forest in Northern Thailand

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Arbuscular Mycorrhizal (AM) status of native plants in the tropical forest of northern Thailand was surveyed. Twenty four framework tree species, used to forest restoration were examined at 3 sites: FORRU's research tree Nursery (FN), Forest Restoration plot (FR) and Natural Forest (NF). Eleven dominant herb species were examined at 2 sites: Degraded Watershed (DW) and Forest Soil extraction area (FS). Rhizosphere soil samples were collected and AM fungal spores were counted and identified morphologically. Most plant species were intensively colonized by AM fungi except *Cyperus cyperoides*. Twenty four AM species were identified: *Glomus* (15 species), *Acaulospora* (6 species) and *Scutellospora* (3 species). *Glomus rubiforme* was the dominant species. Spore density varied from 16.1 to 97.4 per 100 g soil (averaged 59.7). Spore number at DW and FS were 129 and 479 spores, respectively, with species richness of 6 and 8, respectively. Spore number at FN, FR and NF were 1,152, 2,337 and 1,376 spores, respectively, with species richness of 17, 21 and 15, respectively. The AM diversity was lower in the sites dominated by herbs than in sites examined for trees. In the deforested sites, reduced plant diversity was related with reduced mycorrhizal diversity. In contrast, the trial plot had the highest AM fungal community. Therefore, the forest restoration techniques allow tree species grown in nursery to become AM associated. The association is still maintained after planting out trees in restored area. *(Research Journal of Microbiology 3 (2): 51-61, 2008;* ***doi:*** *10.3923/jm.2008.51.61)*

Effect of Phosphate Solubilizing Bacteria on Nodulation and Growth Parameters of Greengram (*Vigna radiata* L. Wilczek)

A. Vikram and H. Hamzehzarghani

Phosphorus is one of important macronutrients and plays an important role in metabolism of crop plants. In vertisols the availability of P is limited due to the problem of P fixation. Phosphate solubilizing microorganisms have the capability to solubilize P and make it available for plant uptake. In the present study ability of 16 isolates of Phosphate Solubilizing Bacteria (PSB) to promote growth parameters in greengram crop was tested under greenhouse conditions. The study consisted of 18 treatments which were replicated three times. Inoculation of greengram seeds with PSBV-14 recorded the highest nodule number, nodule dry weight, shoot dry matter and total dry matter in greengram plants 45 days after sowing. Similarly, treatment receiving the inoculation of PSBV-13 recorded the highest root length, root dry matter, P content and P uptake in root and shoot in greengram plants. Majority of PSB isolates tested in the present study were able to improve the growth parameters of greengram significantly compared to rock phosphate control and single super phosphate control. Among the various PSB isolates tested, PSBV-4, PSBV-9, PSBV-12, PSBV-13, PSBV-14 and PSBV-15 fared considerably better than the remaining ones. The highly efficient PSBs from the pot trial could be tested for their efficacy in field conditions before recommending them for commercial exploitation. *(Research Journal of Microbiology 3 (2): 62-72, 2008;* ***doi:*** *10.3923/jm.2008.62.72)*

Investigation on Lipase Producing Actinomycete Strain LE-11, Isolated from Shrimp Pond

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Actinomycetes (20 strains), isolated from the sediments of shrimp pond were examined for their lipase activity. Strain LE-11, which was tentatively identified as *Streptomyces griseochromogenes*, showed higher lipase activity and it was taken for further study. Effects of various physical and chemical factors such as pH, temperatures, sodium chloride concentrations, carbon and nitrogen compounds on the lipase activity of *S. griseochromogenes* were studied. It was found that at pH 7, temperature 55°C, 0.05% NaCl concentration, carbon compound mannitol and nitrogen compound L-phenelylanine, the enzyme activity was maximum.

Protein content of the crude enzyme was 2.057 μl mL^{-1}. The crude protein and partially purified protein were run in the SDS-PAGE and a band was found on equal position; however molecular weight of the protein was not determined. The study indicated that *S. griseochromogenes* can effectively be used in large scale production of lipase enzyme for commercial purposes, after ascertaining the strain's ability in large scale fermentations. *(Research Journal of Microbiology 3 (2): 73-81, 2008;* ***doi:*** *10.3923/jm.2008.73.81)*

Antibiogram and Plasmid Profile Analysis of Isolated *Escherichia coli* from Broiler and Layer

Masuder Rahman, Bytul M. Rahman and Bahanur Rahman

A total of 17 *Escherichia coli* isolated from 24 fresh faecal samples of broiler and layer were screened for their antibiograms and plasmid profiles. The overall recovery rate of *E. coli* from faecal samples was 70.83%. All *E. coli* strains were analyzed to determine their susceptibility patterns to 8 commonly used antibiotics (ampicillin, cephradine, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, tetracycline and sulphamethoxazole) belonging to different groups. From the antibiogram study it was revealed that 87.50% *E. coli* isolated from broiler were resistant to both ampicillin and sulphamethoxazole. Only 37.50% broiler isolates were highly sensitive to gentamicin and 50% isolates to chloramphenicol. All the *E. coli* isolates of layer were completely resistant (100%) to sulphamethoxazole and about fifty 5% of the isolates (55.55%) were resistant to both streptomycin and tetracycline. *E. coli* isolated from layer were found to be highly sensitive (44.44%) to chloramphenicol and 66.66% were also highly sensitive to gentamicin. Plasmid profile of 17 isolates was analyzed by 0.8% agarose gel electrophoresis. A total of 8 different plasmid bands of different size were estimated by eye comparing to reference marker. The estimated size of the bands were 3.25, 5.20, 6.00, 8.00, 15.0, 30.0, 33.5 and 38.0 kbp. Plasmid profile analysis of the isolated *E. coli* revealed that the isolates carrying multiple plasmids which might be the cause of various degrees of antibiotic resistant. The plasmids were distributed at random in the isolated *E. coli* strains and there was no remarkable interrelationship between antibiotic resistance and plasmid present. In most of the cases, strains having similar plasmid bands but confer resistant to different antibiotics. In some cases, isolates showed resistance to antibiotics but did not harbor any plasmid indicating that chromosomal DNA may carry the genes that confer resistance to antibiotics. *(Research Journal of Microbiology 3 (2): 82-90, 2008;* ***doi:*** *10.3923/jm.2008.82.90)*

Antioxidant and Antibacterial Properties of *Lecaniodiscus cupanioides*

M.O. Sofidiya, F.O. Jimoh, A.A. Aliero, A.J. Afolayan, O.A. Odukoya and O.B. Familoni

Lecaniodiscus cupanioides Planks. ExBth (Sapindaceae) is widely used in Nigerian folk medicine for the treatment of inflammatory conditions, hepatomegaly and bacterial infections. This study investigated the antioxidant and antibacterial activity of the methanolic extract of the leaves to justify its use in traditional medicine. Extract exhibited strong DPPH and ABTS radical scavenging activity greater than BHT and comparable to ascorbic acid. 0.1 mg mL^{-1} extract inhibited DPPH and ABTS radicals up to 99.4 and 98.5%, respectively. Multiple antioxidant activity of extract was evident with moderate reducing power. TAE (37.678±1.66 mg g^{-1} dry extract) was higher than that reported in many other plant extracts. Flavonoid and proanthocyanidin contents were 4.142±0.06 and 2.548±0.32 mg g^{-1}, respectively. Strong correlation recorded; ABTS/TAE (R^2 = 0.89), DPPH/TAE (R^2 = 0.90). Antimicrobial activity was highest on gram +ve organisms *B. cereus*, *S. aureus*, *M. kristine* and *S. pyrogens* (MIC value <1.0 mg mL^{-1}). Gram-ve *S. pooni* and *P. aeruginosa* (MIC value = 2.0 mg mL^{-1}). Results attributed the antioxidant potential of *L. cupanioides* leaf extract to its strong proton donating ability and justified its use for the treatment of bacterial infections in ethnomedicine. *(Research Journal of Microbiology 3 (2): 91-98, 2008;* ***doi:*** *10.3923/jm.2008.91.98)*

Biodegradation Potential of Two *Rhodococcus* Strains Capable of Utilizing Aniline as Carbon Source in a Tropical Ecosystem

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Two closely related strains of *Rhodococcus* species, isolated from aniline polluted tropical ecosystem, were able to utilize aniline as carbon source in 3.0 and 4.0 mM concentrations at 30°C and pH of about 6.4. Rapid increase in turbidity and a sharp decline in pH were observed in the cultures of both organisms within 24 h of incubation. Shortly after the period, growth became slower. Turbidity values obtained at 4.0 mM concentrations of aniline was about twice the values obtained at 3.0 mM concentrations. Aniline concentrations of 10 mM and above were found to be toxic for the organisms. *Rhodococcus* species because of its significant

prevalence in agricultural soils can be used as an effective means of recovering tropical agricultural land polluted with aniline, aniline-based herbicides or its derivatives. *(Research Journal of Microbiology 3 (2): 99-104, 2008; **doi:** 10.3923/jm.2008.99.104)*

Comparison Among Opsonic Activity and Serum Bactericidal Activity Against Meningococci in Rabbit Sera from Vaccines After Immunization with Outer Membrane Vesicle of *Neisseria meningitidis* Serogroup B

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Production of effective vaccine formulations is dependent on the availability of assays for the measurement of protective immune responses. Antibody- and complement-mediated phagocytosis is the main defense mechanism against *Neisseria meningitidis*. Therefore, a newly developed phagocytosis assay based on flow cytometry (flow assay) and the Serum Bactericidal Activity (SBA) assay were using sera obtained from rabbit postvaccination with the Outer Membrane Vesicles (OMVs) of *Neisseria meningitidis* serogroup B was done in order to evaluation of the potential efficacy of (experimental) meningococcal vaccines. The OMVs were injected intramuscularly into of rabbits with boosters on days 14, 28 and 42 after the primary immunization. Phagocytic function of and intracellular oxidative burst generation by rabbit PMN, against *Neisseria meningitidis* serogroup B, was measured with flow cytometer (Coulter Epics-XL-Profile USA), using dihydrorhodamine-123 as probes, respectively. SBA titers are given as reciprocal Log 2 values of the dilution giving at least 50% killing of the inoculum measured as colony forming units. The results of SBA titers and quantitative flow cytometric analysis of rabbit PMN function in hyperimmun sera with the OMVs revealed a highly significant increase in opsonophagocytic responses and bactericidal antibody against serogroup B meningococci after 56 day ($p<0.05$). Both SBA and opsonic activity are crucial for the protection against meningococcal disease. In conclusion, we have shown a very high correlation between opsonic activity and SBA ($r = 0.91$). Present results indicated that the OMVs could be as a candidate for vaccine toward serogroup B meningococci. *(Research Journal of Microbiology 3 (3): 105-113, 2008; **doi:** 10.3923/jm.2008.105.113)*

Optimization of Medium Constituents for the Production of Fructosyltransferase (Ftase) by *Bacillus subtilis* Using Response Surface Methodology

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Fructosyltransferase (FTase) production was maximized by *Bacillus subtilis* NCIM 2439 in submerged fermentation using molasses, which is the renewable carbon substrate. Response Surface Methodology (RSM) involving Central Composite Design (CCD) was adopted to evaluate the activity of fructosyltransferase, by most important factors, such as molasses, glucose and $(NH_4)_2SO_4$. The optimal set of conditions for maximum fructosyltransferase production was as follows: molasses 23.39 (%v/v), glucose 5.23 g L^{-1} and $(NH_4)_2SO_4$ 1.52 g L^{-1}. A maximum Fructosyltransferase activity of 78.92 U mL^{-1} was obtained at these optimal conditions. *(Research Journal of Microbiology 3 (3): 114-121, 2008; **doi:** 10.3923/jm.2008.114.121)*

Phenazine Pigments from *Pseudomonas aeruginosa* and Their Application as Antibacterial Agent and Food Colourants

Samanta Saha, R. Thavasi and S. Jayalakshmi

This study deals with isolation and identification of *Pseudomonas aeruginosa* from marine environment for phenazine pigment production. Pigment production results revealed that, the strain could able to produce two different pigments namely, pyocyanin and pyorubrin. Maximum biomass was observed at 66 h of incubation, but the pigment production seems to be continuous throughout the culture period (72 h). Antibacterial activity of the pigments was evaluated against pathogenic bacteria, maximum growth inhibitory activity was observed with pyocyanin and pyorubrin at 20 µL concentration (1.7 and 1.3 cm, respectively) against *Citrobacter* sp. Hemolytic activity of the pigments inferred that, hemolysis was observed with both pigments at 15, 20 and 25 µL concentration and no hemolysis was found at 5, 10 and 15 µL. The pigments were evaluated for their potential as food colourants with agar. Pleasant colouration was observed with pyocyanin and pyorubrin at 25 mg mL^{-1} concentration. *(Research Journal of Microbiology 3 (3): 122-128, 2008; **doi:** 10.3923/jm.2008.122.128)*

Production and Characterization of Extracellular Amyloglucosidase from *Aspergillus niger* CA-19 by Solid-State Fermentation

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The study was conducted with the aim of producing amyloglucosidase enzyme from *A. niger* using Solid State Fermentation (SSF) and to carry out preliminary characterization of the enzyme produced. Amylolytic *A. niger* CA-19 was isolated from the soil on Remazol Brilliant Blue-starch agar and used for enzyme production using rice bran supplemented with soya bean flour in SSF process. The crude enzyme extract had optimal temperature and pH activities at 60°C and pH 4, respectively. With the exception of cocoyam starch, the enzyme preparation was able to hydrolyse both the cereal (maize) and root starches (yam, cassava, sweet potatoes) tested. Hydrolysis was significantly ($p<0.05$) dependent on starch source. *(Research Journal of Microbiology 3 (3): 129-135, 2008;* ***doi:*** *10.3923/jm.2008.129.135)*

Multiplex PCR Assay for the Detection of Aflatoxigenic and Non-Aflatoxigenic *Aspergilli*

R. Latha, H.K. Manonmani and E.R. Rati

Aflatoxins are potent secondary metabolites produced commonly by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. Primers were designed specifically for *o-methyl transferase* (*omt*) and aflatoxin regulatory gene (*aflR*) of aflatoxin biosynthetic pathway and also to detect the genus *Aspergillus* specific primers (18*s* rRNA genes) using *NS*. Experimental conditions were standardized for optimum multiplex PCR. DNA extracted from mycelia of toxigenic and non-toxigenic *A. flavus*, *A. parasiticus*, other *Aspergilli* and from other genera of fungi were subjected to multiplex PCR using these primers. The *omt* and *aflR* primer pairs gave specific PCR amplification for aflatoxigenic *A. flavus* and *A. parasiticus*. They did not give DNA amplification for non-aflatoxigenic *A. flavus*, *A. oryzae*, *A. glaucus*, *Fusarium*, *Penicillium* and *Rhizopus* spp. *(Research Journal of Microbiology 3 (3): 136-142, 2008;* ***doi:*** *10.3923/jm.2008.136.142)*

Bactericidal Effect of Normal Human Serum of Various Blood Groups Against *Yersinia* species

U.B. Owhe-Ureghe and B.F. Okoh

A total of 150 sera samples from volunteers (30 belonging to each of the A, B, O blood group) with 120 of them without history of diarrhoea infection (asymptomatic) and 30 with diarrhoea (symptomatic) were collected using standard technique. Bactericidal activities of the sera were tested against local isolates of *Yersinia pseudotuberculosis*, *Y. enterocolitica* 0:3, *Y. enterocolitica* 0:8, *Y. kristensenii* 0:11, 23, *Y. intermedia* 0:52, 53 and *Y. intermedia*-like bacteria 0:52, 53, using micro titre plate technique. The *Y. enterocolitica* 0:3 (89.15%) was found to be the most sensitive to the bactericidal effect of sera of blood group A individuals, while *Y. kristensenii* 0:11, 23 (45.58%), was the least susceptible. For group B sera, *Y. pseudotuberculosis* was the most sensitive (97.26%), while *Y. intermedia* 0:52, 53 (48.26%) was the least sensitive. *Yersinia enterocolitica* 0:3 (90.04%) was the most sensitive to blood group AB sera, while *Y. enterocolitica* 0:8 (52.77%) was the least sensitive. For sera of blood group O, only *Y. pseudotuberculosis* (56.77%) and *Y. enterocolitica* 0:3 (64.85) were sensitive, while other *Yersinia* species were resistant. The results of this study suggest that individuals with blood group O whose sera caused a relatively lower bactericidal effect will be more susceptible to Yersiniosis than individuals with other blood groups (A, B and AB). The result further suggests that circulating antibodies and/or lymphocytes induced by diarrhoeic organisms could assist in the elimination of *Yersinia* species from the blood of individuals suffering from *Yersinia bacteraemia*, a finding which is of both epidemiological and clinical significance. *(Research Journal of Microbiology 3 (3): 143-149, 2008;* ***doi:*** *10.3923/jm.2008.143.149)*

Production of Citric Acid by *Aspergillus niger* MTCC 282 in Submerged Fermentation Using *Colocassia antiquorum*

Kishore Kumar Ganne, Venkata Ratna Ravi Kumar Dasari and Hanumantha Rao Garapati

The filamentous fungus, *Aspergillus niger* MTCC 282, was used to produce citric acid in submerged fermentation with *Colocassia antiquorum* (10% w/v) as the substrate. The maximum yield of citric acid (46.5 mg mL^{-1}) was obtained with inoculum age (7 days), inoculum level (2% v/v), temperature (30°C), pH (4.0), sucrose (2.0% w/v) and ammonium nitrate (1.2% w/v). The addition of methanol

to the fermentation medium resulted in substantial increase in the production of citric acid. *(Research Journal of Microbiology 3 (3): 150-156, 2008;* ***doi:*** *10.3923/jm.2008.150.156)*

Isolation of Periplasmic Alkaline Phosphatase from *Rhizobium* Bacteria

Manish Kumar, Pawan Preet Kaur and Deepak Ganjewala

Periplasmic ALP from *Rhizobium* bacteria that catalyzes the efficient hydrolysis of monophosphate ester at alkaline pH was isolated and partially purified. Periplasmic ALP activities have been isolated upon lysozyme (10 mg lysozyme/1 mL Tris-HCl buffer, 30 mM, pH 8.0) treatment of bacterial culture. Based on p-nitrophenyl phosphate as substrate, periplasmic ALP has shown most of the activity in the pH range 6.8 to 11.8 with peak value at pH 9.8. Michaelian kinetic parameters of the enzyme were as follows: V_{max} = 10.91 μmol min^{-1} and K_m = 58 μM. The enzyme had a temperature optimum of 25-65°C and was stable between 0-55°C up to 48 h. Maximum periplasmic ALP activity was obtained by 40% $(NH_4)_2SO_4$ saturation. The ALP activities was affected by metal ions (Ca^{2+}, Mg^{2+}, Co^{2+} and K^+) and inhibitors (sodium fluoride, sodium arsenate, EDTA and THB). Ca^{2+} and K^+ enhanced the ALP activities. Sodium fluoride and sodium arsenate caused drastic inhibition in ALP activity as compared to those of metal chelators (EDTA) and THB (tetra hydro borate). The overall results of the study concluded that *Rhizobium* ALP were similar to that of *E. coli* ALP. *(Research Journal of Microbiology 3 (3): 157-162, 2008;* ***doi:*** *10.3923/jm.2008.157.162)*

Isolation and Identification of Lactic Acid Bacteria and Yeast from Raw Milk in Khartoum State (Sudan)

Zeinab A.M. Elgadi, Warda S. Abdel Gadir and Hamid A. Dirar

Fifty four raw milk samples were collected from cows (farms and venders), goats, ewes and camels of different areas of Khartoum state and microbiologically analyzed. Enumeration and isolation were carried out anaerobically at 37°C on MRS and M17 for lactococci and lactobacilli, respectively, aerobically on PDA at 25°C for yeasts and on nutrient agar for total viable count at 37°C. Sixty three MRS and M17 isolates and six PDA Isolates were purified and kept at 4°C for further identification. The presence of Lactic Acid Bacteria (LAB) and yeast was confirmed by colonial morphology, microscopy in addition to other biochemical

tests. The ability of streptococci to ferment and assimilate sugars was carried out using the API kits. The results obtained showed that the milk samples contained lactobacilli and lactococci in the range of 3.50-6.30 and 3.48-6.21 log mL^{-1}, respectively, yeast in the range of 2.00-3.95 log mL^{-1} and the total viable count in the range of 3.48-7.98 log mL^{-1}. Lactic acid bacteria isolated from milk samples belonged to lactobacillus and streptococcus genera. The homofermentative lactobacilli from cows and camel milk were tentatively identified as *Lactobacillus plantarum* and *Lb. acidophilus*, whereas the hetero fermentative ones from cows, goats and ewes milk were found to be *Lb. fermentum*. The homofermentative streptococci isolated from all milk samples were tentatively identified as *Streptococcus cremoris* and *Streptococcus lactis*, whereas the only heterofermentative strain from camel milk was found to be *Leuconostoc lactis*. Yeasts which were only isolated from cow's milk, were identified as *Debaryomyces hansenii* (4 strains), *Kluveromyces lactis* (one strain) and *Saccharomyces rouxii* (one strain). *(Research Journal of Microbiology 3 (3): 163-168, 2008;* ***doi:*** *10.3923/jm.2008.163.168)*

Mycoflora and Aflatoxin Production in Market Samples of Some Selected Nigerian Foodstuffs

K.O. Jimoh and A.L. Kolapo

Study on the fungi and aflatoxin production in some selected Nigerian foodstuffs was conducted in Ibadan, Nigeria. Foodstuffs studied include dry tatase pepper (*Capsium annum*), cassava chips, yam chips, groundnut and maize. The investigated foodstuffs sold at 4 major markets in Ibadan were contaminated with *Rhizopus nigricans*, *Fusarium oxysporum*, *Aspergillus flavus* and *A. niger*. The rate of occurrence of aflatoxigenic fungi was highest in groundnut while non-aflatoxigenic fungi dominated dry tatase pepper. Aflatoxins B_1 and G_1 were detected only in groundnut and yam chips with their concentrations ranging from 7-24 and 5-27 μg kg^{-1}, respectively. There was a significant difference ($p<0.05$) between the aflatoxin contents of groundnut samples from different market and this was possibly due to the wide variations in the moisture contents of groundnut samples. Result from this study is suggesting that aflatoxin intake in this part of the world may be consequent upon the consumption of staples like groundnut and yam chips. Therefore, resources and efforts should be directed at reducing aflatoxin contents of these culprit foodstuffs so as to produce a more healthy and productive populace. *(Research Journal of Microbiology 3 (3): 169-174, 2008;* ***doi:*** *10.3923/jm.2008.169.174)*

Roles of Uropathogenic *Escherichia coli* Pili in Pathogenesis of Urinary Tract Infection

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Uropathogenic *E. coli* (UPEC) strains account for 90% of all UTI and up to 50% of all nosocomial UTI. Infection is initiated when UPEC binds to the superficial epithelial cells by type 1 pili. In addition to attachment, the presence of type 1 pili can lead to bacterial invasion to bladder epithelial cells. However, P piliation of UPEC is characteristic of strains causing upper urinary tract infection as well as pyelonephritis leading to urosepsis. In this study we determine the roles of type 1 and P pili in interaction of UPEC with human polymorphonuclear leukocyts (PMN_S). Type 1 and P piliated and unpiliated strains of UPEC were used for determining the effects of these adhesins on migration of neutrophils towards bacteria in Boyden chamber. The lectinophagocytosis and intracellular killing of bacteria with purified human neutrophils were estimated by counting of the number of viable bacteria in 45 min. Type 1 piliated UPEC stimulated significantly greater chemotaxis than did P piliated, unpiliated bacteria and bacteria in which the piliation was suppressed. Phagocytosis of type 1 piliated UPEC occurred in the direct and opsonin-independent manner. In contrast, P piliated and unpiliated bacteria failed to bind to PMN_S. The results indicated that type 1 pili have a chemotatic effect and there was a positive correlation between type 1 piliation and bacterial killing by PMN_S. In contrast, PMN_S did not chemotaxis to UPEC with type P pili and unable to react with these bacteria. Therefore the expression of type P pili is critical to UPEC establishment in upper urinary tract. *(Research Journal of Microbiology 3 (3): 175-180, 2008;* ***doi:*** *10.3923/jm.2008.175.180)*

The Assessment of Biofilm Formation in Iranian Meat Processing Environments

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The biofilm is consist of microbial cell clusters with a network of internal channels or voids in the Extracellular Polymeric Substances (EPS) and glycoprotein matrix. Biofilms due to special structure and EPS are more resistant to control measures. Biofilms can remain on various surfaces which may assist the survival of pathogenic and spoilage bacteria in the food processing environment, a contributing factor in foodborne disease outbreaks. In this research bacterial strains were isolated by swabbing method from surfaces of meat processing factory environments. More

than 60 different species of bacteria isolated from various segment of meat processing plant and the hydrophobicity of isolates measured by Microbial Adhesion Test to Hydrocarbon (MATH) method for screening of isolates. The quantity of biofilm of isolates with high hydrophobicity was determined using microtiter plate assay method and ELISA reader machine. Results indicated *Bacillus megaterium* and *Staphylococcus epidermidis* with 45 and 33% of hydrophobicity have the highest potential in biofilm formation. Pathogenic *S. aureus* with 30% of hydrophobicity classified under moderately adherent. *B. subtilis* with 22% of hydrophobicity considered as weakly adherent. *Micrococcus varians* and *M. roseus* with 1 and 5% of hydrophobicity were non adherent. The result from this study highlighted the problems of spread of bacteria. In the development of cleaning and sanitization protocol in meat processing environments, an awareness of these biofilm forming bacteria is essential for the meat processing. *(Research Journal of Microbiology 3 (3): 181-186, 2008;* ***doi:*** *10.3923/jm.2008.181.186)*

Etiology of Acute Hepatitis in Pediatric Patients Referring to a Major City Hospital, Shiraz, Iran

Seddiqe Amini-Ranjbar, Mohammad-Hadi Imanieh, Mahmoud Haghighat and Bita Geramizadeh

On-time diagnosis and consequently early treatment of acute hepatitis have important role in its long-term prognosis. This prospective study was done from November 2002 to January 2004 in Shiraz in order to find the etiology of acute hepatitis in children of Fars Province. For this purpose, 75 children with median age of 8.2 years and the clinical picture of acute hepatitis referring to the Outpatient Clinic and Emergency Room of Namazi Hospital (Shiraz/ Iran) were studied. After taking history, physical examination and recording the pertinent information in a questionnaire, CBC and LFT were requested. In the case of liver enzymes of higher than two times as much as the normal range, IgM HAV, HBsAg, HCVPCR, ANA, AsMAb, urinary copper and serum ceruloplasmin tests and eye examination for KF ring were performed. Ascitic fluid was sent for culture, cytology and further analysis. In some cases, based on the necessity, abdominal sonography and biopsy were performed as well. Fifty five percent were female and 65% gave the history of disease from one week prior to the appearance of icterus. The first leading causes of acute hepatitis were found to be, respectively hepatitis A (45.3%), Wilson disease (17.3%) and autoimmune hepatitis (12%). In 8% of the cases, in spite of extensive work up no cause was

found (unknown cases). Tea color urine (84%), abdominal pain (82%) and anorexia (81%) were the most prevalent complaints. The most common signs were hepatomegaly (90%), icterus (89%) and tender liver (64%), respectively. Ascites (77%) and splenomegaly (61%) were observed more frequently in patients with Wilson disease comparing to other patients. AST and ALT rising to more than 10 times the normal range were more frequently seen in autoimmune hepatitis (89 and 100%) and hepatitis A (62 and 68%) patients, respectively. Considering the relatively high prevalence of treatable causes of acute hepatitis (Wilson, autoimmune hepatitis), attention to the mentioned diseases in facing childhood acute hepatitis is highly recommended. *(Research Journal of Microbiology 3 (3): 187-192, 2008; **doi:** 10.3923/jm.2008.187.192)*

Anti-Microbial Activity of *Tamarindus indica* and *Adansonia digitata* Extracts Against *E. coli* Isolated from Urine and Water Specimens

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The petroleum ether, ethanol and aqueous extracts of the *Tamarindus indica* and *Adansonia digitata* were screened for the presence of possible anti-microbial activity using the cup plate agar diffusion method. They were tested against *Escherichia coli* that isolated from urine and water sources. Each extract was used in concentration of 100, 75, 50 and 25%. *E. coli* isolated from clinical urine samples showed more susceptibility toward both plant extracts. The ethanol extract of both plants was more effective than petroleum ether and water extracts. Ethanol extract showed variation in the antimicrobial activity toward *E. coli* that isolated from water and clinical sources, the zones of their inhibition ranged between (15-60 mm). On the other hand, the petroleum ether extract of *Adansonia digitata* was found to be inactive against all tested organisms. The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics. Ethanol extract of *Tamaridus indica* have more powerful antibacterial activity compared with all antibiotics. The antimicrobial activities of these plants were discussed according to their phytochemical components. *(Research Journal of Microbiology 3 (3): 193-197, 2008; **doi:** 10.3923/jm.2008.193.197)*

Effect of Commercial Probiotics on Large Scale Culture of Black Tiger Shrimp *Penaeus monodon* (Fabricius)

R. Lakshmanan and P. Soundarapandian

The survival rate and growth of both the ponds (High and low dosages of probiotics), which was applied with probiotics, was higher than that of control ponds. Concentrations of nitrite, nitrate and phosphate were higher in control ponds than the probiotics treated ponds. Chlorophyll a was observed maximum in probiotics treated ponds rather than control ponds. The bacterial population decreased at the end of culture in both treated and control ponds but the load of *vibrio* sp. when compared with THB in the control pond was not showing a significant decrease. Black gill, white gut and fungal diseases were predominant in control ponds. But these diseases were meager in probiotics treated ponds. The general conclusion obtained from the present study is that the probiotics plays a vital role in growth, survival and disease resistance of the animal by maintaining good water quality parameters through out the culture period. It is clear from the microbial load data that *vibrio* sp. is dominant only in the control ponds. *(Research Journal of Microbiology 3 (3): 198-203, 2008;* ***doi:*** *10.3923/jm.2008.198.203)*

Dye Degrading Mycoflora from Industrial Effluents

R. Muthezhilan, N. Yogananth, S. Vidhya and S. Jayalakshmi

The present study deals with the distribution of fungal species in Cuddalore dye industrial waste (Lat 11°42'N; Long 79°46'E) and their dye degrading activity. Totally 13 species under 17 genera were isolated and screened for their decolorization activity against methylene blue, gentian violet, crystal violet, cotton blue, Sudan black, malachite green, methyl red and corbol fushion in mineral salt medium and Czepex-Dox broth. In agar medium, decolorization began with the formation of zone of clearance around the colonies. *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum* and *Fusarium moniliforme* decolorized maximum number of dyes to a great extent. *Mucor racemosus*, *Cladosporium cladosporioides*, *Penicillium oxalicum* and *Trichoderma viride* did not decolorize any of the dyes tested. In liquid medium, decolorizing activity was measured spectrophotometrically. *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum* and *Fusarium moniliforme* registered maximum color reduction, where as *Mucor racemosus*, *Cladosporium cladosporioides*, *Penicillium oxalicum* and *Trichoderma viride* expressed very low amount of color reduction. Biomass and the extent of dye removal are directly propositional. Among the 13 species of fungal isolates, *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Fusarium moniliforme* and *Penicillium citrinum* seems to be potential candidates for dye degradation. These strains can be used for the bioremediation of environs polluted with dye effluents. *(Research Journal of Microbiology 3 (3): 204-208, 2008;* ***doi:*** *10.3923/jm.2008.204.208)*

Isolation of Thermotolerant Acetic Acid Bacteria from Fruits for Vinegar Production

Amornrut Moryadee and Wasu Pathom-Aree

Sixty thermotolerant acetic acid bacteria were isolated from 13 kinds of fruit using sterile distilled water supplemented with 4% ethanol (v/v) as an enrichment medium. Successful isolations were obtained from apple, Jamaican cherry, longan, mango, pineapple and rambutan. Morphological and biochemical examinations revealed that 43 isolates were members of the genus *Acetobacter* whereas the remaining 13 isolates were members of the genus *Gluconobacter*. Preliminary screening showed that isolates No. 13, 34, 36 and 37 gave the widest zone of acidity on overoxidation medium. These isolates were identified as *A. aceti* and selected for acetic acid production at 30 and 37°C by shaking culture for 14 days in ethanol-yeast extract medium. It was found that *A. aceti* isolate No. 37 from rambutan gave the highest acetic acid yield of 13.53 and 8.97 g L^{-1} at 30 and 37°C, respectively after 7 days of fermentation. *(Research Journal of Microbiology 3 (3): 209-212, 2008; **doi**: 10.3923/jm.2008.209.212)*

Production of Cellulase and Pectinase from Some Aquatic Hyphomycetes

M.E. Osman, H.K. Om Kalthoum and A.A. El-Shaphy

The highest total activity of cellulases by *Dactylella aquatica* and *Cylindrocarpon heteronemum* was obtained after 9 days incubation at pH 11-14 and 20-25°C, respectively. The enzyme was produced in the presence of cellulose as a sole carbon source. $CaCl_2$ at level 1000 ppm was the most convent for cellulyltic activity of *D. aquatica* where as growth at this concentration was nearly 1/3 that of control. Although $CaCl_2$ was effective in decreasing cellulyltic activity of *C. heteronemum*. Growth of *D. aquatica* and *C. heteronemum* inhibited by the presence of $CaSO_4$. Polygalacturonase (PG), Pectin Lyase (PL) and pectinmethylestrase (PME) were investigated qualitatively in most active of aquatic fungi. *(Research Journal of Microbiology 3 (4): 213-224, 2008; **doi:** 10.3923/jm.2008.213.224)*

Effects of Arbuscular Mycorrhizal Inoculation and Fertilizer on Production of *Castanopsis acuminatissima* Saplings for Forest Restoration in Northern Thailand

P. Nandakwang, S. Elliott, S. Youpensuk and S. Lumyong

Castanopsis acuminatissima is a native tree used to restore forest in Thailand. To accelerate seedling growth experiments were carried out to determine the efficacy of applying to *C. acuminatissima*. Arbuscular Mycorrhizal (AM) fungi, produced on sorghum, were used as inoculum to investigate the symbiosis on seedlings. The effects of AM inoculation (*Acaulospora elegans*, *Glomus etunicatum*, *Glomus mosseae*) together with phosphate fertilization (KH_2PO_4) on seedlings in a P-deficient soil were studied under greenhouse conditions. Increasing P-application rates greatly enhanced seedling growth (maximum at 250 mg kg^{-1} soil). Growth was most rapid with *G. etunicatum*-colonized plants with P application (40.8 cm), whereas much lower height was found with non-AM plants without P added (14.4 cm). The mycorrhizal effective for *C. acuminatissima* in previous experiments were confirmed by growing seedlings in a forest soil with slow-release fertilizer (NPK) and combined with AM species under nursery performance conditions. Plant height was significantly enhanced by fertilizer but not by fungi. The greatest height was found in non-AM plants with fertilization (14.5 cm), whereas lower height was found for non-AM plants with no fertilizer added (10.9 cm). AM inoculation greatly enhanced seedling growth in P-deficient soil more than in forest soil due to differences in abilities of AM species to establish a symbiosis. Therefore, in sapling production, the soil properties and level of fertilization should be evaluated keeping secondary effects caused by changed mycorrhizal association. *(Research Journal of Microbiology 3 (4): 225-236, 2008; **doi**: 10.3923/jm.2008.225.236)*

Synergistic Activities of 4-Arylcoumarins Against Phytopathogenic Fungi

Thongchai Taechowisan, Asawin Wanbanjob, Pittaya Tuntiwachwuttikul, Yuemao Shen and Saisamorn Lumyong

Different extracts of *Streptomyces aureofaciens* CMUAc130 culture were studied as potential antifungal agents for selected phytopathogenic fungi. In a serial agar dilution method, crude ethyl acetate and 10% methanol in ethyl acetate extracts exhibited fungistatic activity against *Aspergillus flavus*, *Colletotrichum musae*, *Fusarium oxysporum*, *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Both ethyl acetate extract and 10% methanol in ethyl acetate extract were highly effective on all tested fungi, with Minimum Inhibitory Concentration (MIC) values ranging from 0.25 to 50 and 10 to 100 mg mL^{-1}, respectively. The major active ingredients from those extracts were purified by silica gel column chromatography and identified to be 5, 7, 4'-trimethoxy-4-phenylcoumarin (1), 4'-hydroxy-5,7-dimethoxy-4-phenylcoumarin (2), 3'-Hydroxy-5,7,4'-trimethoxy-4-phenylcoumarin(3),5,7,3',4'-Tetramethoxy-4-

phenylcoumarin (4) and 4'-hydroxy-5,7,3'-trimethoxy-4-phenylcoumarin (5) by NMR and mass spectral data, respectively. Five compounds (1 to 5) had activity against *F. oxysporum* with MICs of 0.30, 1.00, 0.40, 10.00 and 20.00 mg mL^{-1}, respectively. Compounds 1, 2 and 3 also showed a synergistic effect when combined in different concentrations, displaying four times less concentration to reach complete inhibition in the growth of *F. oxysporum*. *(Research Journal of Microbiology 3 (4): 237-245, 2008; **doi**: 10.3923/jm.2008.237.245)*

Xylanase Production of *Aspergillus niger* and *Penicillium chrysogenum* from Ammonia Pretreated Cellulosic Waste

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Effect of ammonia pretreatment of cellulosic wastes on xylanase production was studied using two microfungi, *Aspergillus niger* ANL301 and *Penicillium chrysogenum* PCL 501. Xylanase activity of culture supernatants of the two microfungi, fermented in basal media containing as sole carbon source pretreated and non-pretreated wastes (sawdust of *Mitragyna ciliata*, sugarcane pulp and wheat bran), was measured at 24 h intervals for 120 h. Ammonia pretreatment of the cellulosic wastes enhanced xylanase production by the organisms, inferred from the activity of extracellular xylanase enzyme (endo-β-xylanase: EC 3.2.1.8). Pretreatment of sawdust increased the optimal specific xylanase activities of *A. niger* ANL301 and *P. chrysogenum* PCL501 by 40.2 and 192.7%, respectively. An increase of 72.9 and 63.5% in optimum activity was obtained for *A. niger* ANL301and *P. chrysogenum* PCL501 respectively by ammonia pretreatment of sugarcane pulp. Pretreatment of wheat bran gave a marginal increase of 3.3% in the optimum xylanase activity of *A. niger* ANL301 and 143.4% activity increase for *P. chrysogenum* PCL501. The present results show that ammonia steeping of the agro-wastes significantly improved xylanase production by the microfungi. The pretreatment method is a cost-effective means for producing xylanases from cellulosic wastes. *(Research Journal of Microbiology 3 (4): 246-253, 2008; **doi**: 10.3923/jm.2008.246.253)*

Influence of Enriched Pressmud Compost on Soil Chemical Properties and Yield of Rice

D. Kalaivanan and K. Omar Hattab

A field experiment was conducted to investigate the effect of enriched pressmud compost on soil chemical properties like pH, EC, major nutrient availability and

yield of rice with five levels of pressmud compost viz., 0, 1.25, 2.50, 3.75 and 5.0 t ha^{-1} in two varieties viz., ADT 36 and ADT 43 and a hybrid ADTRH 1 during the kharif season of 2004 in the farm soil of Pandit Jawarhalal Nehru College of Agriculture and Research Institute, Tamil Nadu Agricultural University (TNAU). The results of the field experiment revealed that the hybrid ADTRH 1 manifested higher grain and straw yield, whereas, the variety ADT 43 and ADT 36 registered lower grain and straw yields, respectively. With regard to the enriched pressmud compost, the application of 1.25 t ha^{-1} of enriched pressmud compost showed its potentiality by providing more available nutrients to promote higher grain yields and it was comparable with 2.50 t ha^{-1} of enriched pressmud compost. However, the straw yield was higher with 2.50 t ha^{-1} of enriched pressmud compost and it was on par with 1.25 t ha^{-1} of pressmud compost. The soil reaction (pH) and Electrical Conductivity (EC) did not show any marked variation with application of enriched pressmud compost. The N, P and K availability in soil was at higher levels and comparable with application of 1.25 and 2.50 t ha^{-1} of enriched pressmud compost, whereas it was lower with control. The outcome of the present investigation revealed that the highest grain yield was obtained, at 1.25 t ha^{-1} of enriched pressmud compost along with inorganic fertilizers for the varieties and hybrid. Hence, the incorporation of 1.25 t ha^{-1} enriched pressmud compost as basal along with required remaining nitrogen through inorganic fertilizer as top dressing in three splits may be recommended for rice crop to realise maximum yield in kuruvai (Kharif) season. *(Research Journal of Microbiology 3 (4): 254-261, 2008; **doi:** 10.3923/jm.2008.254.261)*

Comparative Antimicrobial Activity of Commercial Disinfectants with Naphtholics

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Studies were carried out to determine the disinfectant property of naphthol and its derivatives. The sensitivity of some clinical organisms as compared with the activity of some selected commercial disinfectants was tested. The methods employed for assessing the efficacy of disinfectants in this study are Minimal Inhibitory Concentration (MIC) Test and Capacity Use Dilution Test. The clinical organisms used for the tests are *Pseudomonas aeruginosa, Salmonella typhi and Proteus mirabilis* while the commercial disinfectants used are Dettol (Chloroxylenol), Savlon (Cetrimide/chlorhexidine mixture) and TCP (Trichlorophenol) and the Naphtholics are alpha naphthol and 2-amino-1,4-naphthoquinonimine

hydrochloride. Dettol showed highest antibacterial activity against all the test organisms. Savlon's antibacterial activity was high against the test organisms except *Pseudomonas aeruginosa*. TCP showed low activity against all the test organisms while Purified ∝-naphthol and its derivative, 2-amino-1, 4-naphthoquinonimine hydrochloride were found to exhibit disinfecting properties, with the derivative showing more antimicrobial activity than ∝-naphthol. The compounds have bactericidal effect against the test organisms used in this study. *(Research Journal of Microbiology 3 (4): 262-268, 2008; **doi:** 10.3923/jm.2008.262.268)*

Effect of Mutation on Trehalose-Catabolic-Enzyme Synthesized by a Tropical *Rhizobium* Species F_1

B. Boboye and A. Alao

Rhizobium species F_1 was studied for its ability to grow in the presence of trehalose (Trehalose-Minimal Medium (TMM)) and absence of it (Nutrient Broth (NB)) as sole carbon and energy sources and form Trehalose-Catabolic-Enzyme (TCE). The organism was mutagenized with hydroxylamine. The resultant mutants and the parental strain were grown with and without trehalose. The supernatants of the grown culture alone and lysed cells in supernatants were assayed for the activity of TCE. *Rhizobium* species F_1 and the mutants grew in TMM and NB. Many of the mutants grew better (OD_{670} = 0.36-1.0 in TMM and OD_{670} = 0.005-0.99 in NB) than the wild-type (OD_{670} = 0.51 in TMM and OD_{670} = 0.25 NB). All the strains constitutively and inducibly expressed the trehalose-catabolic enzyme with a range of 0.242-1.42 mg mL^{-1} glucose mg^{-1} mL^{-1} protein for the mutants and 1.025 mg mL^{-1} glucose mg^{-1} mL^{-1} protein for the parental type. In the absence of trehalose in the growth medium, the mutants synthesized higher amount of the TCE with the highest value of 1.091 mg mL^{-1} glucose mg^{-1} mL^{-1} protein and then the wild-type which exhibited enzyme activity of 0.321 mg mL^{-1} glucose mg^{-1} mL^{-1} protein. The enzyme was extracellularly and intracellularly expressed in the TMM and NB. Activity of the total trehalose-degrading enzyme was higher than that of the extracellular. Three classes of the mutants were identified. Low, normal and super-trehalose-catabolic-enzyme producers showed enzyme activity in the ranges of 0 to 30, 31 to 60 and above 60 mg mL^{-1} glucose mg^{-1} mL^{-1} protein, respectively. *(Research Journal of Microbiology 3 (4): 269-275, 2008; **doi:** 10.3923/jm.2008.269.275)*

Production of Indole-acetic-acid by *Rhizobium* Isolates from *Crotalaria* Species

M. Sridevi, N.C.S. Yadav and K.V. Mallaiah

Cultural and nutritional conditions were optimized for Indole Acetic Acid (IAA) production by *Rhizobium* spp. isolated from root nodules of *Crotalaria juncea*, *C. retusa*, *C. laburnifolia*, *C. verrucosa* and *C. alata*. The isolates produced maximum amount of IAA after 72 h of incubation and at 2.5 mg mL^{-1} L-tryptophan concentration. The effect of different carbon and nitrogen sources on IAA production were also studied and it revealed that, mannitol and L-glutamic acid were the best promoters for IAA production. Addition of cell wall affecting agents increased the IAA production over controls. Among the five isolates of *Crotalaria* species, maximum amount of IAA was produced by isolate from *C. retusa*. The compound from *Rhizobium* sp. from *C. retusa* was extracted, purified and structurally confirmed as IAA. *(Research Journal of Microbiology 3 (4): 276-281, 2008;* ***doi:*** *10.3923/jm.2008.276.281)*

Production of Catechol-type of Siderophores by *Rhizobium* sp. Isolated from Stem Nodules of *Sesbania procumbens* (Roxb.) W and A

M. Sridevi, K.G. Kumar and K.V. Mallaiah

The *Rhizobium* sp. isolated from stem nodules of *Sesbania procumbens* (Roxb.) W and A was studied for its ability to produce siderophores on Chrome-Azurol S agar medium. The symbiont was able to produce catechol-type of siderophores in culture after 4 h of incubation. Maximum siderophore production was observed after 24 h. Carbon and nitrogen sources greatly influence the siderophore production. Among the carbon and nitrogen sources, mannitol (2%) and glutamine (0.1%) were found to increase the siderophore production. Thin Layer Chromatography (TLC) of the siderophore extract showed the presence of 2, 3-Dihydroxy Benzoic Acid (DHBA) and 3, 5-DHBA. Arginine, glutamine and proline were identified as conjugated amino acids of siderophore extract. *(Research Journal of Microbiology 3 (4): 282-287, 2008;* ***doi:*** *10.3923/jm.2008.282.287)*

Isolation and Identification of Three Species of Bacteria from the Termite *Coptotermes curvignathus* (Holmgren) Present in the Vicinity of University Putra Malaysia

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In this study the lower termite *Coptotermes curvignathus* (Holmgren) and higher termite *Macrotermes gilvus* (Hagen) were identified from different parts in the vicinity of University Putra Malaysia (UPM). We isolated three enteric bacteria from the hindgut of *Coptotermes curvignathus* (*Holmgren*). All isolates were facultative anaerobes. The isolates were identified as *Enterobacter aerogenes*, *Enterobacter cloacae* and *Clavibacter agropyri* (*Corynebacterium*) by using BIOLOG assay and Bergey's manual. The bacteria were able to assimilate carboxymethylcellulose (CMC) and cellobiose. *(Research Journal of Microbiology 3 (4): 288-292, 2008;* ***doi:*** *10.3923/jm.2008.288.292)*

Utilization of Chlorpyrifos as a Sole Source of Carbon by Bacteria Isolated from Wastewater Irrigated Agricultural Soils in an Industrial Area of Western Uttar Pradesh, India

Ranjan Kumar Bhagobaty and Abdul Malik

In the present study wastewater irrigated agricultural soil with a previous history of chlorpyrifos use was examined for its capacity to harbor bacteria capable of utilizing it as a sole source of carbon. Four bacterial isolates designated as RA-3, RA-5, RA-10, RA-20, isolated from the soil, using enrichment culture technique showed promising capability to utilize chlorpyrifos as a carbon source for their growth. Morphological and biochemical tests performed on the bacteria indicated that they might belong to the genus *Pseudomonas*. Thin layer chromatography and tetrazolium reduction assay showed that the strains were capable of degrading chlorpyrifos. All the chlorpyrifos degrading bacterial isolates were also tested for their antibiotic sensitivity against 10 antibiotics/drugs. All the isolates were sensitive to gentamycin and methicillin. RA-10 and RA-3 were sensitive to ampicillin whereas RA-5 was resistant and RA-20 showed intermediate range of sensitivity. RA-5 and RA-3 were sensitive to chloramphenicol whereas, RA-10 and RA-20 showed intermediate sensitivity. RA-5 and RA-20 showed resistance against co-trimoxazole and nalidixic acid. RA-5 and RA-10 showed intermediate sensitivity to tetracycline whereas RA-20 was resistant and RA-3 sensitive to it. All the bacterial isolates were also found to harbor a single plasmid. This leads us to believe that the soils with previous exposure to chlorpyrifos contain a diverse range of bacteria having novel organophosphorus hydrolase enzyme systems for causing the enhanced biodegradation of this toxic pesticide in the environment. Further elucidation of the enzymatic and molecular mechanisms involved in the process will help in creating possible bioremediation technologies using the soil bacteria. *(Research Journal of Microbiology 3 (5): 293-307, 2008;* ***doi:*** *10.3923/jm.2008.293.307)*

Improving Feeding Strategies for Maximizing Polyhdroxybutyrate Yield by *Bacillus megaterium*

W. Sabra and D.M. Abou-Zeid

The prokaryotic endogenous storage material Poly-β-Hydroxybutyrate (PHB) can be induced to accumulate in bacteria under conditions of unbalanced growth that also stimulate sporulation in endospore forming bacteria. The present study shows that ammonium concentration higher than 0.4 g L^{-1} inhibits growth and may be responsible for the stationary phase onset of *Bacillus megaterium*. Hence, in order to expand the growth rate controlled exponential phase (by delaying stationary phase), ammonium limited fed batch cultures were performed at different feeding rates. Under such conditions, a 2.1 fold increase in the specific PHB productivity was recorded (0.19 $g_{PHB}/g_{biomass}$*h) compared to batch cultivations (0.09 g/g*h). Although the lowest ammonium feeding rate was accompanied with the lowest growth rate, it resulted in the highest PHB yield. Present study demonstrates that the PHB content of the cells growing under optimized fed batch conditions reached 65% of the cell dry weight, a value that has not been recorded before for bacilli using a synthetic medium. *(Research Journal of Microbiology 3 (5): 308-318, 2008;* ***doi:*** *10.3923/jm.2008.308.318)*

Microbial Growth and Chemical Analysis of Mineral Contents in Bottled Fruit Juices and Drinks in Riyadh, Saudi Arabia

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This study aimed to determine the clinically important levels of minerals in bottled fruit juices and drinks and to determine the microbial contamination of commercially available bottled fruit juices and drinks from different supermarkets in Riyadh, Saudi Arabia. Commercially available bottled fruit juices and drinks were brought from different supermarkets in Riyadh, were examined microbiologically and mineral contents were determined by atomic absorption spectrophotometry. A total of 150 specimens (3 replicates of a total 50 samples) were examined for microbial growth on six different culture media (BAP, NA, MacConkey, CAP, Salmonella Agar and PDA). A total of 43 (28.7%) different colonies were seen on different fruit juices. Bacillus cereus was the most common isolate in all types of fruit juices. Other isolates included *Bacillus subtilis*, *Bacillus polymyxa*, *Chryseomonas luteola*, *Tatumella ptyseos*, *Streptococcus lactis* and *Candida* sp. None of the specimens taken from softdrinks and power drinks showed any microbial growth after incubation for 48 h in all six environmental

plates used. Specimens from mixed juice with milk showed microbial colonies in 3 out of 10 specimens with *Lactobacillus* sp., *Streptococcus lactis* and *Lactobacillus casei*. The mineral contents of 8 specimens of fruit juices had iron content within the maximum allowed concentration. As to potassium content, 7 of 8 (87.5%) of the samples had potassium content >10 ppm. Five of 8 (62.5%) samples had sodium content >20 ppm, 7 of 8 (87.5%) had aluminum content >0.2 ppm, 4 of 8 (50%) had lithium content >0.2 ppm, 7 of 8 (87.5%) had magnesium content >30 ppm, 4 of 8 (50%) had manganese content >20 ppm, all 8 contained lead >0.2 ppm and 7 of 8 (87.5%) have zinc content >5 ppm. Commercially sold fruit juices in Riyadh, Saudi Arabia should be further investigated and regulated since they contain dangerous organisms and minerals which are toxic to the body. *(Research Journal of Microbiology 3 (5): 319-325, 2008;* ***doi:*** *10.3923/jm.2008.319.325)*

Formulation and Evaluation of Dehydrated Microbiological Media from Avocado Pear (*Peasea americana* Cmill)

O. Famurewa and O.M. David

Avocado pear (*Peasea amaricana* Cmill) has an excellent nutritional quality that can support the growth of microorganisms. Different media were formulated from both defatted and undefatted dehydrated avocado pear. The proximate analyses of the pear flour show that defatted samples were better in term of minerals contents than their corresponding undefatted samples. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* in that order thrived very well in the composed media. The test bacteria grew better in media composed with defatted pear than their corresponding undefatted samples. Undefatted samples seem to support fungal growth than defatted samples. *Trichoderma* sp. grew better than *Aspergillus flavus* and *Penicillium notatum*. Comparing with the performance of conventional bacteriological and mycological media, avocado pear is a good and cheap media material for the cultivation and isolation of both bacteria and fungi. *(Research Journal of Microbiology 3 (5): 326-330, 2008;* ***doi:*** *10.3923/jm.2008.326.330)*

Regulation of Aspartate Transcarbamoylase Activity in *Pseudomonas putida* Biovar B

Manuel F. Santiago and Thomas P. West

The regulation of aspartate transcarbamoylase activity in cell extracts of *Pseudomonas putida* biovar B was examined. Under saturating substrate

concentrations, ATP, UTP, GTP, ADP, UDP, UMP and pyrophosphate were highly inhibitory of the *P. putida* biovar B transcarbamoylase activity. By examining aspartate transcarbamoylase inhibition by ribonucleotides, it appeared that the *P. putida* biovar B strain could be differentiated from the *P. putida* biovar A strain which is consistent with previous taxonomic analyses that concluded biovar B strains should be reclassified. *(Research Journal of Microbiology 3 (5): 331-335, 2008;* ***doi:*** *10.3923/jm.2008.331.335)*

The Efficacy of Cecure® (CPC Antimicrobial) for Post-Harvest Decontamination of Cantaloupes and Spanish Melons

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Two trials were conducted in Costa Rica in the spring of 2007. One trial was conducted in a cantaloupe processing facility and the other in a facility that processes Spanish melons (piel de sapo). In both trials, the freshly harvested fruits were subjected to a variety of post-harvest treatments in an effort to improve the microbial condition of the fruit. In the cantaloupe trial, there were five different treatments, including fruit from the field, fruit from the field plus a 5 sec dip in 0.5% cetylpyridinium chloride (CPC), fruits that were washed commercially in 100 to 150 ppm total chlorine followed by a water rinse, washed fruits followed by a water rinse plus a 5 sec dip in 0.5% CPC and washed fruits followed by a water rinse plus a commercial fungicide. In the Spanish melon facility, there were four treatments, including fruits from the field, fruits from the field plus a 5 sec dip in 0.5% CPC, fruits that were washed in 100 to 150 ppm total chlorine (plus application of fungicide to the peduncle) and fruits that were washed in chlorine plus a 5 sec dip in 0.5% CPC. In addition, shelf-life studies at the appropriate temperatures were also conducted on both fruits; however, only the cantaloupes were subjected to a detailed sensory evaluation at the end of the shelf-life period. For field cantaloupes, the results indicate that a 5 sec dip in 0.5% CPC will allow for a 99% reduction in APC, no recovery of total coliforms and a 99.9% reduction in yeasts and molds. For Spanish melons from the field, a 5 sec dip in 0.5% CPC resulted in greater than a 90% reduction in APC and a 99% reduction in total coliforms and yeasts and molds. The commercial washing procedure in 100 to 150 ppm total chlorine followed by a water rinse (with or without the application of a commercial fungicide) was not very effective for reducing the levels of any of the groups of organisms in the cantaloupe facility. In fact, total coliforms remained unaffected by the commercial wash process. In the Spanish melon facility, the commercial wash procedure plus application of a commercial fungicide to the

peduncle reduced APC by greater than 99% and total coliforms and yeasts and molds by greater than 90%. In comparison, the commercial washing process plus a 5 sec dip in 0.5% CPC resulted in the greatest reductions with almost complete elimination of APC, total coliforms and yeasts and molds from the cantaloupe and Spanish melons. The results from these trials suggest that the use of a CPC rinse solution as a treatment following commercial washing can significantly improve the overall microbial condition of fresh cantaloupe and Spanish melons. In addition, the sensory quality of cantaloupes at the end of refrigerated and retail storage was significantly improved when the fruit was subjected to a CPC-solution treatment following the commercial washing process. *(Research Journal of Microbiology 3 (5): 336-344, 2008;* ***doi:*** *10.3923/jm.2008.336.344)*

Isolation and Characterization of a *Pseudomonas aeruginosa* Strain DN1 Degrading p-Nitrophenol

Debananda Singh Ningthoujam and Ningthoujam Shovarani

A bacterial strain, DN1, degrading p-nitrophenol (PNP) was isolated from garden soil by selective enrichment in M63 medium. Repeated subculturing in Nutrient Agar (NA) plates, NA slants and Basal Salts Medium (BSM) containing PNP (BSM+ PNP) led to isolation of pure colonies. The organism is Gram negative, aerobic, catalase positive, oxidase positive and rod shaped with mostly single arrangement. It shows bluish green pigmentation on various specialized media such as Pseudomonas P medium, Pseudomonas F medium, Modified F medium, Pseudomonas Isolation Agar (PIA) and HiFluoro Pseudomonas Agar. DN1 gave positive results with motility, citrate utilization, urease, Nitrate Reduction (NR) and gelatin liquefaction tests but negative results with Methyl Red (MR), Voges Proskauer (VP) and indole tests. It was casein hydrolysis and lipase positive but starch hydrolysis negative. Acid production from carbohydrates tested (glucose and lactose) was negative. It can grow at 42°C but not at 4°C and tolerates <5% NaCl concentration. Optimum pH for PNP degradation was found to be 7.0. Among several media tested such as M9, M63 and BSM, BSM was found to be the optimum medium for biodegradation of PNP. DN1 could degrade upto 100 mg L^{-1} PNP using the xenobiotic as sole carbon or carbon and nitrogen sources. On the basis of gross morphological, micromorphological, physiological and biochemical tests DN1 was definitively identified as *Pseudomonas aeruginosa* strain DN1. To our knowledge this is the first report of a *Pseudomonas aeruginosa* strain able to degrade p-nitrophenol (PNP). *(Research Journal of Microbiology 3 (5): 345-351, 2008;* ***doi:*** *10.3923/jm.2008.345.351)*

Optimization of PCR Conditions for Detection of Human Brucellosis from Human Serum Samples

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The conditions of PCR were optimized in order to diagnose brucellosis from human serum samples. For this purpose, 16 serum samples, from confirmed brucellosis cases were examined. The specificity of a polymerase chain reaction assay for detecting *Brucella* DNA using primers specific for the amplification of a 223 bp region of the sequence encoding a 31 kDa immunogenic *Brucella abortus* protein (BCSP31) was evaluated. After modification, factors such as annealing temperature, time, concentrations of magnesium ion, dNTP, Taq and additives like Bovine Serum Albumin (BSA), dimethyl sulfoxide (DMSO), glycerol, gelatin, Tween 20 and Triton X-100 for enhancing PCR reaction were optimized. The optima conditions determined to be: PCR profile with annealing at 60°C for 50 sec optimum concentration of Mg^{2+}(1.5 mM), dNTP(200 µM),Taq(1.25 U), pH 8.3 and the relation between $MgCl_2$ and dNTP concentration, Triton X-100, Tween 20 and BSA were found to be suitable additives. *(Research Journal of Microbiology 3 (5): 352-358, 2008;* ***doi:*** *10.3923/jm.2008.352.358)*

Detection of Diarrheagenic *Escherichia coli* Isolated Using Molecular Approaches

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Escherichia coli strains are among the major bacterial causes of diarrheal illness. There are now seven classes of diarrheagenic *E. coli* (DEC), namely enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diarrhea-associated hemolytic *E. coli* (DHEC) and Cytolethal Distending Toxin (CDT)-producing *E. coli.* Due to the need for costly and labor-intensive diagnostic procedures, identification of DEC is difficult at standard laboratories. Therefore, Polymerase Chain Reaction (PCR) or dot blot has been used for genetic detection of DEC of 25 *E. coli* isolates from different sources. Amplification of *eae* (277 bp), *bfp* (266 bp), *stx1* (154 bp), *EAST* (94 bp), *stx2* (698 bp) and *elt* (450 bp) genes of a single product in separate reactions was produced. PCR showed ability to amplify and detected genes of the most common important categories of diarrheagenic *E. coli* isolates of different sources, it is

possible implementation of this technique to diagnosis water, food-borne outbreaks related to *E. coli*. Dots blot and sequence analysis used to confirm the results of PCR. *(Research Journal of Microbiology 3 (5): 359-367, 2008; **doi:** 10.3923/jm.2008.359.367)*

Endophytic Fungi from Wild Banana (*Musa acuminata* Colla) Works Against Anthracnose Disease Caused by *Colletotrichum musae*

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Screening of endophytic fungi which antagonize *Colletotrichum musae*, the cause of anthracnose disease, was carried out. The *in vitro* screening was studied by dual culture method. The inhibition due to fast competitive growth of *Cordana* sp. (KPP-3) and the antibiotic producing endophyte; *Nodulisporium* sp., reached a high percentage (90% *C. musae* inhibition). Spore germination assay showed 91% *C. musae* germination, while only 0.63 and 1.88% germinated in the conidial suspension of *Cordana* sp. and *Nodulisporium* sp., respectively. There was a significant difference between the Disease Severity Index (DSI) of banana fruits treated with *Cordana* sp. and those treated with *Nodulisporium* sp. The results presented in this research highlight the possibility of using endophytic fungi as biological control agents for anthracnose disease of banana. *(Research Journal of Microbiology 3 (5): 368-374, 2008; **doi:** 10.3923/jm.2008.368.374)*

Studies on Mycological Status of Sundried Jew's-Mallow Leaves and Okra Fruits in Egypt

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Thirty samples of each of sundried jew's mallow leaves and okra fruits collected from six Governorates in Egypt were analyzed for their mould contamination and potential presence of mycotoxins. Mycological investigation revealed that twenty-six species and two varieties belonging to 13 genera of fungi were identified on Czapek's-dextrose and potato-dextrose agar media at 28±2°C using dilution-plating method. Okra fruit samples were highly contaminated with fungal spores (total counts were 47523 and 30563 colonies g^{-1} sample) than jew's mallow leaves samples (16608 and 6045 colonies), while the relative diversity and broad number of fungal genera and species was recorded on jew's mallow leaves (10 genera, 20 species + one variety and 6 genera, 10 species) than okra fruit samples (8, 16 + 2 and 3, 9 + 1) on the two used media, respectively. *Aspergillus*

was the highest occurrence (100% of the samples) and represented by 13 species + one variety of which, *A. flavus*, *A. niger*, *A. fumigatus*, *A. awamori*, *A. foetidus* and *A. ficuum* were the predominant. *Mucor, Rhizopus, Fusarium, Myrothecium*, *Emericella* and *Cochliobolus* were fungal genera isolated with different occurrences in high or/and moderate from the two plants samples tested on the two used media. Mycotoxin analysis proved that jew's mallow leave samples were free from any detectable mycotoxins, while five samples of dried okra fruits out of 30 tested (16.7%) were proved to be toxic. It is the first record of mycotoxins contamination of okra fruits in Egypt. The ability of 347 isolates of recovered fungi was screened for production of mycotoxins and extracellular cellulase enzymes. *(Research Journal of Microbiology 3 (5): 375-385, 2008;* ***doi:*** *10.3923/jm.2008.375.385)*

Helicobacter pylori cagA and *vacA* Genotypes and their Relationships to Peptic Ulcer Disease and Non-Ulcer Dyspepsia

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The aim of this study was to detect cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin gene A (*vacA*) genotypes of *Helicobacter pylori* and to study their relationships to the associated diseases. In the present analytical descriptive study *H. pylori* isolates were collected from 150 patients who underwent gastro duodenoscopy in Imam Khomeini Hospital of Tabriz, Iran. Of the patients 76 (50.7%) were males and 74 (49.3%) were females. The patients were divided into two groups. Group I consisted of 117 (78%) Non-Ulcerative Dyspepsia (NUD) patients and group II consisted of 33 (22%) Peptic Ulcer Disease (PUD) patients. Extracted DNA of *H. pylori* isolates were subjected to PCR tests to detect *cagA*, signal (s) and middle (m) regions of *vacA* genotypes. The designed primers revealed the presence of *cagA* gene in 125 (83.3%) of the isolates. Regarding *vacA* signal sequences 99 (66%) of our isolates revealed s1 type. The proportion of s1a, s1b and s1c subtypes were 76/150 (50.7%), 7/150 (4.7%) and 16/150 (10.6%), respectively while 40/150 (26.7%) presented as s2 type. In further analysis of the m region of *vacA*, m1 and m2 subtypes were detected in 49/150 (32.7%) and 81/150 (54%) of the isolates, respectively. The m1 subtype were further divided into m1a [41/49 (83.7%)] and m1b [(8/49 (16.3%)]. Thirty one isolates (20.7%) showed more than one *vacA* alleles in a single patient. Our results showed that isolates carrying the *cagA* gene were higher in PUD group than in NUD group, but did not substantiate statistically the role of *cagA* as a marker influencing increased virulence ($p>0.05$). Present findings also

showed that s1 and s2 subtypes of *vacA* gene are markers which differentiate between PUD and NUD groups. *(Research Journal of Microbiology 3 (5): 386-394, 2008;* ***doi:*** *10.3923/jm.2008.386.394)*

Isolation and Characterization of a Chitionolytic Enzyme Producing Microorganism, *Paenibacillus chitinolyticus* JK2 from Iran

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Chitinases are glycosyl hydrolases, which catalyze the degradation of chitin. These enzymes are capable of hydrolyzing chitin to its oligomers and monomer, N-acetyl-β-D-glucosamine. Fifty different chitin-degrading microorganisms were isolated in this study. One of these strains with high ability to produce chitinase was selected and identified as *Paenibacillus chitinolyticus* by morphological and biochemical properties along with 16S rDNA partial gene sequence analysis. This strain was able to produce high levels of extracellular chitinase in media containing chitin as sole carbon source. The chitinolytic activity of culture supernatant was maximal after 72 h of culture. The enzyme showed optimal activity at 37°C and a double optimum pH at pH 5 and 7. Chitooligosaccharides were the predominant products throughout the enzymatic hydrolysis of colloidal chitin, indicating that the enzyme was an endochitinase. This enzyme with these properties could be useful for waste treatment, chitooligosaccharides production and other relevant applications. *(Research Journal of Microbiology 3 (6): 395-404, 2008;* ***doi:*** *10.3923/jm.2008.395.404)*

Non-Conventional Method for Evaluation and Optimization of Medium Components for Rapamycin Production by *Streptomyces hygroscopicus*

Yasser Refaat Abdel-Fattah

An evaluation and optimization study of medium components for the production of the immunosuppressant compound rapamycin by *Streptomyces hygroscopicus* was addressed. Plackett-Burman experimental design was applied for screening of the most significant variables affecting production, where $FeSO_4$, mannose, fructose and lysine-HCl were the most positive significant factors. In order to find out the combination among the most significant variables that brings maximum

yield, Response Surface Methodology (RSM) was applied and the rapamycin yield increased to reach a theoretical value of 93 mg rapamycin per liter in the following medium (g L^{-1}): Fructose, 90; mannose, 18.9; lysine-HCl, 16.8 and $FeSO_4$, 0.1. Experimental verification of the polynomial model revealed a rapamycin yield of 95 mg L^{-1}, which is an evidence of more than 98% accuracy of the model under the investigated conditions. *(Research Journal of Microbiology 3 (6): 405-413, 2008;* ***doi:*** *10.3923/jm.2008.405.413)*

Antimicrobial Activity of Dichloromethane-Methanol (1:1 v/v) Extract from the Stem Bark of *Coula edulis* Bail. (Olacaceae)

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In order to confirm the traditional uses of *Coula edulis*, the CH_2Cl_2-MeOH (1:1 v/v) extract of stem bark of this plant and its column fractions were screened for antimicrobial activity. The plant was dried and extracted by maceration in CH_2-Cl_2-MeOH (1:1 v/v). The dry extract was fractionated by silica gel column chromatography. Phytochemical screening was performed using common chemical standard methods. Antimicrobial activity was assayed by disc diffusion method and broth macro dilution method. From the results, it appeared that the crude extract of *Coula edulis* stem bark displayed antibacterial activities against four clinical isolates of bacteria and antifungal activities against six strains of *Candida* species. The Minimum Inhibitory Concentration (MIC) values ranged from 12.5 to 25 mg mL^{-1} for bacteria and 1.56 to 6.25 mg mL^{-1} for yeasts. The fractionation of crude extract gave eight fractions. Fractions F3 and F4 showed higher antibacterial activities while fractions F5 and F6 displayed higher antifungal activity compared to the crude extract. Their MICs ranged from 0.19 to 12.5 mg mL^{-1}. Phytochemical screening indicated that the crude extract contains tannins, flavonoids, anthraquinones, anthocyanins, sterols and phenols. *Coula edulis* crude extract has the ability to inhibit bacterial and yeast growth. Fractionation enhanced the antimicrobial activity in some fractions. These results justify the traditional use of this plant for the treatment of infectious diseases. *(Research Journal of Microbiology 3 (6): 414-422, 2008;* ***doi:*** *10.3923/jm.2008.414.422)*

Monitoring Enterohaemorrhagic *Escherichia coli* O157:H7 in the Vegetable Food Chain in Ghana

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The study was carried out to assess the occurrence of *Escherichia coli* O157:H7 in the vegetable food chain in Accra, the capital city of Ghana. A total of 272 samples of various types of vegetables were screened for *Escherichia coli* and *Escherichia coli* O157:H7 using standard microbiological methods. In addition, 80 samples of water used for irrigation, 40 manure soil samples and 250 stool samples of various livestock were also screened. Overall, a total of 243 (37.9%) *Escherichia coli* isolates were obtained from all the specimens screened. The overall prevalence rates of *Escherichia coli* for vegetables were 35.3 and 29.4% for external and internal vegetable parts, respectively. The prevalence rates of *Escherichia coli* for irrigation water, manured soils and livestock faeces were 26.3, 52.5 and 24%, respectively. Overall, only one *Escherichia coli* isolate from irrigation water was detected to be *Escherichia coli* O157:H7 which translates to an overall prevalence rate of 0.4% among the *Escherichia coli* population. The study shows that *Escherichia coli* O157:H7 is present in the vegetable food chain in Accra but is relatively uncommon. Despite the low prevalence of the organism and its isolation from only irrigation water, the findings of the study call for public health attention owing to the very low infectious dose of *Escherichia coli* O157:H7 and the common practice of vegetable irrigation. *(Research Journal of Microbiology 3 (6): 423-428, 2008;* ***doi:*** *10.3923/jm.2008.423.428)*

Studies on Qualitative and Quantitative Characterization of Alcoholic Beverages from Tropical Fruits

F.O. Omoya and F.C. Akharaiyi

Fermentation of the tropical fruits which involved the activities microorganisms resulted to the alcohol yielded, aroma, taste and the overall acceptability of the products. Six different alcoholic beverages from watermelon, watermelon-banana and watermelon-pineapple mixtures were produced using monoculture and mixed culture fermentation techniques. Three yeast species (*Kleochera apiculata*, *Torulospora delbruckii*, *Saccharomyces cerevisiae*) and six bacteria species (*Aerobacter aerogenes*, *Chromobacterium violacium*, *Lactobacillus* sp., *Leuconostoc oenos*, *Micrococcus luteus* and *Streptococcus lactis* were identified during the study. The daily succession of these organisms in the various fermenting samples, differs in cell mass and occurrence due to their different growth conditions and factors present. A higher bacterial load (4.4±0.3-4.9±0.4 log (cfu) mL^{-1}) than yeast (3.0±00-4.9±0.2 log (cfu) mL^{-1}) counts was observed in the mixed culture fermentation, while in the monoculture fermentation, a higher yeast load (4.1±0.2-4.9±0.3 log (cfu) mL^{-1}) than bacterial loads

(2.5±0.1-4.3±0.3 log (cfu) mL^{-1}) counts was recovered. The monoculture fermented beverages were of better characteristics than the mixed culture fermented alcoholic beverages. *(Research Journal of Microbiology 3 (6): 429-435, 2008;* ***doi:*** *10.3923/jm.2008.429.435)*

Molecular Fingerprinting of Methicillin-Resistant *Staphylococcus aureus* Isolates in Hospital Staff and Patients

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The aims of present investigation were to study the nasal carriage rate of MRSA in hospital staff and in-patients, determination of antibiotic resistant patterns of nasal and clinical MRSA isolates and typing of MRSA isolates by RAPD- PCR. Two hundred and six *S. aureus* isolates were recovered from clinical specimens and noses of 460 staff and in-patients admitted in Imam Khomeini and Pediatrics hospitals by standard methods during 6 months (2004-2005). Disk agar diffusion (using 13 antibiotics disks) and oxacillin agar screening methods for detection of MRSA isolates were performed according to CLSI. PCR was also used to amplify a 310 bp sequence from *S. aureus* genome (*mec*A gene) for detection of MRSA isolates. RAPD-PCR was carried out for fingerprinting of MRSA isolates genome. MRSA isolates were resistant up to 11 antibiotics. All of the MRSA isolates were resistant to penicillin, but sensitive to vancomycin. Of 206 *S. aureus* isolates, 77 MRSA isolates were detected using disk agar diffusion and oxacillin agar screening methods. In contrast, 80 isolates were detected as MRSA by amplification of *mec*A sequence. In RAPD-PCR experiments, 43 different RAPD patterns were obtained from our MRSA isolates. Nasal carrier rate of *S. aureus* was 34.7% and MRSA isolates were high (38.8%) in our hospitals. This study revealed high rate of MRSA, that infected patients and MRSA nasal carriers (staff and in-patients) were the main source of transmission and infection, therefore effective control measures are necessary to avoid nosocomial infection outbreaks. *(Research Journal of Microbiology 3 (6): 436-446, 2008;* ***doi:*** *10.3923/jm.2008.436.446)*

Phenotypic Characteristics of Lactic Acid Bacteria Isolated from Cow's Raw Milk of Bororo Cattle Breeders in Western Highland Region of Cameroon

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A study was carried out in order to screen lactic acid bacteria isolated from cow's raw milk collected from Bororo cattle breeder in the Western Highlands of Cameroon. In order to be assessed for its potential as starter or adjuncts cultures, strains were tested for acid production, aminopeptidase and autolytic activities. Thirty-one gram-positive and catalase-negative isolates were found to be *Enterococcus* sp. (two isolates), *Lactococcus* sp. (fifteen isolates) and *Lactobacillus* sp. (fourteen isolates), using morphological and physiological tests. From these isolates, twenty were selected for species identification using API 50CH and API 20 STREP kits and the SDS-PAGE technique of the whole-cell proteins. The *Enterococcus* strains were *Enterococcus faecalis* (13LC, 14LC). Concerning genus *Lactococcus*, 13 strains were identified as *Lactococcus lactis* ssp. *lactis* (1LF, 2LF, 3LF, 7LF, 1LC, 2LC, 4LC, 10LC, 11LC, 12LC, 15LC, 16LC, 17LC). Five of the *Lactobacillus* strains were identified as follows: four strains were *Lactobacillus plantarum* (6LF, 7LC, 8LC, 19LC); one strain was *Lactobacillus coprophilus* (3LC). According to acidifying activity, only strain 14LC (*Enterococcus faecalis*) presented rapid lactic acid production. Some *Lactococcus* strains (7LF, 10LC, 11LC) produced moderate acidification of milk. These cultures can be used as acidifying starters in dairy industry. Except strain *Enterococcus faecalis* (14LC), all strains tested in this study presented poor leucine aminopeptidase and autolytic activities. *(Research Journal of Microbiology 3 (6): 447-456, 2008;* ***doi:*** *10.3923/jm.2008.447.456)*

Inhibitory Effect of Caffeine on Growth of Various Bacterial Strains

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The study was undertaken to investigate the effect of caffeine (1, 3, 7-trimethylxanthine) on growth, morphology and viability of caffeine degrading *Pseudomonas* sp. and other non caffeine degrading bacterial strains. Growth, morphology and cell viability of the bacterial strains were studied in caffeine medium, minimal medium without caffeine and in minimal medium with caffeine added at log phase of growth. The caffeine degrading *Pseudomonas* sp. achieved a maximum cell dry weight of 1.1 g L^{-1} after caffeine addition at log phase of growth without any change in morphology. The growth and viability of *E. coli* DH5α and other bacterial strains was greatly reduced upon addition of caffeine at log phase of growth. *E. coli* DH5α strain formed long filamentous cells on caffeine exposure and lysis occurred for other Gram negative bacterial species and *Bacillus subtilis*. *E. coli* DH5α transformed with plasmid from caffeine degrading *Pseudomonas* sp. was found to be tolerant to caffeine. This study shows the

susceptibility of non caffeine degrading bacterial strains to caffeine and will be helpful in understanding the basics of the evolution and survival of xenobiotic degrading strains in nature. *(Research Journal of Microbiology 3 (6): 457-465, 2008; **doi:** 10.3923/jm.2008.457.465)*

An Artificial Mouth System (NAM Model) for Oral Biofilm Research

Z.H.A. Rahim, A.R. Fathilah, S. Irwan and W.I. Wan Nordini Hasnor

The objective of this study was to validate NAM model, an artificial mouth system for use in the study of oral biofilms. The NAM model consists of a cylindrical glass chamber (1×6 cm) which was used to mimic the oral cavity and glass beads (3 mm), placed along its length to provide surfaces for biofilm formation. The opening at both ends of the chamber which were fitted with rubber tubing served as an inlet to and outlet from the system. The two tubing were then connected to a reservoir (bacterial reservoir) via a peristaltic pump. The apparatus was kept at 37°C in a water bath. In the formation of oral biofilm, saliva was first pumped into the system to coat the glass beads. Excess saliva was then rinsed with distilled deionised water. Bacterial inoculum (*Strep. mutans*) was then allowed to flow into the system for 24 h. The bacterial population (cfu mL^{-1}) in the biofilm developed on each of the glass beads in different experiments were analyzed and, validated for reproducibility. Its efficiency in maintaining temperature and flow rate for the experiment and sterility prior to the experiment was also determined. The results obtained showed that the bacterial counts of the biofilms between glass beads are not significantly different ($p>0.6$) and demonstrated reproducibility (4-5% standard deviation) between different experiments. It was also observed that the flow rate and temperature are constant and sterility of the apparatus is maintained throughout the experiment. This shows that the NAM model is valid for use in the *in vitro* study of oral biofilm development. *(Research Journal of Microbiology 3 (6): 466-473, 2008; **doi:** 10.3923/jm.2008.466.473)*

Enzymatic Hydrolysis of Palm Oil Mill Effluent Solid Using Mixed Cellulases from Locally Isolated Fungi

Wong Kok Mun, Nor'Aini Abdul Rahman, Suraini Abd-Aziz, Vikineswary Sabaratnam and Mohd Ali Hassan

In order to optimize the enzymatic hydrolysis of POME solid, the effects of substrate pretreatment using varying concentrations of sodium hydroxide and

sulfuric acid, crude enzyme from both strains in different ratio and pH reaction were studied. The best experimental conditions found to degrade POME solids were 12 h incubation time, 0.5% (v/v) sulfuric acid pretreatment, crude enzymes mixture from *Aspergillus niger* EB5 and *Trichoderma* sp. EB6 (1.75 mL Asp+0.25 mL *Tri* with the total cellulase activity equal to 14.76 IU) and incubation pH at 5.0. Under these conditions, the reducing sugar concentration reached 23 g L^{-1} with the hydrolysis yield and productivity at 32% and 1.90 g L^{-1} h^{-1}, respectively. The bioconversion of POME solid to reducing sugar by the mixture of crude enzyme from the strains was relatively higher by almost 2 folds as compared to commercial cellulase. The results suggested that the crude cellulases mixture from locally isolated fungi has potential for hydrolyzing the abundant agriculture residues from the palm oil industry. *(Research Journal of Microbiology 3 (6): 474-481, 2008;* ***doi:*** *10.3923/jm.2008.474.481)*

A Further Characterization of 3-Chloropropionic Acid Dehalogenase from *Rhodococcus* sp. HJ1

Ng Hong Jing, Roswanira Ab. Wahab, Aishah Mohd Taha, Noor Aini Abdul Rashid and Fahrul Huyop

The main aim of the present study is to further characterize a new dehalogenase enzyme found in the crude extracts from *Rhodococcus* sp. The ability of the enzyme to catalyze the dehalogenation of various halogen-substituted organic acids was investigated and the highest activity was found with 3-chloropropionic acid as a sole carbon source in the growth medium. The enzyme followed Michaelis-Menten kinetics and the Km for 3-chloropropionic acid was 0.2 mM. Maximum activity was found at pH 7.6 at 30°C. The enzyme activity in the cell-free extract was unaffected by diaminoethane tetraacetic acid (EDTA), dithiothreitol (DTT) or by Mn and Zn ions but was reduced by $HgCl_2$ (70%) and $Pb(NO_3)_2$ (80%). The enzyme removed the chlorine atom present on a number of 3- and 4-carbon alkanoic acids if the halogen was on the β-position. *(Research Journal of Microbiology 3 (6): 482-488, 2008;* ***doi:*** *10.3923/jm.2008.482.488)*

Extended-Spectrum β-Lactamases-Producing *Escherichia coli* from a Tertiary Hospital in Malaysia: Emergence of CTX-M-Type β-Lactamases Variation

Zamberi Sekawi, Rusmah Yusof and Mariana Nor Shamsudin

A study was conducted to portray a preliminary characteristic of extended-spectrum β-lactamases (ESBLs)-producing *Escherichia coli* in a local tertiary

hospital in Malaysia. Sixteen clinical isolates of ESBLs producing *E. coli* from different sources were examined for $bla_{SHV/TEM/CTX-M}$ ESBL genes by PCR molecular assay. Each strain was found to carry at least one of the genes. This study demonstrated a high prevalence of bla_{CTX-M} (81.3%) and bla_{TEM} genes (75%). Only two strains (12.5%) carried the bla_{SHV} gene. Nucleotide and deduced protein sequences determination showed; 61% produced CTX-M-15, 31% produced CTX-M-14 and 8% produced CTX-M-3. The antimicrobial susceptibility testing data determined that almost all sixteen isolates were resistant to oxyimino-cephalosporins, 46% resistant to gentamicin, 69% resistant to trimethoprim-sulfamethoxazole and 46% resistant to ciprofloxacin. This study preliminary emphasizes the epidemiology of the ESBL-producing *E. coli* particularly SHV, TEM and CTX-M-type producing *E. coli* in Malaysia. *(Research Journal of Microbiology 3 (6): 489-493, 2008;* ***doi:*** *10.3923/jm.2008.489.493)*

Epidemiologic Investigation of an Outbreak of *Tinea capitis*: Experience from the South of Iran

Fateme Nasri, Dadkhoda Sadeghi, Ashraf Bamorovat, Eshrat Bamorovat and Nouzar Nakhaee

The deputy of health affairs of Kerman province encountered a report of *Tinea capitis* outbreak in one of the southern towns of the province in winter 2006 of which stated 1294 cases of *Tinea capitis* among primary school children that 86% of them were boys. The epidemiological and environmental investigation was conducted through a case-control study. The outbreak investigation showed that the risk of infection was significantly higher among subjects with a lower socioeconomic status, poor personal hygiene and a positive personal history of *Tinea capitis*. Improvement of living condition, public attention to personal hygiene and active case finding would be effective in decreasing and preventing *Tinea capitis. (Research Journal of Microbiology 3 (6): 494-498, 2008;* ***doi:*** *10.3923/jm.2008.494.498)*

Isolation and Identification of Biosurfactant Producing Actinomycetes From Soil

Intira Thampayak, Naowarat Cheeptham, Wasu Pathom-Aree, Pimporn Leelapornpisid and Saisamorn Lumyong

Two hundred and twenty-nine soil actinomycete strains were initially screened for extracellular biosurfactant activity by a drop-collapse method in Kim's medium

containing sesame oil as a sole source of carbon. Three isolates, namely S71, S72 and S177, were capable of biosurfactant production. Phenotypic and genotypic analysis strongly suggested that they were members of the genus *Streptomyces*. The isolates S71 and S177 were closely related to *S. griseoflavus* sharing 99% 16S rRNA gene similarities, whereas S72 was closely related to *S. fradiae* sharing only 98% 16S rRNA gene similarities suggesting that this may represent a novel species. The cell-free culture broth of the three isolates had emulsification activity and decreased surface tension. According to emulsification activity (E24) and surface tension values observed in the three isolates, *Streptomyces* sp. S72 was selected for biosurfactant production in larger scale. The cell-free culture broth of the isolate S72 was further extracted with chloroform:methanol (2:1) and two fractions were found positive in producing biosurfactants. To determine structure and molecular weight of the two positive fractions, the Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) will be carried out. *(Research Journal of Microbiology 3 (7): 499-507, 2008;* ***doi:*** *10.3923/jm.2008.499.507)*

Control of *Staphylococcus aureus* Sensitivity to Sweet Pepper (*Capsicum annum*) by a Chemical Mutation

B. Boboye and K. Odekunle

Chemical mutation was carried out on *Staphylococcus aureus* using ethylmethyl sulphonate. The bacterium and its mutants were tested for sensitivity to the extract of *Capsicum annum* (sweet pepper). Mutants with varying degrees of sensitivity to the extract were obtained. On the basis of zone of inhibition, mutants were classified as Non-sensitive (NS), Slightly-sensitive (SS), Fairly-sensitive (FS), Normal-sensitive (NMS) and Super-sensitive (SUS) mutants with zones of growth inhibition on Mannitol Salt Agar (MSA) in the range of 0.00-0.09, 0.10-1.09, 1.10-2.09, 2.10-3.09 and 3.10-4.09 mm respectively. About 26, 7 and 23.5% mutants were screened as NS, SS and FS respectively. Other mutants, NS and SS constituted 27 and 16.5% of the total mutants population. Some of the mutants appeared bacteriostatic, bactericidal and bacteriolytic in actions. Also, mutation caused a change in colour of MSA from red to yellow. Some mutants completely changed the colour (complete colour change, CCC mutants) with zone of colour change of 9.00 mm. Size of colour change exhibited by other mutants ranged from 2.40 to 3.20 mm (slight colour change, SCC), 3.50 to 4.90 mm (strong colour change, STCC) and 0.00 mm (no colour change, NCC) relative to the wild-type which showed 4.70 mm zone of colour change. *(Research Journal of Microbiology 3 (7): 508-513, 2008;* ***doi:*** *10.3923/jm.2008.508.513)*

Characterisation of Extended Spectrum Beta-Lactamase Producing *E. coli* from Secondary and Tertiary Hospitals in South Eastern Nigeria

I.R. Iroha, M.U. Adikwu, E.S. Amadi, I. Aibinu and C.O. Esimone

Clinical isolates of *Escherichia coli* (No. = 109) were collected from Microbiology Laboratory unit of a tertiary hospital (Ebonyi State University teaching hospital, Abakaliki, EBSUTH) and a secondary hospital (Federal Medical Center, Abakaliki, FMC) from four different clinical specimens (urine, stool, blood and sputum) between February to November 2006. Sixty-three clinical isolates of *E. coli* were isolated from EBSUTH while forty-six were from FMC Abakaliki. These organisms were characterized and identified to species level using standard identification technique. Sensitivity studies were carried out on the test organisms using disc diffusion method and later the organisms were characterized phenotypically for ESBL production using the Double Disc Synergy Test (DDST). A preliminary molecular characterization of the ESBL producing isolates were further carried out based on the evaluation of their plasmid profile via agarose gel electrophoresis. The over-all result of the study revealed that the prevalence of ESBL producing organisms was high 18 (16.5%) in our environment. The rate of occurrence varied within the two hospitals with 11 (23.9%) from FMC (urine 2 (18.2%), blood 5 (35.7%), wound 3 (30%) semen 1 (33.3%) and non was isolated from sputum while 7(11.1%) were from EBSUTH (urine 2(9.5%), blood 3 (21.4%), wound 2 (18.2%) respectively and non was isolated from sputum and semen. The plasmid profile studies revealed the presence of low molecular weight plasmid DNA within the ranges of 21.3-29.4 kb. *(Research Journal of Microbiology 3 (7): 514-519, 2008;* ***doi:*** *10.3923/jm.2008.514.519)*

Mesophilic Fungi and Mycotoxins Contamination of Libyan Cultivated Four Fabaceae Seeds

M.S. Youssef, E.M. El-Mahmoudy and Maryam A.S. Abubakr

One hundred and forty three species in addition to 9 varieties belonging to 32 fungal genera were isolated and identified from 15 samples of each of broad bean, chickpea, kidney bean and sweet pea seeds collected from eight Shabias in Libya on 1% dextrose-Czapek's agar medium at 28±2°C using seed-plate method (3792 colonies 25 seeds^{-1}, 29 fungal genera, 111 species and 2 varieties) and dilution-plate method (2330 colonies g^{-1} dry weight sample, 23 genera,

100 species and 7 varieties). The fungal genera of highest occurrence and their respective number of the species were *Aspergillus* (*A. flavus*, *A. niger* and *A. fumigatus*), *Penicillium* (*P. chrysogenum*), *Mucor* (*M. hiemalis*), *Alternaria* (*A. alternata*), *Fusarium* (*F. oxysporum*), *Rhizopus* (*R. stolonifer*) and *Eurotium* (*E. chevalieri* and *E. repens*). Mycotoxin assay proved that 16 samples (26.7%) out of 60 tested were toxic with different toxins and varying degrees of toxicity. No mycotoxins tested were detected in any chickpea seed samples investigated. It is the first report on mycoflora and mycotoxins of Fabaceae seeds in Libya, as well as 85 species in addition to 7 varieties belonging to 20 genera are new records to Libyan mycoflora. *(Research Journal of Microbiology 3 (7): 520-534, 2008;* ***doi:*** *10.3923/jm.2008.520.534)*

Anti-Mycobacterial Activity of Extracts Derived from Australian Medicinal Plants

Michelle Meilak and Enzo A. Palombo

Extracts of seventeen traditional Australian medicinal plants used to treat infections and respiratory conditions were tested for anti-mycobacterial activity against the fast-growing strains, *Mycobacterium fortuitum* and *M. smegmatis*. Four extracts, the aerial parts of *Pterocaulon sphacelatum* (Asteraceae), the bark and leaves of *Acacia ligulata* (Mimosaceae), the leaves and stems of *Eremophila alternifolia* (Myoporaceae) and the leaves of *Eremophila longifolia*, showed activity against *M. smegmatis* only, while the two *Eremophila* extracts were also active against *M. fortuitum*. The minimum inhibitory concentrations ranged from 20-66 mg mL^{-1}. The identification of the anti-mycobacterial compounds from these extracts may yield new and effective agents to combat diseases caused by *Mycobacterium* species. *(Research Journal of Microbiology 3 (7): 535-538, 2008;* ***doi:*** *10.3923/jm.2008.535.538)*

Effect of Biotic and Abiotic Factors on Pathogenic Gram-Negative Bacteria in Lake Qarun, Egypt

S.A. Rabeh and M.F. Fareed

In addition to El-Bats and El-Wadi drains, the main sources of drainage water, six stations were selected and distributed all over Lake Qarun. Total and the most common pathogenic Gram-negative bacteria; *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella choleraesuis*, *Proteus vulgaris* and

Serratia liquefaciens as well as actinomycetes were enumerated and identified. Twenty-one actinomycetes were isolated and screened for their antibacterial activities. All actinomycetes isolated were identified as *Streptomyces* and eleven of them showed antibacterial activities against the pathogenic Gram-negative bacteria isolated from the same tested water sample. The most antibacterial active isolate was subjected to morphological, physiological and biochemical studies and identified as *Streptomyces calvus*. The antimicrobial activity of the identified *Streptomyces calvus* against some indicator organisms was also performed. In addition, the effects of some abiotic factors on the tested bacteria were discussed. *(Research Journal of Microbiology 3 (8): 539-551, 2008;* ***doi:*** *10.3923/jm.2008.539.551)*

Microbiological and Sensory Analysis of Imported Fruit Juices in Kumasi, Ghana

M.G. Addo, W.G. Akanwariwiak, P. Addo-Fordjour and K. Obiri-Danso

A study conducted on some imported fruit juices on microbiological and sensory analysis showed a significant increase in microbial load in the apple and mango fruit juices as they stay over the period in the shelves from June, 2007 to February, 2008. Though significant, the orange juice showed the lowest microbial count of 3.1×10^3 and 9.5×10^3 in June, 2007 and February, 2008, respectively in terms of real numbers. Apple and mango fruit juices showed insignificant increases over the period in yeast growth ($p = 0.062$ and $p = 0.093$, respectively). In general, yeast numbers were relatively lower than bacterial counts in both apple and mango fruit juices. The genus *Bacillus* was the most diverse organism although other Gram positive cocci of the genera *Micrococcus*, *Leuconostocs*, *Lactobacillus* and the yeast *Saccharomyces* were also isolated. Sensory analysis also showed that, consumers preferred the product which had been on the shelf for the shortest period of time (i.e., June, 2007 and October, 2007 products) in terms of aroma, taste and colour. *(Research Journal of Microbiology 3 (8): 552-558, 2008;* ***doi:*** *10.3923/jm.2008.552.558)*

Isolation of Cold Tolerant Antifungal Strains of *Trichoderma* sp. From Glacial Sites of Indian Himalayan Region

A. Ghildiyal and A. Pandey

Three species of *Trichoderma* viz., *T. harzianum*, *T. konengii* and *T. viride* have been isolated from the soil samples collected from forest sites in higher

altitudes of Indian Himalayan Region. The species could grow between 9 to 35°C temperature and 4 to 12 pH on agar plates; the optimum requirement being 24°C and 5.5 pH, respectively. Further incubation of the agar plates showing normal growth of *Trichoderma* sp. at 4°C, induced heavy sporulation in three weeks of time. Induction of sporulation on exposure to low temperature appeared to be a strategy for survival of these species in extreme cold environment experiencing sub zero temperatures. Antifungal activities were demonstrated between *Trichoderma* sp. and phytopathogenic fungi in dual cultures. The antifungal metabolites produced by *Trichoderma* sp., diffusible as well as volatile, caused abnormalities in fungal structures of pathogenic fungi. Plant growth promotion abilities of *Trichoderma* sp. was also demonstrated through a plant based bioassay in greenhouse. The study is important for documentation of microbial diversity of Indian Himalayan Region (IHR) and determination of the associated biotechnological applications. *(Research Journal of Microbiology 3 (8): 559-564, 2008;* ***doi:*** *10.3923/jm.2008.559.564)*

Filter Paper Degradation by Bacteria Isolated From Local Termite Gut

M. Ramin, A.R. Alimon, K. Sijam and N. Abdullah

Bacterial strains isolated from the gut of the local termite *Coptotermes curvignathus* were inoculated into a buffered medium containing minerals and Whatman filter paper as the sole carbon source to observe the ability of the bacteria to digest solid substrate. The bacteria were *Bacillus cereus* strain Razmin A, *Enterobacter aerogenes* strain Razmin B, *Enterobacter cloacae* strain Razmin C, *Acinetobacter* strain Raminalimon and *Chryseobacterium kwangyangense* strain Cb. The Gen Bank NCBI/EMBL accession numbers for the bacterial strains were EU294508, EU305608, EU305609, EU332791 and EU169201, respectively. The ability of bacterial cultures to grow in this medium as well as to digest the filter paper was determined by visual observation after 30 days. All bacterial cultures showed growth as the medium turned cloudy and the filter paper became macerated. *Chryseobacterium kwangyangense* strain Cb showed yellow pigmented colonies on the filter paper. *Bacillus cereus* strain Razmin A showed clumps of degraded filter paper with black dots. *(Research Journal of Microbiology 3 (8): 565-568, 2008;* ***doi:*** *10.3923/jm.2008.565.568)*

Production of Reducing Sugars by *Trichoderma* sp. KUPM0001 during Solid Substrate Fermentation of Sago Starch Processing Waste *Hampas*

Z. Shahrim, V. Sabaratnam, N.A.A. Rahman, S. Abd-Aziz, M.A. Hassan and M.I.A. Karim

Trichoderma sp. KUPM0001 showed good growth during solid substrate fermentation (SSF) of sago pith residue known as *hampas*, supplemented with 10% (v/w) of mineral salts solution containing 0.5% (w/v) (83.3 mM) urea as nitrogen source and an initial moisture content of 80% (v/w). Mycelium suspension of 10% (v/w) density was used as initial inoculum and SSF was carried out at 25±2°C in static condition over a period of 120 h. The parameters optimized included the initial moisture content of the substrate, mineral salts solution, urea concentration, inoculum density, incubation temperature and incubation time. Without optimized condition, the maximum reducing sugar obtained was 24 mg mL^{-1} compared to 46 mg mL^{-1} substrate during optimized SSF after 96 h incubation. The optimum parameters obtained were 80% (v/w) of initial moisture; 10% (v/w) of inoculums size; 1.0% of urea in 20% (w/v) of mineral solution and incubated at 30±2°C. The enzyme activities using optimized condition gave maximum α-amylase, glucoamylase, carboxymethyl cellulase, filter paperase and β-glucosidase of 3.19, 2.22, 1.66, 1.11 and 1.48 U mL^{-1}, respectively. *(Research Journal of Microbiology 3 (9): 569-579, 2008;* ***doi:*** *10.3923/jm.2008.569.579)*

Effect of Inoculation on Root Exudates Carbon Sugar and Amino Acids Production of Different Rice Varieties

U.A. Naher, O. Radziah, M.S. Halimi, Z.H. Shamsuddin and I. Mohd Razi

An experiment was conducted in axenic condition to study the effect of *Corynebacterium* sp. (Sb26) and *Rhizobium* sp. (Sb16) inoculation on the root exudates carbon sugars and amino acid production in three different rice (*Oryza sativa*) genotypes. A total of seven carbon sugars and 16 amino acids were determined from the Mahsuri, Mayang Segumpal and MR219 rice root exudates. The concentration of root exudate sugars, amino acids and its released pattern were significantly different with rice genotypes. Mahsuri released the highest sugar (25.73%) followed by MR219 and Mayang Segumpal (23.14% and 20.85% of plant dry wt.) rice, respectively. Inoculated plants produced different amount of sugar and amino acids in the presence of diazotrophs compared to non inoculated

plants. Mahsuri rice inoculated with *Corynebacterium* sp. released the highest amount of fructose (791 μmol g^{-1} root dry wt.) and arabinose (640 μmol g^{-1} root dry wt.). Mayang Segumpal rice inoculated with *Rhizobium* sp. produced the highest amount of sucrose μmol g^{-1} root dry wt in the root exudate. A significantly higher amount of glycine and isoleucine were detected in the inoculated root exudates of all rice varieties. However, inoculation enhanced production of sugars and amino acids in root exudates. In general rice genotypes inoculated with *Rhizobium* sp. produced higher amount of total sugars and amino acids in root exudates compared to that of *Corynebacterium* sp. *(Research Journal of Microbiology 3 (9): 580-587, 2008;* ***doi****: 10.3923/jm.2008.580.587)*

Anti-Viral Activity of *Cissus repanda* Vahl. Plant Extract on Herpes Simplex Virus

Jiraporn Nikomtat, Narumol Thongwai, Saisamorn Lumyong and Yingmanee Tragoolpua

The effect of *Cissus repanda* Vahl. plant extract on herpes simplex virus type 1 and type 2 was investigated in this study. The cytotoxicity of dichloromethane and methanol extracts of *C. repanda* on GMK cells was determined by MTT assay. Non toxic concentrations were used in the study. Methanol extracts of *C. repanda* showed higher anti-HSV efficacy than dichloromethane extracts, by plaque reduction assay, although, HSV particles were directly inhibited by both extracts. Inhibition of HSV attachment, penetration and replication were also observed, after treatment of HSV with *C. repanda* extracts. The most affected stage was the attachment period. Moreover, *C. repanda* extracts inhibited replication of HSV-2 more than HSV-1. *(Research Journal of Microbiology 3 (9): 588-594, 2008;* ***doi****: 10.3923/jm.2008.588.594)*

Laboratory Containment of Wild Polio Viruses Survey and Inventory in Sudan

M.I. Ahmed, A.H. Aldoma, M.A. Alzohyrey, J.A. Bilal and N.S. Saeed

The aim of the present study was to evaluate phase-one containment of wild polio virus and laboratory inventory in Sudan according to the WHO plan. A questionnaire was designed for phase one poliomyelitis virus laboratory containment; this questionnaire was used to collect data from 488 laboratories form the whole Sudan. The present study showed that the Sudan country followed all steps of WHO guidance in laboratory containment. The country has succeeded

in fulfilling the phase one polio virus laboratory containment; survey and inventory. *(Research Journal of Microbiology 3 (9): 595-599, 2008;* ***doi:*** *10.3923/jm.2008.595.599)*

In silico Analysis of *Chlorobium* Genomes Divulge Insights into the Lifestyle of the Bacteria

Saubashya Sur, Asim K Bothra, Manprit Bajwa, Louis S. Tisa and Arnab Sen

The finished sequences of three *Chlorobium* genomes were examined and compared to each other for their synonymous codon usage. Codon usage by *Chlorobium* was moderately biased but a considerable amount of variation was observed. GC3 composition plays an important role in the codon usage variation among the genes in the studied genomes. Similar homologs of horizontally transferred nitrogen fixing and photosynthesis related genes having high identity levels indicated their co-evolution within the genus. Correlation of codon usage bias with tRNA content in *Chlorobium* genomes revealed the inability of the translation machinery in these bacteria to co-evolve with higher codon usage resulting in moderate bias. Arrangement of the genes in leading strand and lagging strand of replication had virtually no role in influencing synonymous codon usage variation in these bacteria. Whole genome alignment revealed the conserved nature of the genomes. Using codon adaptation index, a set of potentially highly expressed genes in *Chlorobium* was determined taking ribosomal protein genes as a reference. A sizeable fraction of the potentially highly expressed (PHX) genes in the COG categories were related to metabolism. Quite fascinatingly, some of the genes associated with nitrogen fixation and photosynthesis like hydrogenases, nitrogenase iron protein complexes, bacteriochlorophylls, chlorosomes etc. were also PHX. These results offer insights into the survival patterns of these bacteria thriving under stressed conditions and efficiently carrying out two important metabolic processes especially under reduced light and anoxic environments. *(Research Journal of Microbiology 3 (10): 600-613, 2008;* ***doi:*** *10.3923/jm.2008.600.613)*

Diversity of Coral *Eunicea fusca* Associated Bacteria Using Culture Dependent Techniques

Abdelnasser S.S. Ibrahim

Invertebrates are known to be associated with diverse bacterial population, however, very little is known about the structure, composition and maintenance of

these bacterial communities. In the current study, we characterize the culturable bacterial community associated with gorgorian coral *Eunicea fusca*. This was achieved using culture-based methods and molecular techniques for the identification of the bacterial isolates. The culturable heterotrophic bacterial community of this coral is composed mainly of the bacterial groups Alphaproteobacteria (65.5%), Gammaproteobacteria (20.7%), Betaproteobacteria (6.9%), Cytophaga-Flexibacter Bacteroids (3.4%) and Firmicutes (3.4%). This study provides evidence of specific bacterial association with the coral in comparison to bacterial community in the coral surrounding seawater. Furthermore, bacterial isolation using oligotrophic conditions,. at slightly alkaline pH, was found to increase the culturability and diversity of *Eunicea fusca* associated bacteria. *(Research Journal of Microbiology 3 (10): 614-621, 2008;* ***doi:*** *10.3923/jm.2008.614.621)*

Newly Isolated *Pandoraea* sp. Capable of Phenol Biodegradation

R.A. Amer

The aim of this study was to isolate and characterize new strains capable of phenol bioremediation. A new strain of *Pandoraea* sp. was isolated from Red Sea soil contaminated with hydrocarbon. Morphological and molecular characterization were performed to identify the isolated strain, it was designated as *Pandoraea* sp. phen16 and located in the database under accession number EU549818. The isolated strain could remove 100% of 50 mg phenol L^{-1} in culture as sole carbon source after 3 days of incubation, where 100 mg phenol L^{-1} in culture inhibited the growth, only 15% from total phenol was removed. For further support a PCR product was obtained from amplification of phenol hydroxylase, suggesting the possible existence of the ring-hydroxylating mono-oxygenases genes responsible for phenol degradation. *(Research Journal of Microbiology 3 (10): 622-629, 2008;* ***doi:*** *10.3923/jm.2008.622.629)*

Dynamics and Diversity of Bacterial Communities of Fermented Weaning Foods via Denaturing Gradient Gel Electrophoresis PCR-DGGE

S.M. Wakil, A.A. Onilude and A.S. Ball

Different cereal-legume weaning blends were formulated and subjected to spontaneous fermentation for a period of 72 h. The analysis of the DGGE pattern,

of the fermented blends, obtained with bacterial primer targeting the 16S rDNA genes clearly demonstrated that there was a major shift in the community structure within the first 24 h. The species richness R for total bacterial community varied from a low value 6.50 for maize-based blends to a higher value 9.50 for sorghum-based blends. The biodiversity index H' as well as concentration of dominance S according to Shannon and Weaver and Simpson's index, respectively varied significantly with the sample type. Statistical analysis showed a significance difference $p<0.05$ in the total bacterial diversity within samples with increase in fermentation time but with no significant difference in the diversity among the different fermented samples. *(Research Journal of Microbiology 3 (11): 630-640, 2008; **doi:** 10.3923/jm.2008.630.640)*

The Relation of the Cytokines and the CD Markers to the Antibody Titers in Patients with Brucellosis

A.M. Al Ali, A.I. Al Haroon and A.M. Alluwaimi

Human Brucellosis is one of the highly reported zoonotic diseases in the world. In Saudi Arabia, it is a highly reported zoonotic disease. Human immune response to Brucellosis is controversial. The relation of antibody titers to cytokines and CD markers of cells subpopulations was seen important to reveal the immunopathological changes at different stages of Brucellosis. The level of the cytokines, IL-1β, IL-2, IL-4, IL-8, IL-12, INF-γ and TNF-α and the CD markers, $CD4^+$, $CD8^+$, $CD19^+$, NK/$CD18^+$, MHC-II and γδ TCR were monitored 32 patients with serum titres 1/80, 1/160, 1/320 and 1/640 (8 samples/each patient). The level of the cytokines IL-2, IL-4, IL-12, INF-γ and TNF-α were decreased significantly in all serum titres. However, the CD markers $CD8^+$, NK/$CD18^+$ and γδ TCR as well as MHC-II increased significantly in the patients with 1/320 titer, Whereas $CD19^+$ cells decreased significantly. The significant decrease of IL-2, IL-4, IL-12, INF-γ and TNF-α even in the titres 1/80 and 1/160 implies that organism could inflicts major defects on the resistance to human Brucellosis. However, increased expression of CD markers, $CD8^+$, NK/$CD18^+$ and γδ TCR could refer to the nonspecific polyclonal activation during acute Brucellosis. The dichotomy of the Th1/Th2 type cytokines activity was not observed. *(Research Journal of Microbiology 3 (11): 641-647, 2008; **doi:** 10.3923/jm.2008.641.647)*

Bacteriocidal Activity of Some Plants Essential Oils Against *Bacillus cereus*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Yersinia enterocolitica*

M. Bonyadian and H. Moshtaghi

This study was conducted to determine the effects of some plant essential oils on *B. cereus*, *S. typhimurium*, *L. monocytogenes* and *Y. enterocolitica*. In the first step Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of plant essential oils were determined by Tube Dilution Method in Luria-Bertani broth medium. In the second step the growth rate of each bacterium was assessed in presence of plant essential oils in concentration of less than MIC. The results showed that the essential oils of the plants used in this study have the acceptable antimicrobial activities against tested bacteria, the Caraway seed oils showed the most antimicrobial activity followed by Pennyroyal and Peppermint but Tarragon oils showed the least antimicrobial activity on tested bacteria. The results of the second step showed that, the plant essential oils were affected not only on lag phase but also on logarithmic phase of growth of bacteria. *(Research Journal of Microbiology 3 (11): 648-653, 2008;* ***doi:*** *10.3923/jm.2008.648.653)*

Antimicrobial Susceptibility Pattern and Characterization of Ciprofloxacin Resistant *Salmonella enterica* Serovar Typhi Isolates in Kerala, South India

N. Ayana and K. Surekha

The aim of the study was to determine antimicrobial susceptibility pattern and to examine the mechanism of increasing quinolone resistance of *S. typhi* isolates in Kerala, South India. Ciprofloxacin resistant isolates were characterized by mutation analysis of quinolone resistance determining region (QRDR) of *gyrA*, *gyrB*, *parC* and *parE* genes and the results showed that majority of the isolates were resistant to nalidixic acid. There was an increase in the number of isolates showing resistance to ciprofloxacin, ceftriaxone and ofloxacin. Multi drug resistant (MDR) *S. enterica* serovar Typhi were not isolated. Majority of ciprofloxacin resistant isolates carried mutation in quinolone resistance determining region (QRDR) of *gyrA* and strains with high level ciprofloxacin resistance carried double mutation in quinolone resistance determining region (QRDR) of *gyrA* and single mutation in quinolone resistance determining region (QRDR) of *parC*. In conclusion we reported that there was an increase in the number of *S. typhi* isolates showing resistance to flouroquinolones and ceftriaxone. We have found out that flouroquinolone resistance in *S. typhi* isolates was not plasmid encoded. We had also found out that mutations in quinolone resistance determining region (QRDR) region of *gyrA* play an important role in flouroquinolone resistance of

S. typhi isolates and *parC* mutation facilitate increasing level of resistance. *(Research Journal of Microbiology 3 (11): 654-660, 2008;* ***doi:*** *10.3923/jm.2008.654.660)*

Microbial Proteases and Application as Laundry Detergent Additive

D. Kumar, Savitri, N. Thakur, R. Verma and T.C. Bhalla

Proteases represents one of the major groups of industrial enzymes and a number of detergent stable proteases have been isolated and characterized because of its widespread use in detergents. It is worthwhile to screen microbes from new habitats for proteases with novel properties to meet the needs of rapidly growing detergent industry. High-alkaline serine proteases have been successfully applied as protein degrading components of detergent formulations and are subject to extensive protein engineering efforts to improve their stability and performance. Protein engineering has been extremely used to study the structure-function relationship in proteases and led to deeper understanding of the factors influencing the cleaning performance of detergent proteases. This study, discusses the types and sources of proteases with an overview on applications of proteases as laundry detergent additives and some advances in improving the stability and performance of detergent enzymes. *(Research Journal of Microbiology 3 (12): 661-672, 2008;* ***doi:*** *10.3923/jm.2008.661.672)*

Studies on the Bioactivity of Different Solvents Extracts of Selected Marine Macroalgae Against Fish Pathogens

Sahar Wefky and Mary Ghobrial

Selected species of marine benthic algae belonging to the Phaeophyceae and Rhodophyceae, collected from different coastal areas of Alexandria (Egypt), were investigated for their antibacterial and antifungal, activities against fish pathogens. *In vitro* screening of organic solvents extracts from the marine macroalgae, *Laurencia pinnatifida* (Hudson) Stackhouse, *Pterocladia capillaceae* (Gmelin), *Halopteris scoparia* (Linnaeus) Kützing, *Stepopodium zonale* (J.V. Lamouroux) and *Sargassum hystrix* var. *fluitans* Børgesen, showed specific activity in inhibiting the growth of five virulent strains of bacteria pathogenic to fish *Pseudomonas fluorescens*, *Aeromonas hydrophila*, *Vibrio anguillarum*, *V. tandara*, *Escherichia coli* and of two fungi *Aspergillus flavus* and *A. niger*. Acetone and ethanol extracts of all test macroalgae exhibited antibacterial activity,

while acetone extract of *S. hystrix* exhibited the highest antifungal activity. Macroalgal extracts inhibited bacteria more readily than fungi, besides, the extracts of the Rhodophyceae species showed the greatest activity against current test bacteria rather than fungi. Cluster analysis revealed the general response of the tested pathogens to the action of the different algal extracts. Composition of the most potent algal extracts included acetone extracts of *L. pinnatifida*, *P. capillaceae* and *S. hystrix* and ethanol extract of *P. capillaceae* was determined using GC-MS. The present study provides the potential of red and brown macroalgae extracts for the development of anti-pathogenic agents for use in fish aquaculture. *(Research Journal of Microbiology 3 (12): 673-682, 2008;* ***doi:*** *10.3923/jm.2008.673.682)*

Microbial and Heavy Metals Contamination of Herbal Medicines

S.S. Alwakeel

This study was conducted to evaluate the microbial contaminants and presence of toxic heavy metals on some herbal medicines. Twenty-seven samples (3 kg each) of well-known herbs and 5 kinds of henna available in herb markets around Riyadh, Saudi Arabia were collected for microbial and toxic metal contamination. Twenty-one (60%) of the samples showed the presence of fungi. *Aspergillus flavus* and *Aspergillus fumigatus* were the most common isolates (9/35, 35%). *Astralagus sarcocolla* had the highest TPC count; *Matricaria chamomilia* had the highest total coliform count and fecal coliform count. Two henna samples showed more than 1 ppm lead content. Mercury was the highest in *Lepidium sativum,* aluminum in *Zingiber officinale*, calcium in *Artemisia herba alba*, cadmium in *Lepidium sativum*, *Vigna radiata* and *Zingiber officinale.* Copper was the highest in *Cinnamomum zeylanicum*, iron in *Zingiber officinale*, zinc in *Salvia officinalis*, potassium in *Matricaria chamomilia*, whereas sodium was the highest in *Pimpinella anisum*. Microbial determination showed that *Bacillus* species was seen in 3 (9.7%) of the isolated microorganisms with the predominance of *Bacillus cereus* (14/31, 45.2%). Other microbial isolates were *Aeromonas hydrophilia*, *Shigella* spp., *Enterobacter agglomerans*, *Enterobacter* spp., *Vibrio fluvialis*, *Escherichia coli*, *Pasteurella multocida*, *Enterobacter cloacae*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *Acinetobacter iwoffii* and *Klebsiella.* Tests showed the sensitivity of most isolated bacteria to amoxicillin, gentamicin, imipinem, tobramycin and trimethoprim-sulfamethoxazole. *(Research Journal of Microbiology 3 (12): 683-691, 2008;* ***doi:*** *10.3923/jm.2008.683.691)*

Application of Random Amplification of Polymorphic DNA, Antibiogram and Serotyping for Differentiating *Streptococcus agalactiae* Clinical and Environmental Isolates from Kuwait

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The aim of this study was to assess the phenotypic and genotypic diversity among human and environmental isolates of *S. agalactiae* from Kuwait. A total of 87 isolates were collected from clinical and environmental samples. Conventional typing methods were performed by stereotyping test (slid agglutination) and antimicrobial susceptibility test (disk diffusion) method. Molecular typing method was performed by RAPD analysis to study genetic variability at the molecular level. Fifty six of the isolates were positive for *S. agalactiae* by PCR and culture method. The bacterial isolates showed 100% sensitivity to the ampicillin and ciprofloxacin antibiotics, but 75% sensitivity to chloramphenicol and 66% sensitivity to the erythromycin antibiotics. Serotype III was predominant 26.7%, followed by serotype V, Ia and VI. Serotypes found among isolates from environment samples included V 60%, III 40%. Twelve genotypic patterns were generated using a single arbitrary RAPD primer, conventional phenotypic typing methods presented less significant discriminatory power comparing to molecular. Serologic analysis data showed to certain extent correlation with molecular data using genetic clustering and similarity indices generated by RAPD-PCR. The detection of DNA polymorphism between isolates within a serotype confirmed earlier reports of the heterogeneous nature of individual GBS serotypes. *(Research Journal of Microbiology 4 (1): 1-12, 2009;* ***doi:*** *10.3923/jm.2009.1.12)*

Helminth Contamination of Lettuce and Associated Risk Factors at Production Sites, Markets and Street Food Vendor Points in Urban and Peri-Urban Kumasi, Ghana

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The study assessed contamination levels of lettuce with helminth parasites and associated practices that may influence contamination levels at farm, market and street food vendor points in urban and peri-urban Kumasi. Three farms, three market sites that sold lettuce purchased from the selected farms and 20 street food vendors, who purchase their lettuce from these markets, were studied. Samples

of lettuce, irrigation water and refreshing water (water used for keeping lettuce fresh throughout the day) were collected from these sites and analyzed for helminths eggs/larvae using standard methodology. Helminths on the lettuce leaves, irrigation water and refreshing water in the farms and markets were mostly *Ascaris lumbricoides*, with some *Shistosoma*, *Hookworm*, *Trichuris trichura*, *Taenia*, *Clonorchis* and *Strongyloides* larvae. Helminths eggs on lettuce leaves ranged between 4 and 14 100^{-1} g wet weight and 3 and 25 eggs L^{-1} in irrigation water on the farms and between 2 and 7 100^{-1} g wet weight and 4 and 15 eggs L^{-1} in refreshing water in the markets. Helminths egg counts on lettuce leaves on two farms were 40-52.9% more when compared with the farms' irrigation water but one farm had 40.5% more in irrigation water when compared with the lettuce leaves and these differences were significant. Helminths eggs on lettuce from the two farms were 50 and 60% higher when compared with its corresponding market samples and 23.5% higher in one market when compared with its farm source. Helminths eggs in street food lettuce samples analysed from the selected areas were only *Ascaris* and *Shistosoma* eggs ranging between 0 to 2 eggs 100^{-1} g wet weight. Helminths eggs for both farm and market samples exceeded the recommended level of <1egg L^{-1}. Education on farm practices, post harvest handling and washing methods at both market and street food vendor sites and improved hygienic practices at consumer level may help reduce their numbers and minimize the risk. *(Research Journal of Microbiology 4 (1): 13-22, 2009;* ***doi:*** *10.3923/jm.2009.13.22)*

Nosocomial Legionnaires' Disease Outbreak in Tehran

R.H. Doust, A.M. Mobares, S. Mirkalantar, J. Aslani and A.I. Fuladi

The study took placed during the summer time of 2007. A 20 years old university hospital with 600 beds equipped with central air conditioning. No special disinfection program was achieved for the hospital water supplies at the time of investigation. The hospital is supplied by city water and sewages organization and is treated with standard chlorination. To analysis the first nosocomial outbreak of Legionnaires' disease in a major university hospital of Iran. Seventy Broncho Alveolar Lavage specimens were obtained from patients with pneumonia. In addition 20 water samples of various hospital points were screened for the presence of *Legionella* species and free-living amoebae. Six nosocomial cases occurred over an 8 weeks period, between the first and last case detection. *Legionella* isolates from the patients matched the water sample isolates. *L. pneumophila* were grown up from only 3 out of 70 samples, while the bacteria *mip* gene were detected from additional three cases. *L. pneumophila* (serogrup

1) were isolated from two hospital sites. Since, *Legionella* positive patients had been admitted to the hospital at least 2 weeks prior to sampling, the cases could be assumed as hospital acquired Legionnaire's disease, originated from hospital water supplies which should be treated for effective disinfection. *(Research Journal of Microbiology 4 (1): 23-30, 2009; **doi**: 10.3923/jm.2009.23.30)*

Production and Freeze-Drying of Leben Lactic Starter

Z. Manel, M'hir Sana, M. Abdeslam, T. Philippe and H. Mokhtar

The production of two strains of lactic acid bacteria isolated from Tunisian fermented milk (Leben): *Lactococcus lactis* var. *lactis* (SLT6 and SLT10) was investigated in fed-batch process. The final biomass production after 8 h was upper than 10^{10} cells mL^{-1} for both strains. The strains present an important growth rate ($0.95\pm0.03\ h^{-1}$) and short generation time. The conversion yield ($Y_{x/s}$) is 0.12 and 0.14 g g^{-1} for SLT6 and SLT10, respectively. The survival after freeze-drying is 22 and 37% for SLT6 and SLT10, respectively. *(Research Journal of Microbiology 4 (1): 31-37, 2009; **doi**: 10.3923/jm.2009.31.37)*

Bio-Control of *Vibrio fluvialis* in Aquaculture by Mangrove (*Avicennia marina*) Seeds Extracts

Gehan M. Abou-Elela, Nermeen A. El-Sersy, Mohamed A. El-Shenawy, Hanan Abd-Elnabi and Hassan A.H. Ibrahim

The microbial community associated with mangrove plant (*Avicennia marina*) in Safaga (Red Sea) was studied, the heterotrophs (TVC), *Vibrio*, *Aeromonas* and *Staphylococcus* counts in sea water were 56000, 200, 300 and 160 cfu mL^{-1}, respectively. The mangrove stems harboured lower values and the roots harboured higher values. The dominant heterotrophs isolated from the roots and stems were: *Bacillus*, *Vibrio*, *Aeromonas* and *Pseudomonas*. Different extracts of the different parts of the plant (seeds, leaves, stems and roots) were applied on different bacterial pathogens such as: *P. aeruginosa*, *V. fluvialis*, *V. vulnificus*, *S. faecalis*, *E. coli*, *S. aureus* and *B. subtilis*. The chloroform extracts showed considerable activities against the different pathogens, while the activity of the ethanol extracts showed lower values. The chloroform seeds extracts inhibited the growth of all pathogens efficiently and recorded the highest activity unit (AU = 25.0) against the fish pathogen *V. fluvialis*. Chemical composition of the extract contained carbohydrates, proteins and lipids (2.58, 0.74 and 0.074 mg), respectively, in addition to flavonoids, triterpenoids, lignin and tannin (8.6, 3, 11

and 8%), respectively. The study extended to apply these extracts on *Nile tilapia* sp. (*Oreochromis niloticus*) aquaculture, 2.5 and 5 ml L^{-1} of the chloroform seeds extracts were applied, 5 ml L^{-1} showed satisfied results while the efficiency ranged from 64.1% in the second day to 79.4% in the six day. *(Research Journal of Microbiology 4 (1): 38-48, 2009;* ***doi:*** *10.3923/jm.2009.38.48)*

Isolation and Characterization of 3-*N*-Trimethylamino-1-Propanol Degrading *Arthrobacter* sp. Strain E5

Isam A. Mohamed Ahmed, J. Arima, T. Ichiyanagi, E. Sakuno and N. Mori

The aim of this study was to screen for microorganism that able to utilize 3-*N*-trimethylamino-1-propanol (homocholine) as sole source of carbon and nitrogen and to see which mechanism is followed in the degradation of this compound by soil microorganisms. A gram-positive bacterium, designated, as strain E5 was isolated from soil. The strain was identified as *Arthrobacter* sp. strain E5 based on the phenotypic features, physiologic and biochemical characteristics and phylogenetic analysis. The cells of strain E5 displayed primary branching at the exponential phase and fragmented into irregular rod and coccoid elements at the stationary phase. The colonies were yellow in color, convex, round and entire with smooth and regular margins on both homocholine and nutrient agar medium. Comparative 16S rDNA sequencing studies indicated that strain E5 fall into *Arthrobacter nicotinovorans* subclade where it forms a monophyletic group with the type strains of *Arthrobacter nicotinovorans* and *Arthrobacter histidinolovorans*. Metabolites analysis by capillary electrophoresis and gas chromatography-mass spectrometry showed trimethylamine as a major metabolite beside β-alanine betaine and trimethylaminopropionaldehyde. Therefore, the possible degradation pathway of homocholine in *Arthrobacter* sp. strain E5 is through consequence oxidation of alcohol group (-OH) to aldehyde (-CHO) and acid (-COOH), respectively and thereafter cleavages of C-N bond providing trimethylamine and alkyl chain. *(Research Journal of Microbiology 4 (2): 49-58, 2009;* ***doi:*** *10.3923/jm.2009.49.58)*

Occurrence of Antibiotic-Resistant and Plasmid DNA Harbouring Bacterial Pathogens in Stressed Polluted Water Environment of Lake Manzala, Egypt

Mahmoud M.M. Zaky

This study aims at characterization of microbial pollution of Lake Manzala, bacteriological investigation of water and fish and isolation of antibiotic resistant

and plasmid harbouring bacterial strains. The study revealed high levels of pollution in the water and fish samples taken from the most important sites (Kapoty, Bashtier and Mataryia areas), representative of human activity and the different ecosystems in the Lake water environment. The testing for total suspended solids (TSS), ammonia and nitrates, demonstrated that figures exceeded national and international standards. The fish-tissue samples gathered from two different sites yielded high concentration of bacterial count by plate count method. Total viable bacteria (TVB) reached more than 10^4 cfu mL^{-1} in water samples and 10^5 cfu g^{-1} in fish samples, particularly in Kapoty and Mataryia areas. Faecal coliform counts reached 10^2 cfu mL^{-1} in water samples and 10^3 cfu g^{-1} in fish samples. The API-20E test kit was used for the identification of eighty isolates of different bacteria strains. The bacterial strains *Stenthorpohomonas maltophilia, Proteus mirabilis, Escherichia coli* and *Erwinia* sp. were common species found in the samples of the study and demonstrated multi-drug resistance. These strains harbored β-Lactamases and plasmid DNA; characteristics that can be attributed to the stressed water environment of the polluted Lake Manzala. *(Research Journal of Microbiology 4 (2): 59-66, 2009;* ***doi:*** *10.3923/jm.2009.59.66)*

Cellulase Production by *Trichoderma longi*, *Aspergillus niger* and *Saccharomyces cerevisae* Cultured on Plantain Peel

P.F. Omojasola and O.P. Jilani

In this study, three fungi: *Trichoderma longibrachiatum*, *Aspergillus niger* and *Saccharomyces cerevisiae* were cultured on plantain peel, a cellulosic waste. The waste was dried, pre-treated with alkali and steam, re-dried and then blended. The powdered waste was then used as substrate in shake-flasks which contained Mineral Salts Medium (MSM) and inoculi of the three test fungi. Fermentations were initially carried out in flasks containing the MSM, waste substrate and the inoculum at pH 5.0, 1% substrate concentration, 10% inoculum size and cultured on a rotary shaker at 29±1°C for 5 days to verify cellulase production by the organisms from the waste substrates, then for 7 or 9 days while varying different fermentation parameters. Cellulase activity and amount of glucose produced by the three test organisms from the waste substrate was determined and compared. Glucose production was optimized by varying the fermentation parameters: Time, pH, Substrate concentration, Inoculum size and Temperature. The results obtained from the fermentations showed that *Trichoderma longibrachiatum* produced the highest amount of glucose among the cultures tested (1.64 mg mL^{-1}). This was produced from plantain peel at pH 5.0 and temperature of 45°C on day 7 of fermentation. The highest amount of glucose produced by *Aspergillus niger* from

plantain peel was 1.18 mg mL^{-1} at pH 4.5 and temperature of 45°C on day 7 of fermentation. The highest amount of glucose produced by *Saccharomyces cerevisiae* was 1.00 mg mL^{-1} at pH 3.5 and temperature of 45°C on day 5 of fermentation. *(Research Journal of Microbiology 4 (2): 67-74, 2009;* ***doi:*** *10.3923/jm.2009.67.74)*

Construction of pcDNA/*fim*H Cassette as a DNA Vaccine Candidate Against Urinary Tract Infection and Evaluation of *fim*H Transcripts in COS7 Cell Line

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Uropathogenic *Escherichia coli* is one of the major agents of urinary tract infection. Since it has intracellular propagation, cellular immune response is so important in this case. Accordingly, a genetic construct for inducing of cellular immune system was designed. At first, chromosomal DNA extracted from *E. coli* 35218 and *fimH* gene amplified with this template by PCR. PCR product inserted to pcDNA.1 eukaryotic expression vector and confirmed the recombinant vector by sequencing. The COS7 cell line transfected with a complex of pcDNA/*fim*H and ExGen 500 poly cationic polymer. Expression of *fimH* gene in COS7 was confirmed by RT-PCR. Consequently, pcDNA/*fim*H cassette could express inserted *fim*H gene in eukaryotic cells and is a valuable DNA candidate cassette for urinary tract infection vaccination. This is the first prompt to designing a DNA vaccine against urinary tract infection that caused by Uropathogenic *Escherichia coli*. *(Research Journal of Microbiology 4 (2): 75-81, 2009;* ***doi:*** *10.3923/jm.2009.75.81)*

Biocatalytic Production of a Commercial Textile Dye (Indigo) from a Xenobiont

S. Mutnuri, C. Bandi and A. Ganguly

A Gram negative rod SCV1 was isolated from oil contaminated garage soil. This bacterial strain was used for the production of indigo-a commercial textile dye after induction on xenobiotics like diesel, naphthalene and salicylate. The specific rates of indigo formation are 0.30, 0.38 and 0.35 mg $mL^{-1}\times h$ for diesel, salicylate and naphthalene induced bacterial strain SCV1. The bacterial strain SCV1 was hydrophobic in nature as evident from hydrophobicity measurements. Hydrophobic nature gives the advantage to the bacterial strain in adhering to the

hydrocarbons. The results of the indigo production by different substrates induced bacterial strain SCV1 suggest that the diesel induced the maximum at 1.75 and 2 mM concentrations. It is also suspected that the uninduced culture i.e., SCV1 enriched on nutrient broth produced other indigoid compounds other than indigo. *(Research Journal of Microbiology 4 (3): 82-88, 2009; **doi:** 10.3923/jm.2009.82.88)*

PHA Production Using Low-Cost Agro-Industrial Wastes by *Bacillus* sp. Strain COL1/A6

M.C. Santimano, Nimali N. Prabhu and S. Garg

Recycling of wastes generated from agro based industries for polyhydroxyalkanoate production is not only crucial for waste management but also in economizing and commercializing the polymer. In this study, the heterotrophic bacterium *Bacillus* sp. strain COL1/A6 isolated from humus was biologically characterized and explored for its potential to synthesize PHA using agroindustrial wastes. Qualitative analysis using Nile blue A staining revealed that starch, wafer residue, citrus pulp and cane molasses proved to be excellent carbon substrates for PHA accumulation. Growth and PHA producing ability of the isolate on cane bagasse and rice chaff improved after dilute acid hydrolysis. Highest cellular PHA content was obtained using wastes such as hydrolyzed wafer residue (62.41±1.04% of dry cell wt.) followed by cane molasses (54.68±1.36% of dry cell wt.) and hydrolyzed citrus pulp (47.5±1.01% of dry cell wt.). This is the first report wherein a *Bacillus* sp. has been reported to grow and utilize wastes such as wafer residue and citrus pulp as carbon feedstock for PHA production. *(Research Journal of Microbiology 4 (3): 89-96, 2009; **doi:** 10.3923/jm.2009.89.96)*

Antimicrobial Activity of Titanium Dioxide Nanoparticles Synthesized by Sol-Gel Technique

Vilas S. Desai and Meenal Kowshik

The process of Heterogeneous Photocatalysis (HP) using titanium dioxide photocatalysts is a field of immense research potential for researchers worldwide. TiO_2 as a photocatalyst has been widely applied for air and water remediation. This study reports the synthesis of a visible light responsive nanosized TiO_2 photocatalyst by a modified sol-gel process. The synthesized TiO_2 photocatalyst exhibits photocatalytic activity against some common pathogenic microorganisms

such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* under visible light illumination. TiO_2 is known to exhibit photocatalytic activity under UV light irradiation, the results obtained in this study using solar irradiation are very promising and enables the use of cheaply available solar energy for the process of photocatalysis. *(Research Journal of Microbiology 4 (3): 97-103, 2009;* ***doi:*** *10.3923/jm.2009.97.103)*

Construction and Testing of EGFP Based Bacterial Biosensor for the Detection of Residual Tetracyclines in Milk and Water

J. Scaria, S. Ramachandran, P.K. Jain and S.K. Verma

A plasmid containing a transcriptional fusion between *tetR* regulated *tet* promoter from plasmid pOT182 and Enhanced Green Fluorescent Protein (EGFP) gene was created and was transformed into *E. coli* JM109 and this strain was used as whole cell bacterial biosensor for detection of tetracyclines in milk and water samples. The sensor strain *E. coli* JM109 (pJSKV41) was able to detect tetracycline in the range of 10-60 ng mL^{-1} sample and oxytetracycline in the range of 25-125 ng mL^{-1} of sample. When employed for detecting residual tetracyclines in pond water samples, the biosensor strain showed high sensitivity. Also the biosensor strain was able to detect residual tetracycline in goat milk even after 4 days of tetracycline treatment. *(Research Journal of Microbiology 4 (3): 104-111, 2009;* ***doi:*** *10.3923/jm.2009.104.111)*

Genome Wide Single Nucleotide Polymorphism Analysis of *Mycobacterium* Species and Subspecies

S.K. Srivastava, M. Agrawal and M. Grover

In this study we report the reannotation of the genome of seven *Mycobacterial* species and subspecies. We have used bioinformatics tools for annotation and reevaluated each of the Protein-Coding Sequences (CDS) previously annotated and presented the combined results of recent database searches. We have also used comparative genomic tools to focus on comparative analysis as an effective strategy. Pair wise comparison between the various *Mycobacterium* strains was performed so as to predict the relationships between them. Among the wide variety of mycobacterium strains present, we selected seven and showed how their genome is interrelated by studying synteny with the genomes of various strains studied. The genome wide SNP analysis in the seven genomes of *Mycobacterium* sp. was also done in this study and the base by base changes in the genome of

these seven subspecies were identified. The gene based SNPs were further classified into the marker SNPs (SNPs which are unique amongst all the seven studied species). Out of a total of 2073 SNPs, 966 were identified as marker SNPS. This study may be used for further analysis of host pathogen interactions at the pathway and product level. The present investigation will also be useful for study of evolutionary relationship. *(Research Journal of Microbiology 4 (3): 112-121, 2009;* ***doi:*** *10.3923/jm.2009.112.121)*

Antibacterial Activity of *Leuconostoc lactis* Isolated from Raw Cattle Milk and its Preliminary Optimization for the Bacteriocin Production

Ram Lal Thakur and Utpal Roy

Leuconocin, a bacteriocin like inhibitory substance produced by *Leuconostoc lactis* an isolate from fresh raw cattle milk was inhibitory against *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and interestingly to the gram-negative species like *Pseudomonas putida*, *E. coli* DH5α and *E.c oli* DH5α with pUC 18 vector. The inhibitory potential was confirmed both by spot assay and cut well agar assay as well with the cell-free supernatant of the test culture in Elliker's broth. MRS broth adjusted to pH to 7.0 and 6.8, respectively produced an inhibitory zone of 15-16 mm against *B. cereus*. This promising wild-type isolate was identified up to a species level by 16S rDNA-based PCR which showed a band at about 692 bp. The set of primer used appeared to be specific as it did not amplify the closely related species. The cell-free supernatant upon concentration by 5 fold (approximately) showed a much stronger biological activity and showed heat stability. This isolate thus appears to be novel as no bacteriocin so far has been reported from *Leuconostoc lactis*. Moreover, the bacteriocin was active against both gram-positive and gram-negative organisms. *(Research Journal of Microbiology 4 (3): 122-131, 2009;* ***doi:*** *10.3923/jm.2009.122.131)*

Graph Theoretic Approach on Metabolomic Networks of Mycobacterial Strains for Potential Drug Targets

V. Baths, V.V. Rohit Kumar, G.V.R. Praneeth and U. Roy

A special strain of *Mycobacterium tuberculosis*, H37Rv's Gluconeogenesis pathway is analyzed for clusters in the pathway using the principles of spectral graph theory to find out a drug target for tuberculosis. The software named Visant

was used and the data set was obtained from KEGG. The large-scale properties of chemical reaction systems, such as metabolism, can be studied with graph-based methods. To do this, one needs to reduce the information, lists of chemical reactions, available in databases. There are several ways by which this reduction can be done even for the simplest type of graph representation. Present study is aimed to apply the knowledge of graphs and graph theoretic concepts to compare the metabolic network in *Mycobacterium tuberculosis*. The study is done on the gluconeogenesis pathway, a pathway that is important for the growth of *M. Tuberculosis* H37Rv strain. Each metabolite of the pathway is taken as node of a network with the edge between the nodes representing the reaction. Spectrum and spectral radius of this network were obtained using spectral graph theory, manually. The spectral radius of this network is found out to be 0.9254. *(Research Journal of Microbiology 4 (3): 132-137, 2009;* ***doi:*** *10.3923/jm.2009.132.137)*

Inhibition of *Candida albicans* and Two Selected Gram-Negative Pathogens by Polar *Enterococcus faecalis* and *Carnobacterium* sp.

R. Shekh, K. Upadhyay, S.M. Singh and U. Roy

The current study has the objectives to identify the polar microorganisms with the ability to produce antimicrobial substances with wide-spectrum potential to antagonize the multi-drug resistance *Candida albicans*, *Pseudomonas aeruginosa* and *putida*. As many as 218 bacterial strains were screened and isolated from 6 Antarctic Penguin rookery faecal samples at Larsemann Hills, East Antarctica and from arctic sea-water-glacier stream convergence samples for checking the production of antimycotic and antibacterial substances using the cut well agar assay. Seven selected bacterial isolates were grown at 15°C for 48 h and the cell free supernatant showed activity against either *Pseudomonas aeruginosa* and *putida* or four strains of *Candida albicans*. Three selected isolates produced antimicrobial substances (AMS) which inhibited 4 strains of multi-drug resistant *Candida* sp. and two other species of *Bacillus* inhibited one Candida strain. The isolates PR 210 and 211 were found to demonstrate a very strong fungicidal agent when concentrated. The present investigation led to the findings of the three AMS producers which were identified *Enterobacter hormaechii*, *Carnobaterium maltaromaticum*, *Enterococcus faecalis*, based on *16S rRNA* gene sequences and fatty acid compositions, respectively. The other two isolates were *Bacillus megaterium* and *B. mycoides* identified by 16 S rDNA phylogenetic analysis. *(Research Journal of Microbiology 4 (3): 138-142, 2009;* ***doi:*** *10.3923/jm.2009.138.142)*

Application of PCR-Based Fingerprinting for Detection of Nontuberculous Mycobacteria among Patients Referred to Tuberculosis Reference Center of Khuzestan Province, Iran

A.D. Khosravi, S. Seghatoleslami and M. Hashemzadeh

The present study was conducted to determine the frequency of NTM by application of PCR-based Restriction Fragment Length Polymorphism (PCR-RFLP) among suspected tuberculosis patients. In total 150 clinical isolates from patients referred to TB reference laboratory were screened. Culture and biochemical tests were performed. The PCR-RFLP method based on amplification of a 439 bp fragment of *hsp* gene involving genus specific primers was performed and the PCR products were digested with *HaeIII* and *Bst EII* restriction enzymes. Of total isolates tested, 100 isolates were culture positive (66.6%). Eighty out of 88 isolates that were subjected to RFLP, showed the identical restriction patterns similar to *Mycobacterium tuberculosis* (90.9%). Eight clinical isolates (9.1%) showed different restriction patterns, six isolates identified as *Mycobacterium intracellularae* and two isolates were *Mycobacterium gordonae* I. In conclusion, RFLP as a fast, cheap and accurate technique is a valid alternative for phenotypic identification of pathogenic and potentially pathogenic mycobacteria in the routine laboratory. *(Research Journal of Microbiology, 4 (4): 143-149, 2009;* ***doi:*** *10.3923/jm.2009.143.149)*

Spectra of Antibacterial Activity of Propolis (Promax-C) Samples from Two Localities of Adamaoua Province (Cameroon)

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Fifteen samples of Promax-C, ethanolic extracts of propolis collected from different hives situated in two localities of the Adamaoua Province of Cameroon were tested each against seven strains of bacteria namely *Samonella enterica*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Pseudomonas fluorescens* and *Bacillus subtilis*. The aim of this study was to evaluate the antibacterial activity of those Promax-C samples. Antibacterial activity essays were investigated by the determination of the zones of growth inhibition using the well diffusion method on agar medium and the evaluation of the Minimal Inhibitory Concentration (MIC) using the macrodilution method. All the Promax-C samples were active against the Gram positive bacterial strains except *E. faecalis*. On the other hand, there was no activity of those samples on the Gram negative bacterial strains studied. Considering the

diameter of the inhibitory zones and the MIC values, the susceptibility of bacterial strains to the Promax-C samples decreased as follows: *L. monocytogenes*> *S. aureus*>*B. subtilis*. The most active sample was Promax-C8 from the Martap locality and the most susceptible bacteria was *L. monocytogenes*. The areas of the minor and major peaks of the phenolic compounds obtained by HPLC analysis were more important for the Promax-C8 sample, showing that the greatest activity of these antimicrobial components was probably linked to their higher contents in the samples. *(Research Journal of Microbiology, 4 (4): 150-157, 2009;* ***doi:*** *10.3923/jm.2009.150.157)*

Study of Bacteria Isolated from Orthopedic Implant Infections and their Antimicrobial Susceptibility Pattern

A.D. Khosravi, F. Ahmadi, S. Salmanzadeh, A. Dashtbozorg and E. Abasi Montazeri

The aim of the present study was, to determine the bacteriology of orthopedic implant infections and susceptibilities of isolated bacteria to the commonly used antimicrobial agents. One hundred and sixty five patients were investigated for early or late postoperative infections of orthopedic bone implants using conventional microbiological procedures. Antimicrobial susceptibility testing were then performed for the isolated bacteria according to the standard guideline. A total of 155 isolates were recovered (152 aerobes and 3 anaerobes). *Staphylococcus aureus*, *Klebsiella ozaenae* and *Pseudomonas aeruginosa* were the most common causative agents. In relation to onset of infection, about 72.9% of patients were with early; 22.6% with delayed and 4.5% with late infections. The correlation between infection onset and total number of isolated bacteria was found to be statistically significant. The majority of isolated bacteria were sensitive to vancomycin, ciprofloxacine and imipenem. In conclusion, present study showed that *S. aureus* was the most common recovered bacterium with high sensitivity to vancomycin as expected. knowledge of the commonly isolated organisms and their antimicrobial susceptibility patterns within a given hospital assists in the selection of appropriate antimicrobial treatment. *(Research Journal of Microbiology, 4 (4): 158-163, 2009;* ***doi:*** *10.3923/jm.2009.158-163)*

Antibacterial Potential of Herbal Formulation

Archana A. Bele, Varsha M. Jadhav, S.R. Nikam and Vilasrao J. Kadam

Natural drugs are boon to mankind. They have few side effects as compared to allopathic medicine. This invention relates to herbal composition, having potent

anti-bacterial and wound healing property. The formulation prepared is a gel, which is used for effective treatment of wounds and exhibits broad spectrum antibacterial action. Crude extracts of *Punica granatum* pericarp and *Curcuma longa* showed antibacterial activity against different strains of gram positive such as *Staphylococcus aureus*, *Bacillus subtilis* and gram negative microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Enterobacter aerogenes*. The MIC is recorded as the lowest concentration of drug which showed clear fluid without turbidity. Minimum inhibitory concentration of *Punica granatum* peel ranged from 0.05 to 3.2 mg mL^{-1} and for *Curcuma longa* MIC ranged from 5 to 320 mcg mL^{-1}. Formulation containing these extracts, showed significant zone of inhibition for 0.5, 1, 2.5, 5% of which 5% showed maximum zone of inhibition (ranging from 20.2 to 26 mm) as compared to marketed preparation. The present investigation revealed that gel formulation has potential antibacterial activity. *(Research Journal of Microbiology, 4 (4): 164-167, 2009;* ***doi:*** *10.3923/jm.2009.164.167)*

L-Glutaminase Production and the Growth of Marine Bacteria

P. Jeya Prakash, E. Poorani, P. Anantharaman and T. Balasubramaniam

The search of salt-tolerant and thermo-stable bacterial L-glutaminase in the marine environment was done from Coleroon estuary, Muthupet mangrove and Mullipallam lagoon which possess different marine biotopes. The isolated and identified high potent strains were subjected in to comparative study between their growth and production to select the industrially potent strains. Within that the Mullipallam lagoon strain *Vibrio* sp. SFL-2 (Sethusamudharam Field Laboratory) had produced 352.4±0.23 IU (International Unit) of L-glutaminase in the 96 hrs of culture but their growth rate was more or less same as other strains. *(Research Journal of Microbiology, 4 (4): 168-172, 2009;* ***doi:*** *10.3923/jm.2009.168.172)*

A Real-Time Polymerase Chain Reaction Based Assay for the Detection of *Escherichia coli* in Patients with Urinary Tract Infection in the Sudan

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This study was undertaken in Khartoum State, Sudan, during the period May 2007 to March 2008. A detection system based on real-time PCR has been

developed for detection of *Escherichia coli* strains in patients' urine. The optimized assay format included two PCR primers. Urine specimens (46) were collected from patients attending different hospitals in Khartoum State. Bacterial DNA was extracted from each urine specimen using the Phenol-Chloroform method. Real time PCR technique was adopted to detect *E. coli*. The study revealed that 45.7% of the specimens were positive for *E. coli*. The bacterium was more prevalent in female patients than in male patients. Adult age group was more exposed to the pathogen than the children age group. Real-time PCR technique facilitated detection of *E. coli* directly in patients' urine without a need for bacterial culture. The technology could be easily adopted in hospital settings in the Sudan. *(Research Journal of Microbiology, 4 (4): 173-177, 2009;* ***doi:*** *10.3923/jm.2009.173.177)*

Thiamine (Vitamin B_1) Plays a Critical Role on Sugar Utilization by the Phytopathogenic Fungus, *Ustilago esculenta*

Kuang-Ren Chung and Dean D. Tzeng

Ustilago esculenta, inducing edible galls in its host *Zizania latifolia*, exhibits an obligate requirement for thiamine (vitamin B_1) in axenic culture. The function of thiamine for growth in *U. esculenta* was investigated and compared with two closely related species, *U. maydis* (corn smut) and *U. scitaminea* (sugarcane smut). Sucrose was readily broken into glucose and fructose, independent of thiamine, by all three fungal species tested. Growth of *U. maydis* and *U. scitaminea* was apparently not affected by thiamine when glucose or fructose was used as the sole carbon source. By contrast, *U. esculenta* was incapable of utilizing glucose and fructose in the absence of thiamine. Addition of thiamine into a synthetic medium drastically enhanced the growth of *U. esculenta*. In all cases, *Ustilago* species preferentially utilized glucose prior to fructose. Fructose uptake in *U. esculenta* exhibited a saturated kinetic, indicative of carrier protein-mediated process. The uptake of fructose by *U. esculenta* was highly influenced by the amounts of glucose, and was likely via., a noncompetitive mode. Taken together, the results strongly indicate that thiamine plays a key role for glucose and fructose metabolisms and energy production by *U. esculenta*. *(Research Journal of Microbiology, 4 (4): 178-185, 2009;* ***doi:*** *10.3923/jm.2009.178.185)*

Sero Diagnosis of *Bluetongue virus* Infection and Isolation of Virus in Embryonated Chicken Egg and BHK-21 Cell Line

N. Ramesh, V. Rajesh Kannan, K. Karthikeyan, K. Nanthakumar and R. Karthik Raja

Isolation of *Bluetongue virus* from blood samples of sheep and goat was carried out in the present study. Out of one fifty blood samples screened for seroprevalance of BTV antibodies by Agarose Gel Precipitation Test (AGPT) 42 gave positive results. The overall percentage of virus isolation was 28% from Embryonated Chicken Eggs (ECE). The identities of the isolates were confirmed by cytopathogenicity. All the isolates were passaged twenty one times in embryonated chicken eggs and further passaged in BHK-21 cell lines. The viral isolates adapted well to the cell culture system and produced cytopathic change like grouping of cells, polycaryon, syncytica formation, acidophilic and intracytoplasmic inclusion bodies in BHK-21 cells. This study confirms the BTV incidence in the tested blood sample with a possible means showing that the virus can easily adapt to ECE and BHK-21 cell line. *(Research Journal of Microbiology, 4 (5): 186-193, 2009;* ***doi:*** *10.3923/jm.2009.186.193)*

Bacterial Isolates from Ethiopian Soda Lake Producers of Alkaline-Active β-Glucanases Resistant to Chelating and Surfactant Compounds

M. Minig, D. Walker, P. Ledesma, M. Alejandra Martínez and Javier D. Breccia

β-glucanase activities were screened from isolated bacteria of the Ethiopian Shala Lake. Five isolates were selected according to the highest production of alkaline-active β-glucanase. By sequence analysis of 16S rDNA and physiological tests, four strains (SES01, SES22, SES4 and SES05) were related to *Bacillus halodurans* specie and the strain SES33 was identified up to genus level as *Bacillus* sp. Intergenic spacer regions fingerprinting showed different patterns among selected strains, having DNA amplicons of high molecular weight characteristic of alkaliphilic *Bacillus*. Herein, *B. halodurans* SES01 produced a highly stable β-glucanase in presence of surfactant and chelating compounds (sodium lauryl sulphate, Triton X-100 and EDTA) indicating its potential as additive for laundry technologies. *(Research Journal of Microbiology, 4 (5): 194-201, 2009;* ***doi:*** *10.3923/jm.2009.194.201)*

Effect of Yeast Extract Supplementation on Curdlan Production from Condensed Corn Distillers Solubles

Thomas P. West

The effect of yeast extract supplementation on bacterial curdlan production using a medium containing corn syrup and the corn-based ethanol coproduct condensed corn distillers solubles was determined. Curdlan was produced by *Agrobacterium* sp. ATCC 31749 on a medium containing selected solubles concentrations as a source of nitrogen and corn syrup as a carbon source. The presence of yeast extract in the medium was found to enhance bacterial curdlan production at all three concentrations of solubles tested after 120 h of growth. Bacterial biomass production was also noted to be higher after 120 h when the cells were supplemented with yeast extract. It was concluded that the observed increase in curdlan production by the yeast extract-supplemented ATCC 31749 cells was due to the yeast extract stimulating biomass formation. *(Research Journal of Microbiology, 4 (5): 202-207, 2009;* ***doi:*** *10.3923/jm.2009.202.207)*

Date-Palm Fruit Spoilage and Seed-Borne Fungi of Saudi Arabia

Hashem Al-Sheikh

The seeds and fruits of different date palm varieties were collected from local market and brought to the laboratory of the Department of Biology, College of Science, King Faisal University, in Al-Hassa, Saudi Arabia, where further experiments for isolation of fruit spoilage and seed-borne fungi were conducted by using common technique of wet blotter method. A total number of 100 seeds and 100 cubes (1 cm^3) obtained from the fruits (10 pieces per plate) were put on wet filter paper and incubated at 25°C to allow the growth of fungi for a period of 1 week. Fungal species developed on seeds and fruit pieces were isolated on potato dextrose agar for identification. This study was carried out during year from May 2007 to April 2008. Twenty species from 14 genera of fungi have been isolated from 13 different varieties of date-palm as seed-borne fungi while 39 species of 16 genera of fungi were isolated as fruit spoilage fungi. *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum* and *F. solani* were the predominant species in both seed-borne and fruit spoilage fungi. *(Research Journal of Microbiology, 4 (5): 208-213, 2009;* ***doi:*** *10.3923/jm.2009.208.213)*

Screening of the Efficacy of Some Commonly Used Antibiotics in Ghana

G.K. Helegbe, L.Y. Anyidoho and F.N. Gyang

The objective of this study was to screen some commonly used antibiotics in Ghana for their efficacy in treating diseases so as to select sensitive organisms that can be used to design an assay in assessing their biological activity. The disc susceptibility test was used to screen stock antibiotics such as ampicilline, chloramphenicol, kanamycin and penicillin based antibiotics from different manufacturers (both local and foreign) which were obtained from different pharmacy shops against some bacteria species such as *Salmonella typhi*, *Staphyloccus auresus* and six strains of *Escherichia coli*. It was observed that both stock and field antibiotics (Antibiotics obtained from pharmacy shops for study) zone of inhibition were similar and compared with literature values. J916 (an *E. coli* isolate) and *Salmonella typhi* were found to be less sensitive to the penicillin-based antibiotics similar to literature values for both stock and pharmacy shop samples. This study revealed that the antibiotics produced by local and foreign pharmaceutical companies appear to be effective. In as much as this study demonstrate that, local and foreign pharmaceutical industries appear to be producing quality drugs, further studies are needed to substantiate this claim observed by this study, which was on a small scale. *(Research Journal of Microbiology 4 (6): 214-221, 2009;* ***doi:*** *10.3923/jm.2009.214.221)*

Captive Dogs as Reservoirs of Some Zoonotic Bacteria

Maha A. Sabry

The present study is an attempt to clarify the role of captive dogs as a source of some zoonotic bacteria to their contacts or vise versa. Bacteriological examination of fecal swabs evidenced infection by 3 enteric bacteria in attendants, puppies and dogs. *Salmonella* (20, 33.3 and 41.67%), *Campylobacter* (13.33, 33.3 and 33.3%) and *Enteroinvasive E. coli* (46.66, 46.67 and 58.33%). Serotyping of these bacteria revealed presence of *S. typhimurium* in dogs (60%) and attendants (66.67%), *S. enteritidis* in one of the worker as well as four untyped strains. Two serotypes of *Campylobacter* as *C. jejuni* in two workers and four dogs, *C. coli* in three dogs, while two untyped isolates were recorded in dogs. Three serotypes of *E. coli* (O 26, O 76 and O 55) and two untyped strains were isolated from workers and dogs. Moreover two isolates (O 5 and O 111) were diagnosed from dogs only. The isolates showed high sensitivity for *Gentamycin* (10 μg) and

Tetracyclin (30 µg). The study recommended some precautionary measures to minimize the role of captive dogs as a potential source of zoonotic pathogens. *(Research Journal of Microbiology 4 (6): 222-228, 2009;* ***doi:*** *10.3923/jm.2009.222.228)*

Epidemiology of Dermatophytes in the Eastern Province of Saudi Arabia

Hashem Al Sheikh

This study was conducted for one year period during March 2008 to February 2009 in the Eastern Province of Saudi Arabia. Out of a total 250 samples collected during this period 178 (71.54%) were found positive. The dermatophytes causing different types Tinea were *Epidermatophyton floccossum*, *Microsporum canis*, *M. gypseum*, *Trihophyton mentagrophytes*, *T. rubrum*, *T. schoelneinii*, *T. soudanense*, *T. violaceum* and *T. verrucossum.* Besides these non-dermatophytes fungi *Candida albicans*, *C. krusei*, *C. tropicalis* and *Fusarium solani* were also isolated causing infection at different sites of human body. Samples from females yielded higher percentage of dermatophytes as compared to males. The percentage of infection of T. capitis and T. corporis were found to be higher in the age group of 0-15 years, while, T. pedis and T. cruris dominates in the age group of 16-30 years. Orychomycosis was dominated among the age group of 31-45 followed by 46-60 years. While, above 60 years yielded very low percentage of dermatophytes. Present study showed that more females were affected by dermatophytes (almost double in number) than males. Result of present study clearly indicates that the epidemiology of dermatophytes significantly differs from other regions of Saudi Arabia. *(Research Journal of Microbiology 4 (6): 229-234, 2009;* ***doi:*** *10.3923/jm.2009.229.234)*

Rock Phosphate Solubilization by Two Isolates of *Aspergillus niger* and *Penicillium* sp. and their Promotion to Mung Bean Plants

W.I.A. Saber, K.M. Ghanem and M.S. El-Hersh

Isolation and identification of rock phosphate (RP) solubilizing fungi were studied under laboratory conditions. Fungal isolates that displayed the highest ratio of clear

zone/colony diameter on plates of phosphate solubilization medium, were selected and identified as *Aspergillus niger* and *Penicillium* sp. The optimum condition for RP solubilization were found to be at the 6th (*A. niger*) and 7th (*Penicillium* sp.) day of incubation with shaking (150 rpm) at 30°C and pH ranging from 5.6 to 6.0. Glucose followed by fructose and xylose supported the RP solubilization process in the presence of 2.5 g L^{-1} RP as the optimum concentration. The overall soluble P after optimization studies on RP were 99.7 (*A. niger*) and 77.5 mg L^{-1} (*Penicillium* sp.). During the fermentation process, there was remarkable reduction in the final culture pH. The titratable acidity was positively correlated with RP solubilization. Under NaCl salt stress both fungi were able to solubilize RP, in which, *A. niger* was more tolerant than *Penicillium* sp. The dual and individual cultures of fungi solubilized sources of phosphate commonly exist in soil and also, possessed phytase activity. Under *in vivo* conditions, the inoculation of mung bean seeds with *A. niger* and/or *Penicillium* sp. in the presence of RP or calcium superphosphate (CSP), increased significantly the growth (except for branches No. $plant^{-1}$), seed yield and P-uptake, as well as, improved the nodulation status and population of total and phosphate dissolving fungi in the rhizospheric soil of mung bean. These inoculations saved about 1/3 phosphate fertilizer dose. Hereby, these combined effects encourage the potential use of the isolated fungi in the biosolubilization of RP in soil plant system. *(Research Journal of Microbiology 4 (7): 235-250, 2009;* ***doi:*** *10.3923/jm.2009.235.250)*

Optimization of Media and Cultivation Conditions for Alkaline Protease Production by Alkaliphilic *Bacillus halodurans*

Abdelnasser S.S. Ibrahim and Ali A. Al-Salamah

Media and cultivation conditions were investigated to optimize alkaline protease production by alkaliphilic *Bacillus halodurans*. This includes different carbon, nitrogen and metals sources in addition to different pH, incubation temperature and aeration level. The specific enzyme activity was increased by about 48.8 fold by optimizing different nutrient sources and cultivation conditions. The maximum specific enzyme activity was obtained in a medium containing 15 g L^{-1} lactose as the carbon source, 6 g L^{-1} soybean as the nitrogen source and a 5 mM mixture of Mg, Mn and Ca as trace elements, fermentation for 48 h at 37°C and agitation at 200 rpm. This study indicated the significance of nutrient source and cultivation conditions on the alkaline enzyme production by *Bacillus halodurans*. *(Research Journal of Microbiology 4 (7): 251-259, 2009;* ***doi:*** *10.3923/jm.2009.251.259)*

Isolation of Extreme Halotolerant Bacteria from Asian Desert Dust; Molecular Phylogeny and Growth Properties of their Cells

H. Sasaki, E. Iwata, A. Oshima, A. Ishida and S. Nagata

We tried the isolation of halophilic bacteria from Asian desert dust falls in Japan and growth property of these bacteria and their molecular phylogeny were analyzed. Two Gram-positive bacteria designated as IMU-1 and IMU-2 were isolated from Asian desert dust. These two strains were adapted with 0-3 and 0-4 M NaCl under nutrient medium culture conditions, respectively, showing the properties of halotolerance. Under the Davis minimal medium culture condition, IMU-1 attained to the similar level of growth as that of nutrient medium culture and growth was observed at 0-2.5 M NaCl. On the other hand, IMU-2 showed the different growth as that of nutrient medium culture condition and growth was observed at 0-1.2 M NaCl. Phylogenetic analysis using 16S rRNA gene sequences revealed that IMU-1 and IMU-2 belong to *Bacillus licheniformis* and *B. megaterium*, respectively. It was first study about the isolation of *B. licheniformis* as the halophilic bacteria in Japan. *(Research Journal of Microbiology 4 (7): 260-268, 2009;* ***doi:*** *10.3923/jm.2009.260.268)*

Antimicrobial Activity of the Methanolic and Crude Alkaloid Extracts of *Acalypha wilkesiana* cv. *macafeeana* Copper Leaf

C.N. Ezekiel, C.P. Anokwuru, E. Nsofor, O.A. Odusanya and O. Adebanjo

The antimicrobial activity of methanolic leaf extracts and crude alkaloid extracts of *A. wilkesiana* cv. *macafeeana* was evaluated after a preliminary phytochemical screening of the leaf extracts. The standard agar well diffusion method was used in the bioassay involving test bacteria and yeast isolates, while percentage inhibition of extracts on radial growths of the molds was determined. The Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) were also determined by the broth microdilution assay technique. The microorganisms used were clinical strains of *Escherichia coli*, *Salmonella typhi*, *Streptococcus pyogenes*, *Strept. pneumonia*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), non-methicillin resistant *Staph. aureus*,

Candida albicans, *Aspergillus fumigatus* and *A. flavus*. Alkaloids, tannins, terpenoids and cardiac glycosides were extracted by the methanol solvent. The crude alkaloid extracts inhibited only the Gram-negative bacteria with mean inhibition zones of 10.0±0.00 to 12.3±0.03 mm while the methanol extracts inhibited all other test organisms, a broad spectrum activity. The water extracts had no activity against the non-MRSA strains. The MIC was 0.4 mg mL^{-1} for all unicells except strains of *C. albicans* which both had MICs of 0.8 mg mL^{-1}. The MBC was 0.4 mg mL^{-1} for tested isolates except the non-MRSA and *C. albicans* which had MBCs of >12.0 mg mL^{-1} and 1.0 mg mL^{-1}, respectively. The methanolic extract totally inhibited all tested aspergilli while the water extract had a varying inhibitory effect (63.0±2.50 to 81.0±2.90%) on the tested fungi strains. The alkaloid had no effect on the molds. *(Research Journal of Microbiology 4 (7): 269-277, 2009; **doi**: 10.3923/jm.2009.269.277)*

Klebsiella pneumoniae Producing CTX-M-15 Genes from Neonatal Intensive Care Unit in Saudi Arabia

M.H.M. Al-Agamy, A.M. Shibl, A.F. Tawfik and A.R. Elbannai

Reports on outbreak of extended-spectrum β-lactamases (ESβLs) by Enterobacteraiceae and especially *Klebsiella pneumoniae*, are few in Saudi Arabia. This study was therefore devoted to describe the outbreak which occurred by ESβL-producing *K. pneumoniae*. Sixteen *K. pneumoniae* isolates were isolated from 16 neonatal patients hospitalized from September 2007 to December 2007 in the neonatal intensive care unit during the outbreak in Al-Qatif Hospital, Eastern Province, Saudi Arabia. These isolates were sent to microbiological laboratories, College of Pharmacy, King Saud University, for investigation. *Klebsiella pneumoniae* strains were found to produce antibiotic resistance and produce extended spectrum beta-lactamase. Genotypic characterization of extended spectrum beta-lactamase producing *K. pneumoniae* showed that all isolates carried TEM-1, SHV-1 and CTX-M-15 genes. Matting out assay revealed that all third generation cephalosporins were located on transferable plasmid. An outbreak which occurred in neonatal intensive care unit was due to CTX-M-15-producing *K. pneumoniae* isolates either single or in multiple clones. This is the first report of $bla_{CTX\text{-}M\text{-}15}$ gene in Saudi Arabia from *K. pneumoniae* and the first outbreak in Saudi hospitals due to CTX-M-15 producing *K. pneumoniae*. *(Research Journal of Microbiology 4 (7): 278-285, 2009; **doi**: 10.3923/jm.2009.278.285)*

Synergistic effect of *Trichoderma* and *Rhizobium* on Both Biocontrol of Chocolate Spot Disease and Induction of Nodulation, Physiological Activities and Productivity of *Vicia faba*

W.I.A. Saber, K.M. Abd El-Hai and K.M. Ghoneem

Experiments were carried out to correlate the biochemical features of *Trichoderma* species and *Rhizobium leguminosarum* to both biocontrol of *Botrytis fabae* and improving the productivity of faba bean. Of several *Trichoderma* species, isolated from phyllosphere of faba bean, six isolates, which grew considerably faster than *B. fabae* and have moderate to very good antagonism against this pathogen, were selected. *Trichoderma*'s growth inhibiting properties of *B. fabae* were due to the combined action of non-volatile and volatile metabolites (with antibiotic nature) and the secretion of cell-wall degrading enzymes. *Trichoderma viride* (tag3 and tag4) and *T. harzianum* tag7 have shown to be efficient mycoparasites on *B. fabae* (in which the mycelium appeared to be fragmented hyphae, vacuolated and disrupted as a result of *Trichoderma* parasitism). These three *Trichoderma* isolates were further applied in field of faba bean combined with *R. leguminosarum* which, the chromatographical analysis of its supernatant showed activity in growth promoter substances. The dual inoculation of seeds with a mixture of *R. leguminosarum* and *T. viride* tag4 then foliar spraying of the developed plants with the spore suspension of the same *T. viride* tag4 at the 35th and 55th day from sowing reduced chocolate spot disease and enhanced nodulation, nitrogenase activity and nitrogen fixing bacterial population in the rhizosphere. In addition to the improvements in the physiological activities (photosynthetic pigments, total phenol and polyphenol oxidase), plant growth and yield. On average, this treatment recorded about 57% reduction in chocolate spot disease and 23% increase in faba bean yield, compared to control plants. Therefore, a commercial production of an inoculum based on a mixture of *Rhizobium* and *Trichoderma* is very encouraged. *(Research Journal of Microbiology 4 (8): 286-300, 2009;* ***doi****: 10.3923/jm.2009.286.300)*

Single Cell Oil Production by an Oleaginous Yeast Strain in a Low Cost Cultivation Medium

Husain A. El-Fadaly, Noura El-Ahmady El-Naggar and El-Sayed M. Marwan

An oleaginous yeast strain, *Cryptococcus curvatus* NRRLY-1511 was used for the production of single cell oil (SCO) using a low cost cultivation medium

containing beet molasses and corn gluten meal as carbon and nitrogen sources. Obtained results showed that 125 and 0.130 g L^{-1} showed to be the optimum concentrations for carbon and nitrogen, respectively. In addition, 28°C, 72 h, 5.5, 200 rpm were the favorable values of growth temperature, incubation period, pH value of cultivation medium and agitation speed, respectively. The extracted lipids were mainly 30.68% linoleic acid (C18:2), 22.66% oleic acid (C18:1) and 16.74% palmitic acid (C_{16}:0). Furthermore, the GC analysis also showed that the total saturated fatty acids (n = 9) represented 41.96% while the value of the total unsaturated fatty acids (n = 6) was 58.04%. These results giving possibility to use such this yeast strain to produce SCO in a low cost medium from economic point of view. *(Research Journal of Microbiology 4 (8): 301-313, 2009;* ***doi:*** *10.3923/jm.2009.301.313)*

Antibacterial Activity of Seagrass Species Against Biofilm Forming Bacteria

P. Mayavu, S. Sugesh and V.J. Ravindran

The present study was carried out on antimicrobial properties of seagrass species against biofilm forming bacteria's from boat hull during the period April 2008 to March 2009. Seagrass species have a very potential groups were producing several secondary metabolites. The bioactive potential of two different seagrass species viz., *Cymodocea serrulata* and *Syringodium isoetifolium* occurring commonly along the Tuticorin coastal area were selected and preliminary effort has been made against the marine biofilm forming bacteria's *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus vulgaris*, *P. mirabilis*, *E. coli*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococus aureus* and *Vibrio paraheamolyticus*, which also the human pathogens. The seagrasses of *C. serrulata* and *S. isoetifolium* were extracted with four different solvents such as ethanol, methanol, acetone and dichloroethane. Ethanol and methanol extracts of *S. isoetifolium* was inhibited the biofilm forming bacteria such as *E. coli* (14 mm), *P. aeruginosa* (8 mm) and *V. paraheamolyticus* (7 mm) and it showing Minimum activity against *S. aureus* (2 mm). The crude extract of ethanol and methanol of *C. serrulata* was inhibited the growth of all the 9 species of the biofilm forming microbes. The results of present study were concluded that seagrasses have potential bioactivity against marine biofilm forming microorganisms. *(Research Journal of Microbiology 4 (8): 314-319, 2009;* ***doi:*** *10.3923/jm.2009.314.319)*

In vitro Susceptibility of *Naegleria fowleri* Trophozoites to Amphotericin B-combined Chlorpromazine

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The objective of this study was to study the susceptibility of *Naegleria fowleri* trophozoites to Amphotericin B-combined chlorpromazine investigated the activities of single drugs used in combination with amphotericin B compared to those of each drug alone *in vitro*. The 50% inhibitory concentrations (IC_{50}) and 100% minimal concentrations (MIC_{100}) were calculated for single drugs and the drugs combination with fixed combination ratios of IC_{50} of amphotericin B. Single drugs, amphotericin B had the best IC_{50} and MIC_{100} scores against *N. fowleri* trophozoites. chlorpromaxine, Artesunate and azitromycin had following IC_{50} and MIC_{100} scores against trophozoites. However, we found that chlorpromazine in combination with amphotericin B was the best synergistic drug against *N. fowleri* trophozoites. According to single drugs, chlorpromaxine, artesunate and azitromycin plus amphotericin B had also been synergistic drugs against *N. fowleri* trophozoites. It was suggested that the combined use of these agents may be beneficial in treating Primary amoebic meningoencephalitis. *(Research Journal of Microbiology 4 (9): 320-333, 2009; **doi**: 10.3923/jm.2009.320.333)*

Enteric Bacteria Associated with Farmed Freshwater Fish and its Culture Environment in Kerala, India

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A study was designed to investigate the enteric bacterial population associated with farmed freshwater fish and its environment, limnological quality of carp farm and the existing association between these parameters. Enteric indicator bacterial counts were determined following the United States Food and Drug Administration (USFDA) methods and the physico-chemical parameters according to the standard methods of American Public Health Association (APHA). Fish samples yielded mean microbiological counts in the range of 4.19 to 4.85 log CFU g^{-1}, sediment in the range of 5.18±0.01 to 6.34±0.01 log CFU g^{-} ,^{1} pond water in the range of 3.64±0.03 to 6.10±0.04log CFU mL^{-1}. Fish and feeder canal water showed higher count for all indicator bacterial count. Sediment showed 2 log cycle higher count of sulphite reducing *clostridia*. Emerging pathogen *E. coli* O157:H7 were absent in all the samples analyzed. *Aeromonas* (26.2%) followed by *Enterobacter* (24.6%) were the dominant flora recovered. *Escherichia*, *Klebsiella*, *Serratia*, *Hafnia*,

Plesiomonas, *Shigella*, *Salmonella*, *Morganella* and *Yersinia* were the other opportunistic enteric bacterial pathogens detected from this system. The rearing practices such as natural fertilization and feeding could have influenced the enteric flora. Study on the various physico-chemical parameters of pond water revealed that they were within the suitable range for the freshwater fish culture throughout farming phase. Correlation analysis revealed a significant positive correlation between physico-chemical parameters such as total organic carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) with that of Total Plate Count (TPC), Total *Enterobacteriaceae* Count (TEC), Total Coliforms (TC), Faecal Coliforms (FC) and *E. coli* (EC). Presence of bacteria of public health significance in the aquaculture ponds envisages a strict hygienic handling and processing of fish from the culture systems for ensuring public health safety. *(Research Journal of Microbiology 4 (9): 334-344, 2009;* ***doi:*** *10.3923/jm.2009.334.344)*

Swimming Motility in *Agrobacterium tumefaciens* is Controlled by Quorum Sensing and Inhibited by Garlic Bulb Extract

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Bacteria can produce and sense signal molecules, allowing the whole population to initiate a concerted action once a critical concentration (corresponding to a particular population density) of the signal has been reached; a phenomenon known as Quorum Sensing (QS). The current study was conducted to examine the possible role of QS in the regulation of swimming motility of *Agrobacterium tumefaciens*. In addition, we investigated the anti-QS or Quorum-Quenching (QQ) activity of garlic bulb and *Salvadora persica* extracts. We found that treatment of *A. tumefaciens* culture with different exogenous QS compounds induced swimming motility. C4 AHL, C6 AHL, C7 AHL, C8AHL, C10 AHL and C14 AHL induced bacterial swimming motility by about 3.5, 4, 4.5, 4.5, 3.5 and 4 fold, respectively, providing strong evidence that quorum sensing in *A. tumefaciens* controls cell motility, or at least plays a major role in its regulation. We also found that different QS compounds affect the bacterial phenotype, including the colony pattern and morphology. In addition, garlic bulb and *Salvadora persica* extracts were investigated for their QQ activity. While *S. persica* extract did not show any significant QQ activity, garlic bulb extract showed QQ activity against C4 AHL, C8 AHL, C10 AHL and C14 AHL, repressing the *A. tumefaciens* swimming motility induced by these QS compounds. To the best of our knowledge, this is the first report of a possible role for QS in the regulation of swimming motility in A. tumefaciens. *(Research Journal of Microbiology 4 (9): 345-354, 2009;* ***doi:*** *10.3923/jm.2009.345.354)*

Microbiological Evaluation of the Quality of Tap Water Distributed at Khartoum State

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This study was aimed to evaluate the microbial quality of drinking water distributed at Khartoum state- the capital of the Sudan. Water distributed at piped system was investigated using two different standard methods (MPN and chromogenic media- based techniques), 47.5-90% showed positive isolation of bacteria. The results revealed isolation of faecal coliform (*E. coli*), coliform group (*Klebsiella* sp., *Citrobacter* sp., *Enteriobacter* sp.), some pathogenic and potential pathogenic bacteria (*Staphylococcus aureus*, *Salmonella* sp., *Yersienia enteriocolitica*, *Proteus* sp., *Bacillus* sp. and *Pseudomonas aeruginosa*) were isolated. Other bacteria with significant importance were detected. The quality of drinking water, types and number of isolated bacteria were evaluated and discussed according to seasons and locations. *(Research Journal of Microbiology 4 (10): 355-360, 2009;* ***doi:*** *10.3923/jm.2009.355.360)*

Viability of Antifungal Metabolite Producing *Pseudomonas* Bacteria

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The objectives of this study were to determine the suitability of transport medium (ice jells) and estimate the duration of viability of *Pseudomonas* in the transport medium. Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites. These include simple metabolites such as 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid and pyrrolnitrin [3-chloro-4-(2-nitro-3-chlorophenyl)-pyrrole], as well as the complex macrocyclic lactone, 2, 3-de-epoxy-2, 3-didehydro-rhizoxin. Pyrrolnitrin is active against *Rhizoctonia* sp., *Fusarium* sp. and other pathogenic fungi and it has been used as a lead structure in the development of a new phenylpyrrole fungicide. The survival rates of four different pseudomonad strains after continuous incubation for 4 h in the cold temperature (4°C) were: 94.8% for *P. putida* strain CBD, 94.5% for *P. aeruginosa* No. BRCH and 62.1% for *Pseudomomas* species *(fluorescent)* with lowest survival rate of 33.5% for *P. aeruginosa* strain H. Since, there were no drastic reductions in the survival rates, the study findings suggest that the transport medium would be generally suitable for these cold-sensitive bacteria. *(Research Journal of Microbiology 4 (10): 361-365, 2009;* ***doi:*** *10.3923/jm.2009.361.365)*

In vitro Activity of Some Antimicrobial Agents against *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* in Khartoum, Sudan

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Staphylococcus aureus is a causative agent of many types of diseases throughout the world. Patients hospitalized for long period of time usually are predisposed to infection by methicillin resistant *S. aureus* (MRSA). The objectives of the present study were to evaluate the efficacy of some antimicrobial agents against *S. aureus* and MRSA and to select the most effective antibiotic. Clinical specimens were collected from patients with wounds and/or urinary tract infections. The specimens were proceeded for isolation of the pathogens. Identification was done by conventional methods. Antimicrobial sensitivity test was carried out using modified Kirby-Bauer Disc Diffusion Technique in accordance with National Committee on Clinical Laboratory Standards (NCCLS). Of 163 *S. aureus* recovered, 15 (9.2%) isolates were MRSA. The most effective antimicrobial agent against both *S. aureus* and MRSA was vancomycin (99%). The activity of the rest antimicrobial agents was cephalexin, 92%, methicillin, 90%, cloxacillin 33%, penicillin 14% and amoxicillin 10%. It is concluded that vancomycin may be an alternative antibiotic for patients with wound and/or urinary tract infections caused by *S. aureus* or MRSA. *(Research Journal of Microbiology 4 (10): 366-369, 2009;* ***doi:*** *10.3923/jm.2009.366.369)*