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Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* Strains Isolated in Kerala, South India

J. Jeshina and K. Surekha

The aim of the present study is to report the prevalence, antimicrobial susceptibility pattern molecular characteristics of methicillin resistant *Staphylococcus aureus* strains and the emergence of vancomycin intermediate *Staphylococcus aureus* (VISA) strains in Kerala, India. The study was conducted during January 2006 to December 2007 on 70 strains obtained from pus cultures of patients from various hospitals in Kerala, India. Organisms were isolated, cultured and identified as per standard routine procedures. Susceptibilities to thirteen commonly used antibiotics were tested by agar diffusion method as recommended by CLSI. Minimum inhibitory concentrations of oxacillin, ciprofloxacin and vancomycin were determined using standard protocol. Plasmid profile analysis of the strains carried out and the central resistance determinant *mec*A and internal control gene *fem*A were isolated and sequenced. Cassette chromosome typing carried out as per standard procedures. Among the 70 strains isolated 13 of them showed reduced susceptibility to vancomycin and two isolates were resistant. All the strains were resistant to oxacillin and ampicillin and uniformly sensitive to gentamycin. *mec*A gene was isolated from 88% strains and sequence analyzed. The strains were found to be Hospital Associated-MRSA (HA-MRSA) with type III cassette chromosome. This study reveals the high prevalence of MRSA and a gradual emergence of VISA strains in Kerala. This is greatly due to the irrational and overuse of antibiotics like vancomycin and partly due to negligence on the part of health care workers in acknowledging the prevalence of MRSA and VISA strains and initiating appropriate strategies to control their spread. Careful use of existing antibiotics and regular monitoring of strains circulating in a particular hospital at regular intervals is necessary to control the spread of multidrug resistant strains and to prevent the emergence of even more serious strains. *(Current Research in Bacteriology 2 (1): 1-6, 2009;* ***doi****: 10.3923/crb.2009.1.6)*

Bacterial Symbionts of Reef's Invertebrates as a Sustainable Source of Marine Natural Products

Ocky Karna Radjasa and Agus Sabdono

Marine invertebrates are mainly accumulating within coral reef ecosystems such as soft corals, sponges, tunicates and bryozoans have long been recognized as the prolific sources of structurally unique and diverse natural products since they

provide a large proportion of bioactive compounds with different biological activities. Unfortunately, the supply of these bioactive natural products is usually insufficient to meet the ultimate development of most marine natural products. The concentrations of many highly active compounds in reef's invertebrates are often minute, accounting for less than 10^{-6}% of the wet weight. This problem has been viewed as the most significantthreat regarding the development of pharmaceutical from reef's invertebrates. The secondary metabolites from bacterial symbionts, on the other hand, are a rapidly growing field, due to the suspicion that bioactive metabolites obtained from invertebrates may be produced by their bacterial symbionts. In particular, from sustainability point of view, isolating bioactive-producing bacteria is obviously offers a much better approach than cultivating and harvest invertebrates, which are in most cases extremely difficult. Bacteria isolated from living surfaces, in particular from reef's invertebrates, are a promising source of natural products. It is expected that still quite a few parts of unexplored culturable bacterial symbionts exists in the reefs. Such information might be desirable, as these bacterial symbionts may serve beneficial purposes as the source of secondary metabolites including novel marine natural products. *(Current Research in Bacteriology 2 (1): 7-13, 2009;* ***doi****: 10.3923/crb.2009.7.13)*

Phylogenetic Diversity of the Causative Agents of Vibriosis Associated with Groupers Fish from Karimunjawa Islands, Indonesia

Sarjito, O.K. Radjasa, A. Sabdono, S.B. Prayitno and S. Hutabarat

A molecular-based study was conducted to estimate the richness of the causative agents of vibriosis associated with groupers from Karimunjawa islands, North Java Sea, Indonesia. Moribound grouper fish were collected from the cage cultures and a total of 32 isolates were isolated from external wound and kidney of groupers. Based on the repetitive sequence-based PCR (rep-PCR) and Koch postulate test, eight isolates were chosen for further sequencings. On the basis of the sequence analysis, the data showed that the causative agents are closely related with *Vibrio natriegen*, *V. oliviceaus*, *V. fortis*, *V. alginolitycus*, *V. harveyi*, *V. parahemolitycus*, *V. damsela* and *V. carchariae*, respectively. Present study highlighted the effectiveness of rep-PCR in rapid grouping and estimating the richness of the causative agents of vibriosis associated with the groupers. *(Current Research in Bacteriology 2 (1): 14-21, 2009;* ***doi****: 10.3923/crb.2009.14.21)*

Two Pathotypes of *Xanthomonas oryzae* pv. *oryzae* Virulence Identified in West Africa

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Pathotyping analysis of 50 Xanthomonas oryzae pv. oryzae (Xoo) isolates from seven West African countries against 18 rice cultivars was carried out to identify and characterize Xoo virulence. The study revealed two major pathotypes (Pta and Ptb) of Xoo virulence. Pta has 29 virulence (Vr) Xoo isolates while Ptb has 21 mildly virulence (MVr) Xoo isolates. Pta has three subgroup pathotypes (Pta1, Pta2 and Pta3) and Ptb has two subgroup pathotypes (Ptb1and Ptb2). At country level the study revealed the presence of Pta1, Ptb1 and Ptb2 in Niger, Pta3, Ptb1 and Ptb2 in Benin and Nigeria, Pta1, Pta3 and Ptb1 in Burkina Faso, Pta1, Pta3, Ptb1 and Ptb2 in Mali, Pta1, Pta2, Pta3, Ptb1 and Ptb2 in Guinea and Pta1, Pta2, Ptb1 and Ptb2 in the Gambia. The existence of five subgroups was likely due to mutations and interactions among isolates that originally constituted Pta and Ptb pathotypes. The study revealed information on Xoo virulent population structure in West Africa as well as possible Xoo pathogen migration between these countries and this provide useful information for selection and deployment of cultivars with durable resistance to BLB disease in West Africa. *(Current Research in Bacteriology 2 (2): 22-35, 2009;* ***doi****: 10.3923/crb.2009.22.35)*

Antibiotic Susceptibility and Genetic Analysis of *Vibrio* Species Isolated from Reverine Environment

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The resistance profile and its correlation with mobile genetic elements were investigated in 11 *Vibrio cholerae,* 10 *V. parahaemolyticus*, 12 *V. vulnificus,* 11 *V. fischeri,* 10 *V. proteolyticus* and 5 *V. mimicus* isolated from River Narmada. All the 59 isolates of *Vibrio* species were examined for their susceptibility/resistance against 14 commonly used antibiotics against *Vibrio* species. More than 50% isolates showed resistance against five commonly used antibiotics viz., ampicillin, ceftadizime, erythromycin, chloramphenicol, cefuroxime. Plasmid of 6 kb was detected in 11 resistant isolates and class 1 integron was detected in 16 resistant isolates. SXT element was not found among resistant isolates. The present study indicated that plasmid and Class 1 integron mainly contributed to the circulation of multidrug resistance determinants in *Vibrio* species isolated from river Narmada. *(Current Research in Bacteriology 2 (2): 36-49, 2009;* ***doi****: 10.3923/crb.2009.36.49)*

Ceftriaxone-Sulbactam Combination: Microbial Analysis by Variation of Ratios and Comparative Disc Diffusion

S.M. Shrivastava, S. Kumar and M. Chaudhary

Development of β-lactamase provides resistance to bacteria against cephalosporins. Ceftriaxone, a third generation cephalosporin has also lost it's effectiveness in clinical practices. However, it is the current trend to use combinations of β-lactam antibiotics and β-lactamase inhibitors as they have come up as the ideal solution. The potential combination with ceftriaxone is of sulbactam, a β-lactamase inhibitor. This combination is used in clinical practice for achieving better therapeutic value. In present study, comparative microbial analysis of various ratios of ceftriaxone, sulbactam and sulbactomax, a Fixed Dose Combination (FDC) of ceftriaxone and sulbactam has been performed by Minimum Inhibitory Concentration (MIC) analysis. Comparative evaluation of susceptibility discs of FDC of ceftriaxone and sulbactam with ceftriaxone is done under time stress to find out possibility of development of resistance in *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Escherichia coli* and Methicillin Resistant *Staphylococcus aureus* (MRSA). In the results of MIC, 2:1 ratio of ceftriaxone: sulbactam has shown better bactericidal activity than the ratio of 1:6.66 and 1:3.33. Antibiotic Susceptibility Test (AST) demonstrated that ceftriaxone-sulbactam, apart from being more bactericidal, has less chances of resistance development, when compared with ceftriaxone alone. It may be concluded that ceftriaxone-sulbactam in the ratio of 2 :1 has better bactericidal properties and reduces the probability of resistance development. *(Current Research in Bacteriology 2 (2): 50-55, 2009;* ***doi****: 10.3923/crb.2009.50.55)*

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±0.03 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

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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

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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

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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 h 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

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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 $bla_{\text{CTX-M-15}}$ 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

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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)*

Cyclodextrin Glycosyltransferase Production by the *Bacillus* sp., Subgroup *alcalophilus* using a Central Composite Design

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Cyclodextrin glycosyltransferase (CGTase) activity was produced by the *Bacillus* sp., subgroup *alcalophilus* in a culture medium containing cassava starch. A central composite design and response surface methodology were used to study the influence of carbon source (cassava starch), nitrogen sources (yeast extract and tryptone) and sodium carbonate in the production medium. Assays were performed in 300 mL Erlenmeyer flasks containing 100 mL of production medium maintained in a shaker at 150 rpm at 35±1°C for 72 h of fermentation. The independent variables [0.75% cassava starch, nitrogen sources (0.375% yeast extract and 0.375% tryptone) and 1% Na_2CO_3] produced an enzyme activity of 96.07 U mL^{-1}. *(Research Journal of Microbiology 4 (11): 450-459, 2009;* ***doi****: 10.3923/jm.2009.450.459)*