

World Congress and Expo on

Applied Microbiology

August 18-20, 2015 Frankfurt, Germany

Enterovirus 71: Candidates for vaccines and antivirals

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Hand, foot and mouth disease (HFMD) is commonly caused by a group of Enteroviruses such as Enterovirus 71 (EV71) and Coxsackievirus CVA5, CVA8 and CVA 16. Coxsackieviruses generally cause mild symptoms such as high fever, rashes and vesicles in the hand, foot and mouth but EV71 can produce more severe symptoms such as brainstem encephalitis, leading to cardiopulmonary failure and death. China experienced over 2.7 million cases of HFMD infections with 384 deaths in 2014. The lack of vaccines and antiviral drugs against EV71 highlights the urgency of developing preventative and treatment agents against EV71 to prevent further fatalities. The inactivated vaccine (IV) is well advanced in development and has good clinical trial data to support the use of the vaccine. It is ready for production in China but it remains to be investigated if the immunogenicity of the IV is able to confer protection against all EV71 sub-genotypes. Although there is data to support broad protection for some genotypes/sub-genotypes at varying efficacies, more studies need to be carried out on whether the neutralizing levels induced by IV are sufficient to protect against serious HFMD infections. New developments of experimental vaccines and antivirals are presented.

Biography

Chit Laa Poh completed her PhD from Monash University, Australia in 1980 and returned to Malaysia and Singapore to pursue her academic career. Initially trained as an environmental bacteriologist, she started to focus on Medical Virology research since 1999 and worked on the development of rapid molecular diagnostics, novel antivirals and vaccines against Enterovirus 71 which can cause serious hand, foot and mouth disease (HFMD). She has achieved a Google H-index of 33. She is on the editorial board of Journal of Bioscience and Bioengineering (Elsevier) and Austin Journal of Tropical Medicine and Hygiene. She has graduated 12 PhDs, 8 MScs and many BSc (Hons) students. She has published 87 papers in reputed journals and co-authored 3 book chapters in books published by ASM and Humana Press. She has served as Ad Hoc reviewers for papers submitted to PLoSOne. After working for 25 years in the National University of Singapore (NUS), she is currently engaged as a Distinguished Professor by Sunway University. In her current role, she hopes to attract good graduate students and provide them with excellent supervision in research. She is often invited by reputable journals to contribute review papers and original research papers.

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Influence of anti estrogen therapy in patients with breast cancer in the bearing of *Candida spp.*

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Objective: To determine the prevalence of yeast species in the evolution of patients with breast cancer treated with antiestrogen therapy.

Materials & Methods: 30 postmenopausal patients who attended the Southern OMI Medical Center were included. The following groups were formed:

Group 1 patients diagnosed with Breast Ca. treated with anti estrogens for less than one year

Group 2 patients diagnosed with Breast Ca. treated with anti estrogen for 1-2 years

Group 3 patients diagnosed with Breast Ca. treated with anti estrogen for 2-5 years

Group 4 patients diagnosed with Breast Ca., who have completed their treatment with anti estrogen. Patients were surveyed about their symptoms, periodontal indices and then oral mucosa sample were taken. Conventional Microbiological examinations for *Candida* species as well as the molecular biology study data were performed.

Results: Microbiological findings showed that a greater variety of species of *Candida* were isolated from patients who used the drug during the first two years (Group 1 and 2). Only 2 species were isolated in patients who used the drug more than two years (Group 3) and those who have completed treatment (Group 4).

Conclusion: The length of intake of anti estrogens influences the growth and species of *Candida*, having a cumulative beneficial effect on the population studied.

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Evaluation of the rabbit as a laboratory model for bovine viral diarrhea virus infection

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Bovine Viral Diarrhea Virus (BVDV) is an important pathogen of even-toed ungulates. It is endemic in cattle herds in most parts of the world. It belongs to the family Flaviviridae, genus *Pestivirus*. Until now, there is no standard lab host for BVDV. Although some reports mentioned that rabbit could propagate certain strains of BVDV upon intravenous exposure, lack of lab host is an obstacle faced by most of the researcher. Our work aimed to evaluate rabbits as lab host for BVDV using NADL BVDV strain in white New Zealand rabbits. Successful multiple passages of BVDV in rabbits using mixture of splenic homogenate and buffy coat were achieved followed by re-isolation and molecular identification of the virus from infected animals. Later, the re-isolated virus has been intravenously administered in rabbits; animals developed signs of depression and were off-food for 3 days followed by diarrhea in some of them. Also, transient leukopenia, lymphocytopenia and thrombocytopenia were observed. Post-mortem and histo-pathological examination showed typical picture of *Pestivirus* transient infection. Demonstration of the viral antigen has been done on splenic tissues using immune-histochemistry. Moreover, virus propagation has been followed up and confirmed over 10 days using quantitative Real-Time PCR technique on tissue samples. On the other hand, saliva and feces were virus negative. From our work, we discovered that adapted NADL BVDV strain have the capability of inducing transient *Pestivirus*-like infection in rabbits, which makes rabbits suitable lab animals for BVDV pathogenicity and virocidal studies.

Biography

Mostafa El-Gaffary has completed his MVSc in 2010 from Cairo University and his PhD from Cairo University, Faculty of Veterinary Medicine. He is a Lecturer and the Director of Clinical Pathology Lab in his Faculty. He was a Trainer for postgraduate students on biomedical application of nanotechnology, molecular biology and immunology at biotechnology center for research located in his Faculty during 2007 – 2014.

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Alkanna orientalis (L.) Boiss. plant callus cultures as novel source with antibacterial activity

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The ever-increasing demand for herbal medicine requires searching for new sources of biologically active compounds. *Alkanna orientalis* (L.) Boiss. is known as a plant with high biological activity. *A. orientalis* plant callus culture was isolated, antimicrobial activity of its aqueous extracts was studied against a number of Gram-negative (*Escherichia coli*, *Salmonella typhimurium*), Gram-positive (*Bacillus mycoides*, *B. mesentericus*, *B. megaterium*, *B. subtilis*, *Brevibacterium lavum*, *Enterococcus hirae*, *Micrococcus luteus*, *Staphylococcus aureus*, *St. citreus* WT, *St. roseus*, *Lactobacillus acidophilus*, *L. rhamnosus*) bacteria and yeasts (*Debaryomyces hansenii*, *Pichia guilliermondii*) by the method of diffusion in the agar. A Minimal Inhibitory Concentration (MIC) for callus extracts was determined against *E. hirae*. The extracts with different concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.625 µg/ml) had been used. As a positive control, shikonin was used with the same concentrations, as those of the extract. According to our studies, callus extracts possessed marked bacteriostatic activity against gram-positive bacteria and bactericidal activity - against lacto acid bacteria. MIC was corresponding to 250 µg/ml dry weight of extract in comparison with purified shikonin, which MIC was 31.25 µg/ml. Hence, *A. orientalis* callus tissue extracts have the rather high antimicrobial activity, which will be useful for development of new medicinal preparations as well as for food industry.

Biography

Sahakyan Naira has completed her PhD from Supreme Certification Commission of the Republic of Armenia (of Ministry of Education and Science of the Republic of Armenia). She is the researcher of Yerevan State University, Armenia. She has published more than 25 papers in reputed journals and over 20 participations on scientific meetings.

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In-vitro inhibition of BVDV as surrogate model for HCV using novel gold nanoparticles

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In this study, we evaluated *in-vitro* cytotoxic effect and antiviral properties of gold nanoparticles, which are previously reported to possess *in-vitro* antiviral properties against HIV and multi strains of influenza virus. To investigate the antiviral activity of gold nanoparticles against cytopathic strain (NADL) bovine viral diarrhea virus (BVDV): citrated gold nanoparticles of 7 ± 2 nm were prepared and PEG functionalized. Evaluation of the cytotoxicity of prepared gold nanoparticles did not show toxic effects to MDBK cells with concentration of 2 and 4 ppm. Afterward the antiviral activity of nanoparticles was evaluated by the inhibition of the cytopathic effect on infected MDBK cells by means of (MTT) based colorimetric assay and was found that 4 PPM is the optimum concentration for virus inhibition. The results of the *in-vitro* antiviral activity and cytotoxicity showed that prepared gold nanoparticles has limited *in-vitro* toxic effect at concentration of 4 PPM also has strong affinity to BVD virus and reasonable inhibitory effect on BVDV.

Biography

Mostafa El-gaffary has completed his MVSc 2010 from Cairo University and his PHD from Cairo University Faculty of Veterinary Medicine. He is lecturer and director of Clinical pathology Lab in his Faculty; he was Trainer for postgraduate student on Biomedical application of Nanotechnology, Molecular biology and Immunology at Biotechnology center for research located in his Faculty 2007 – 2014.

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Malnutrition, infection & disease

ECG Muchaneta Kubara

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Malnutrition is the condition that results from taking an unbalanced diet which certain nutrients are lacking, in excess (too high an intake), or in the wrong proportions. A number of different nutritional disorders may rise, depending on which nutrients are under abundant in the diet. In most of the world, malnutrition is present in the form of undernutrition, which is caused by a diet lacking adequate calories and protein. The World Health Organization cites nutrition as the greatest single threat to the world's public health. Improving nutrition is widely regarded as the most effective form of aid. Nutrition-specific interventions, which address the immediate cause of undernutrition, have been proven to deliver among the best value for money of all development interventions. Malnutrition is responsible directly or indirectly for 54% of the 10.8 million deaths per year in children under five and contributes to every second death (53%) associated with infectious diseases among children under five years of age in developing countries. Infection causes energy loss on the part of the individual, which reduces productivity on the community level and perpetuates the alarming spiral of malnutrition, infection, disease and poverty.

Biography

ECG Muchaneta –Kubara completed her PhD as a mature student, mother wife and bread winner at Sheffield University in 1998 and has worked as a Senior Scientist in the Department of Chemical Pathology, Lecturer Immunology and Microbiology in the Department of Medical Laboratory Sciences and currently Senior Lecturer in the Department of Medical Microbiology. She has over 21 International publications in reputed journals.

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Coinfection of malaria and intestinal parasites among school children in Ajagba, Southwestern Nigeria

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Concomitant parasitic infections in the developing world are increasing, yet most studies are focused on single parasite. In this study, the extent of co-infections was investigated. Three hundred consenting individuals consisting of 136 males and 164 females participated in this study. Faecal specimens and venous blood were collected from the participants. The formal-ether concentration method were used to screen the faecal samples for helminths and protozoans, while Giemsa-stained blood smears was used for malaria parasite and packed cell volume (PCV) was determined by hematocrit. Demographic information from all the participants and data were analyzed using Chi-square test. The prevalence of Malaria parasite, Hookworm, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Entamoeba histolytica* were 27.3%, 24.6%, 8.7%, 6.6%, 6.6% respectively. Females (55.0%) were generally more infected with all parasite than the males (45.1%) and it is statistically significance ($p=0.000$). Co-infection of parasites were observed as follows; Hookworm, *Ascaris lumbricoides* and Malaria parasite (2.7%), Hookworm, *Entamoeba histolytica* and Malaria parasite (0.7% histolytica), Hookworm, *Ascaris lumbricoides*, Malaria Parasite and *Entamoeba histolytica* (1.3%), *Entamoeba* and *Ascaris lumbricoides* (0.7%), Hookworm and *Strongyloides stercoralis* (2.0%), *Ascaris lumbricoides* and hookworm (3.3%), Hookworm and Malaria Parasite (3.3%), *Ascaris lumbricoides* and Malaria (2.7%), *Entamoeba histolytica* and Malaria (2.0%), *Ascaris lumbricoides* and *Strongyloides stercoralis* (0.7%) and *Ascaris*, *Strongyloides stercoralis* and Malaria Parasite (0.7%). The overall Mean Packed cell Volume (PCV) of the population was 29.40 ± 5.16 and it statistically significant ($p=0.029$). These result showed the existence of polyparasitism in Ajagba community and it is a major public health problem hence there is need for improved environmental condition which includes clean water supplies, periodic de-worming of children in the community should be initiated and action against deficiency in sanitary facilities, poor personal hygiene should be addressed by the government.

Biography

O.S. Bolaji started his career in 1990 at Obafemi Awolowo University Teaching Hospital School of Medical Laboratory Sciences where he obtained Associate Certificate of Medical Laboratory Science Council of Nigeria (AMLSCN- bacteriology option) in 1994. He proceeded to Imo State University, Owerri, Nigeria and obtained in 2002 Post Graduate Diploma in Medical Laboratory Science- Microbiology option (PGDMLS), M.Sc. Medical Parasitology and Entomology in 2005 and finally Ph.D Medical Parasitology in 2011 from Ladoke Akintola University of Technology (LAUTECH), Ogbomoso-Nigeria. His thesis titled 'Molecular Epidemiology of Urinary Schistosomiasis among School children in Osun State, Nigeria'. He joined LAUTECH as an Assistant Lecturer in 2006 and is presently a Senior Lecturer in the Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, Osogbo, Osun State, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. He lectures Medical students (MBBS Degree), Medical Laboratory Science students (B.MLS), Nursing students (B.NSc.), PostGraduate Diploma and M.Sc. students in Medical Parasitology. He is a Lecturer, Practising Medical Laboratory Scientist and Research Scientist. He is currently on research activities 'Genetic Diversity of *Schistosoma haematobium* among humans in endemic areas of Osun State'. He is presently designated as a Visiting Scholar (Scientist) to Johns Hopkins University Bloomberg School of Public Health, Baltimore, USA in the Department of Molecular Microbiology and Immunology for 3months.

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Primary cutaneous actinomycosis: A first case report from Kurdistan- Iraq

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A case of primary cutaneous actinomycosis was diagnosed on clinical and bacteriological grounds. A fifty-five year woman presented with multiple discharging sinuses on both legs since 9 years with slowly progressive course; from rural area in Kurdistan Region-Iraq. Bacteriological study including macroscopical and cultural examination of the discharge and crust taken deep from the lesions, revealed actinomyces as the causative organism. Good response with complete healing was noticed after 4 months of treatment with benzathine penicillin. Primary cutaneous actinomycosis is a rare variety of actinomycosis and this is the first case reported in Iraq. Good awareness of the full clinical spectrum of the disease aided by bacteriological study is needed to minimize the misdiagnosis of the case.

Biography

HushamBayazed has completed his PhD from University of Mosul, College of Medicine. He is now Consultant at the Scientific Research Center, University of Zakho / Kurdistan Region, Iraq. He is specialist in Microbiology & Immunology and has published more than 25 papers in reputed journals and has been serving as scientific reviewers of many local and international medical journals. In addition he has a Fellowship of ISC, Infection, Cancer, Immunology Advisory Board Member (EUROMDnet) (Belgium), Membership of World Stroke Organization, Membership of Metabolomics (USA), and Membership of American Association of Science & Technology.

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Paratuberculosis in Egypt and Arab area

Diea Abo El-Hassan
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Mycobacterium avium subsp. *Paratuberculosis* is the etiological agent of a severe gastroenteritis in ruminants, known as Johne's disease. Johne's disease is prevalent in domestic animals worldwide and has significant impact on the global economy. It is considered to be one of the most serious diseases affecting cattle, camels, sheep and goats in Egypt and Arab area. Isolation of *M. paratuberculosis* from intestinal tissue of Crohn's disease patients has led to concern that it may be pathogenic for humans. Thus, the pathogenic role of *M. paratuberculosis*, early diagnosis and efficient control in animal population are topics of intense debate.

Biography

Diea Abo El-Hassan has been a professor of infectious diseases in, Faculty of Veterinary Medicine, Cairo University since 1987. He received his PhD in Animal infectious diseases at Cairo University, Texas A & M University and Plum Island Institute, USA in 1986, and obtained both his B.V.Sc. and M.V.Sc. degrees at Faculty of Veterinary Medicine, Cairo University in 1979 and 1983 respectively. Professor of Animal Infectious diseases and Clinical laboratory diagnosis in Qassim University Saudi Arabia since 2006 - 2010, Director of Publications and Publishing Center College of Veterinary Medicine, Cairo University, since 2010. Currently, he is the head of Animal Internal Medicine & Infectious Diseases in Cairo University and consultant for many dairy & beef farms. He worked in many international projects in cooperation with Germany, USA and Saudi Arabia as well as other national projects.

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Towards rapid detection of *Staphylococcus aureus* during blood culture

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The presence of viable bacteria in the blood is commonly known as bacteremia. It can be a very localized and transient event with no consequences but for the immune-suppressed or seriously wounded people. The most severe cases can develop into sepsis, septic shock and sometimes death. Faced with suspected bacteremia, a practitioner is forced to use a broad spectrum antibiotic treatment while awaiting the results of microbiological analyses of blood samples which can last for 24 hours to 72 hours. Despite numerous efforts to shorten the time required for diagnosis, in most techniques the organism identification begins only after the blood culture turns positive. *Staphylococcus aureus* is one of the most frequent strains causing bacteremia. For this reason, its detection is a major challenge for health issues. We propose here to carry out the microorganism identification directly from blood culture phase. To achieve this, live bacteria are detected on an antibody based biochip without any labeling. This approach relies on a simple to operate optical technique named Surface Plasmon Resonance imaging (SPRi), recently described for pathogen detection in complex samples (ground meat, milk). Biological samples are diluted in a media specifically dedicated to this application and in accordance with the recommendations for blood cultures. Then, samples are spiked with a known amount of *S. aureus* and loaded on the biochip. Interactions are then recorded in real time until a positive signal appears on specific antibody due to antibody-antigen recognition. In general, a few dozens of bacteria are detected in less than ten hours in human serum. We are now focusing on the methicillin-resistant strain (MRSA versus MSSA), by the identification of the PBP2a protein, which is anchored at the cell surface and therefore, is accessible to antibodies, using the recognition capability of this antibiotic resistance marker.

Biography

V Templier completed his engineering studies at Institut National des Sciences Appliquées de Toulouse as a biochemical engineer. He is currently a PhD student in the CEA Grenoble (Institut Nanosciences et Cryogénie). His research interests include biosensors with focus on pathogenic bacteria detection.

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Fever-like temperature is a virulence regulatory cue controlling the motility and host cell entry of typhoidal Salmonella

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Human infection with typhoidal Salmonella serovars causes a febrile systemic disease, termed enteric fever. Here we establish that in response to a temperature equivalent to fever (39°C–42°C) Salmonella enteric serovars Typhi, Paratyphi A, and Sendai significantly attenuate their motility, epithelial cell invasion, and uptake by macrophages. Under these fever-like conditions, the residual epithelial cell invasion of *S. paratyphi* A occurs in a type III secretion system (T3SS) 1–independent manner and results in restrained disruption of epithelium integrity. The impaired motility and invasion are associated with down-regulation of T3SS-1 genes and class II and III (but not I) of the flagella-chemotaxis regulon. In contrast, we demonstrate up-regulation of particular Salmonella pathogenicity island 2 genes (especially *spiC*) and increased intraepithelial growth in a T3SS-2–dependent manner. These results indicate that elevated physiological temperature is a novel cue controlling virulence phenotypes in typhoidal serovars, which is likely to play a role in the distinct clinical manifestations elicited by typhoidal and non-typhoidal salmonellae.

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Isolation, characterization and genome analysis of *Paenibacillus polymyxa* CR1, a bacterium for biocontrol, biomass degradation, biofuel and chemical production

Ze-Chun Yuan

University of Western Ontario, Canada

Non-food biomass holds great promise as sources of clean and renewable fuels and chemicals. However, lignin depolymerization is the bottleneck for lignocellulosic biofuels and chemicals. Understanding the bacterial metabolic pathways and regulatory mechanisms underpinning lignin degradation is very important for developing cost effective techniques to revitalize the biorefinery industry. We recently isolated and characterized *Paenibacillus polymyxa* CR1 (Corn Rhizobacterium) exhibited multifaceted plant-beneficial traits including phytohormone (indole-3-acetic acid) production, nitrogen fixation, and antagonistic activities against crop pathogens. *P. polymyxa* CR1 significantly promotes the growth of corn, potato, cucumber, tomato and *Arabidopsis thaliana*. *P. polymyxa* CR1 also demonstrated strong ability in degrading and utilizing lignin, cellulose and hemi-cellulose as sole source of carbon and produce valuable chemicals and fuels. In addition, *P. polymyxa* CR1 degrades saw dust directly and produces phenylacetic acid (also called benzeneacetic acid) which is widely used in perfumes, penicillin G production and other purposes. To facilitate understanding its metabolic pathways implicated in biomass degradation and bioproduction, we sequenced the complete genome of *P. polymyxa* CR1 (~6Mbs). We also compared the *P. polymyxa* CR1 genome with the other 3 *P. polymyxa* genomes fully sequenced. Our genomics and comparative genomics analysis revealed many conserved genes/functions relevant to antimicrobial production, biomass degradation and bioproduction, which lays a solid foundation for our future work, e.g., using genetic and metabolic engineering to rewire bacterial metabolic flux networks and synthetic pathways for efficient conversion of lignocellulosic biomass (forestry and agricultural residues, municipal biosolids and wastes of paper industry) into value-added biofuels and chemicals.

Biography

Ze-Chun Yuan has completed his PhD from McMaster University in Canada and Postdoctoral studies from University of Washington (Seattle, USA). He is a research professor at University of Western Ontario (Canada), and a Principal Investigator (research scientist) at Agriculture and Agri-Food Canada (London, Ontario). He has published several interesting papers in reputed journals and is serving as routine reviewers for many important scientific journals.

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Piezophilic bacteria from the West Iberian margin: Hydrocarbons degradation under high hydrostatic pressure

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Microbial community shifts from an uncontaminated environment to an alkane-polluted one were investigated using deep-sea sediment from the West Iberian Margin. Samples were collected at 1 km water depth and supplied with long-chain aliphatic hydrocarbons. Because of their nature such hydrocarbons will hardly dissolve at ambient temperature and pressure, rather become emulsified and be dispersed as small droplets in the water column. Hence, their chances to reach the seafloor are higher with respect to gaseous or liquid short-chained ones and their supply will determine a more realistic piezophile-enriched microbial community. Deep-sea samples were incubated in the laboratory using either icosane (C20) or triacontane (C30) as unique carbon source, at 3 hydrostatic pressures (HP) (1, 100 or 200 bars). The latter were selected to separate piezotolerant bacteria (growing well at both 1 and 100 bars) from piezophilic ones (growing better at 100 and 200 bars). Reactors were inoculated with the same microbial community collected at deep-sea and 9 consecutive incubations of 10 d each were conducted. At the end of any incubation, aliquots of growing bacterial cells were transferred to a new reactor (final enrichment, 90 d). An effective microbial community shift was observed with both C20 and C30 (by DGGE and Illumina sequencing) being high HP a main driver for the final community structure. Both C20 and C30 were degraded, for pH values dropped constantly along the enrichment, values being $1 < 100 < 200$ bars. Despite O_2 respiration was always very high, SO_4^{2-} concentration was not significantly lower than controls, meaning that reactors were maintained under aerobic conditions. A rather constant cell number increase during the enrichment was obtained with C20 ($1 > 100 > 200$ bars), while a profile it was less clear when using C30. In all reactors, we could observe cells smaller than $1.5 \mu m$. Hydrophobicity, *i.e.*, the capability by the cells to move towards oil droplets, was generally high with C20 and extremely low with C30. Water-soluble traces of C20 or C30 were detected (by GC-FID) in few cases and always at 200 bars using C20. Irrespective of the carbon source, PO_4^{3-} consumption increased as HP increased. Characterization of the lipid content of the enrichments and strain isolation procedures are underway.

Biography

Alberto Scoma obtained his PhD within a joint project between the CNR and Bologna University (Italy) in 2010 while working on the biophotolytic H_2 production achieved with green microalgae. His work led to the first solar-driven H_2 production in these oxygenic microbes. During his first postdoc, he focused on integrated, stand-alone 2nd generation biorefineries working in Italy, Germany, Portugal and Hungary. He published 31 papers in 13 different domains in the field of green- and bio-technology and served as Guest Editor in 3 reputed journals. He is now a postdoc at LabMET, Gent University (Belgium), where he is developing a research vision on marine biotech.

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Analysis of bacterioplankton community by illumina sequencing of 16S rRNA genes during a field-scale bioremediation test in a Tunisian tourist port

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²University of Florence, Italy

Marine legislation requires monitoring programs to assess ecological integrity and marine health status at different spatial and temporal scales. Bacteria are an important component of biota in marine environments where they play a fundamental role in element cycling and functioning of the ecosystems. In consideration of the fast growth rate and the consequently rapid responses, prokaryotic communities are suitable ecosystem component for the ecological quality assessment of the marine environment over very fine spatial and short temporal scales. This study was carried out in the framework of the project MAPMED, a multidisciplinary project aimed at improving the environmental sustainability of tourist ports in the Mediterranean Sea by the optimization, validation and transfer of tools for monitoring and reduction of marine pollution. The present work was directed to define the structure and composition of the bacterioplankton community during a multidisciplinary physico-chemical and ecological monitoring of a field-scale demonstration of (bio)remediation technology in the water compartment at the tourist port of El Kantaoui (Tunisia). A bimonthly monitoring program was implemented over one year. The bacterioplankton communities are currently under characterization by Next Generation Sequencing with the MiSeq platform. The present study will define the seasonal variation as well as the effect of treatments on bacterioplankton communities.

Biography

Elena Tamburini has a PhD in Genetics at the University of Pavia with an experimental work on cellulolytic streptomycetes, carried out at the University of Florence. From 2006, she is Senior Researcher at the Department of Biomedical Sciences at the University of Cagliari. Her main research topics are microbial surfactants and emulsifiers for environmental applications, microbial communities involved in bioremediation and phytoremediation of hydrocarbons and heavy metals. She published 25 articles in international peer-reviewed journals and book chapters.

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Biosurfactants production by bacillus strain from an environmental sample in Egypt

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With increasing environmental awareness and emphasis on a sustainable society in harmony with the global environment, biosurfactants are gaining prominence and have already taken over for a number of important industrial uses. They are produced by living organisms, for examples *Pseudomonas aeruginosa* which produces rhamnolipids, *Candida* (formerly *Torulopsis*) *bombicola*, which produces high yields of sophorolipids from vegetable oils and sugars and *Bacillus subtilis* which produces a lipopeptide called surfactin. The main goal of this work was to optimize biosurfactants production by an environmental Gram positive isolate for large scale production with maximum yield and low cost. After molecular characterization, phylogenetic tree was constructed where it was found to be *B. subtilis*, which close matches to *B. subtilis* subsp. *subtilis* strain CICC 10260. For optimizing its biosurfactants production, sequential statistical design using Plackett-Burman and response surface methodology, was applied where 11 variables were screened. When analyzing the regression coefficients for the 11 variables, pH, glucose, glycerol, yeast extract, ammonium chloride and ammonium nitrate were found to have a positive effect on the biosurfactants production. Ammonium nitrate, pH and glucose were further studied as significant independent variables for Box-Behnken design and their optimal levels were estimated and were found to be 7.328 pH value, 3 g% glucose and 0.21 g% ammonium nitrate yielding high biosurfactants concentration that reduced the surface tension of the culture medium from 72 to 18.16 mN/m. Next, kinetics of cell growth and biosurfactants production by the tested *B. subtilis* isolate, in bioreactor was compared with that of shake flask where the maximum growth and specific growth (μ) in the bioreactor was higher by about 25 and 53%, respectively, than in shake flask experiment, while the biosurfactants production kinetics was almost the same in both shake flask and bioreactor experiments.

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Environmental factors influencing antibiotic resistant bacterial pathogens in polluted lake Manzala, Egypt

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Port-Said University, Egypt

Lake Manzala is considered one of the most important Coastal lakes, in the northern part of Egypt. It suffers from high load of pollutants from different sources such as sewage, industrial and agricultural wastes. In this study, physicochemical characterization of lake water revealed high level of pollution in different sampling sites such as, pH, T.S.S, T.D.S, ammonia, nitrates, sulfates, alkalinity, chlorides, calcium and magnesium. Bacterial count such as TVB and fecal coliform of water and fishes of the lake revealed high contamination in lake Manzala, a total of 90 isolates were identified and resulted in different bacterial pathogens such as, *E. coil*, *Proteus mirabilis*, *Sphomonas paucimobilis*, *Citrobacter freunii*, *Erwinia sp*, *Pasturella sp.* and *Pseudomonas sp*. Antibiogram was done for all isolates using eight antibiotics such as penicillin, ampicillin, cefotaxime, chloramphenicol, rifampicin, tetracycline, streptomycine and gentamicin. The result showed high resistant pattern among different species which are harboring plasmid DNA. This is an indication that these bacterial pathogens have risk factors on the communities around lake Manzala.

Biography

Mahmoud.M.M.Zaky has completed his Ph.D at the age of 36 years from Mansoura University and postdoctoral studies from Port-Said University Faculty of science. He is lecturer and consultant of microbiology ,Botany department, Faculty of science, Port Said University a He has published more than 13 papers in reputed journals and serving as a reviewer of many journals.

Notes:

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Assessing the microbiological and heavy metal burden in some fresh water and marine fishes in a segment of the bight of bonny, Niger delta area of Nigeria

Alfred Y Itah and Favour A Eyo
University of Uyo, Nigeria

The microbiological, physico-chemical and heavy metal burden in fresh water and marine fishes were carried out using standard analytical procedures. Five fin and shell fishes were purchased from fishers at different fishing communities in Akwa Ibom State coastline, Niger Delta Area of Nigeria. Micro-organisms isolated included *Chromobacterium violaceum*, *Salmonella enterica*, *Bacillus subtilis*, *Alcanivorax borkumensis*, *Micrococcus varians*, *Porticoccus hydrocarbonoclasticus*, *Marino bacter*, *Marino monas*, *Cladosporium resinae*, *Penicillium italicum*, *Saccharomyces estuari* and *Candida marina*. The microbiological burdens were $4.9 (\pm 0.02) \times 10^5$ cfu/ml (fresh water), $6.4 (\pm 0.03) \times 10^5$ cfu/ml (marine water), $4.0 (\pm 0.01) \times 10^5$ cfu/g (fresh water sediments) and $5.2 (\pm 0.02) \times 10^5$ cfu/g (marine sediments). Densities of heterotrophs in the fishes ranged from $7.0 (\pm 0.04) \times 10^5$ to $7.8 (\pm 0.03) \times 10^5$ cfu/g (intestine), $6.4 (\pm 0.02) \times 10^5$ to $6.9 (\pm 0.10) \times 10^5$ cfu/g (gills) and $7.1 (\pm 0.04) \times 10^5$ to $7.8 (\pm 0.03) \times 10^5$ cfu/g (skin) from fresh water fin fishes; $5.1 (\pm 0.2) \times 10^5$ to $5.9 (\pm 0.01) \times 10^5$ cfu/g (intestine), $4.5 (\pm 0.04) \times 10^5$ to $4.9 (\pm 0.04) \times 10^5$ cfu/g (gills) and $6.1 (\pm 0.04) \times 10^5$ to $6.9 (\pm 0.02) \times 10^5$ cfu/g (skin) from fresh water shell fishes; $7.5 (\pm 0.02) \times 10^5$ to $8.6 (\pm 0.05) \times 10^5$ cfu/g (intestine), $7.1 (\pm 0.03) \times 10^5$ to $7.9 (\pm 0.04) \times 10^5$ cfu/g (gills), $6.1 (\pm 0.03) \times 10^5$ to $9.8 (\pm 0.5) \times 10^5$ cfu/g (skin) from marine water fin fishes; $5.3 (\pm 0.03) \times 10^5$ to $6.1 (\pm 0.2) \times 10^5$ cfu/g (intestine), $4.1 (\pm 0.04) \times 10^5$ to $4.9 (\pm 0.02) \times 10^5$ cfu/g (gills) and $7.1 (\pm 0.03) \times 10^5$ to $7.9 (\pm 0.05) \times 10^5$ cfu/g (skin) from marine water shell fishes. Comparable trends in heavy metal concentrations were: $FE > Cu > Al > Zn > Ni > Pb = Cd$ (fresh water sediments), $Fe > Al > Ni > Pb > Cd > Cu > Zn$ (marine sediments), $Cu > Fe > Zn > Al > Pb = Ni = Cd$ (fresh water) and $Al > Fe > Ni > Cu > Zn > Pb > Cd$ (marine water). Although densities of hydrocarbonclastic micro-organisms 10^5 cfu/g and above are considered significant, their presences in high numbers in fishes present some ecological advantage in the event of oil spill as they could metabolize and biodegrade the pollutants in fishes for their survival. Shell and fin fishes are promising candidates in bio-monitoring and as pollution indicators.

Biography

Alfred Y Itah did his BSc (Hons.) in Microbiology (1983), Calabar, Nigeria and PhD (1987) at Graduate School Board and Senate of the University of Calabar following his excellent performance in MSc course work examinations. He worked as a Professor of Environmental and Public Health Microbiology (2004); Head, Department of Microbiology (2001-2006). He was elected as Dean, Faculty of Science (2008-2010) and re-elected as Dean (UNOPPOSED, 2010-2012). He is a member of 10 learned societies including the Nigerian Society for Microbiology and American Board of Research Advisors. He has more than 51 scientific publications in reputable national and international journals with high impact factor. He is a Consultant Environmental and Public Health Microbiologist (Since 1998) and Litigation Expert Witness on crude oil pollution matters (Since 2000). He has attended more than 22 scientific conferences and is the Editorial Board Member and Editor-in-Chief to some reputable journals in Nigeria.

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Induction of Shiga toxins in *Escherichia coli* O157:H7 isolated from groundwater in the North West Province, South Africa intended for human consumption using ampicillin and tetracycline

Daphney P Shandukani, Namathamsaqua P Sithebe and Collins N Ateba
North West University – Mafikeng Campus, South Africa

A total of 67 isolates from groundwater were used to determine their susceptibilities against 7 antibiotics and the Multiple Antibiotic Resistance (MAR) patterns were compiled. Most isolates were resistant to amoxycillin, ampicillin, chloramphenicol and penicillin G. MAR phenotype A-AP-K-NE-OT-C-PG was dominant among isolates from Rustenburg. However, in Carltonville and Delaryville the phenotypes A-AP-C-PG and A-AP-OT-PG were obtained at 87.5% and 80%, respectively. The isolates were screened for the presence of shiga toxin genes by PCR analysis and none was positive. Moreover, when the *E. coli* O157:H7 isolates were subjected to antibiotic treatment for the induction of shiga toxins using both ampicillin and tetracycline in broth cultures, no shiga toxins were detected with an ELISA assay after 24 hours of incubation. However, after 72 hours of treatment with these antibiotics shiga toxins were detected in a large proportion (89.6%) of *E. coli* O157:H7 isolates with ampicillin when compared to tetracycline in which only one of the isolates produced shiga toxins. Tetracycline and ampicillin are readily available over the counter and is most often used in animal medicine. The consumption of these antibiotics when suffering from *E. coli* O157:H7 infections may worsen the complications.

Biography

Collins Njie Ateba has completed his PhD from the North West University - South Africa. Collins also received professional training in the Centre for Medical Genetics, Yerevan State University, Yerevan – Armenia in 2006; Department of Microbiology- Tartu University Tartu – Estonia in 2007 and the Lethbridge Research Station – Lethbridge Alberta, Canada in 2014. He is currently an Associate Professor in the Department of Biological Sciences, Microbiology Division, North West University –Mafikeng Campus and is head of the Water, Food Safety and Phage Therapy/Biocontrol Research Laboratory. Collins is actively involved in research training and lecturing at both undergraduate and postgraduate levels. He has been serving as a host mentor for the DST/NRF internship program from 2011 till date. He has published more than 30 papers in reputed journals and serving as an editorial board member of repute. Collins has presented research papers in a number of conferences locally and internationally.

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Biotechnological potential of the microflora associated with the brown alga *Ascophyllum nodosum*

Marjolaine Martin, Renée Martin, Tristan Barbeyron, Daniel Portefelle, Gurvan Michel and Micheline Vandenberg
University of Leige, Belgium

Bacteria associated with algae are underexplored despite their huge biodiversity and the fact that they differ markedly from those living freely in seawater. These bacterial communities are known to represent great potential for the production of diverse bioactive compounds, such as specific glycoside hydrolases, as they interact in multiple complex ways with their host. Furthermore, enzymes from marine bacteria have original properties, like cold-adapted, halotolerant and highly stable, which are constantly searched out by bio-industries. The aim of our study was to identify bacteria, associated with the brown alga *Ascophyllum nodosum*, showing diverse polysaccharolytic activities. To isolate cultivable microorganisms, algal thalli of *Ascophyllum nodosum* were swabbed with sterile cotton tips and marine agar plates were inoculated. Three-hundred isolated bacteria were screened for agarase, kappa- and iota-carrageenase, and sulfatase activities on specific marine media. Thirty-two bacteria with polysaccharolytic activities were isolated and a part of their 16S rDNA (8F-1492R) were amplified and sequenced. Twenty-seven were classified as *Flavobacteria* and five as Gamma proteobacteria. Putative new strains and species of *Zobellia*, *Maribacter*, *Cellulophaga*, *Shewanella*, *Glaciecola*, *Pseudoalteromonas* and *Colwellia* were identified by phylogenetic analysis. All those genera are well-known to colonize algal surface but only some of them are famous to degrade algal polysaccharides (*Zobellia*, *Maribacter*, *Cellulophaga*, and *Pseudoalteromonas*). However, all those novel bacterial strains/species showed multiple and diverse enzymatic activities (agarase, iota- and kappa-carrageenase, cellulase, beta-glucosidase, sulfatase and/or amylase activities). Genomics libraries with their DNA were constructed in *Escherichia coli* and *Bacillus subtilis* and are screened to identify the genes coding for the observed enzymatic activities. Those novel glycoside hydrolases from unknown marine bacteria should have original and innovative properties with great biotechnological potential.

Biography

Marjolaine Martin started her education at Gembloux Agro-Bio Tech (University of Liège) in 2005. She ended her studies in 2010, with a 4 months stay in Bolivia where she realized a part of her master thesis regarding the prevalence of the Bovine Viral Diarrhea Virus in bovine and lama farms. After graduation, she was immediately hired to start a PhD as a research and teaching assistant at the Microbiology and Genomics Unit of her university. In 2012, she realized a 7 months stay at the Biological Station of Roscoff (Brittany, France). There, she worked on the microflora associated with brown and red algae and got to know to work with this new environment. She presented her results at the National Symposium on Applied Biological Sciences (NSABS18 in Ghent (Belgium)), where she received the best oral presentation in the "Human health and Biotechnology" session. She published a review in Applied Microbiology and Biotechnology dealing with the diversity and the biotechnological potential of those communities. A second paper followed quickly concerning the identification and purification of an interesting cold adapted and halotolerant glucoamylase in the prestigious American review of Applied and Environmental Microbiology. She is now, ending her PhD while working on cultivable microorganisms associated with the brown alga *Ascophyllum nodosum* and their biotechnological potential.

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Cellulosomic and proteomic analyses of *Clostridium clariflavum*

Lior Artzi

Weizmann Institute of Science, Israel

Clostridium clariflavum is an anaerobic, cellulosome-forming thermophile, containing in its genome, a large number of cellulosomal enzymes and a complex scaffolding system. The major cohesin-dockerin interactions of the cellulosome components were characterized, and on this basis a model of diverse cellulosome assemblies was derived. Further on, we cultivated *C. clariflavum* on cellobiose-, microcrystalline cellulose- and switchgrass-containing media, and isolated cell-free cellulosome complexes from each culture. Gel-filtration separation of the cellulosome samples revealed two major fractions, which were analyzed by label-free LC-MS/MS in order to identify the key players of the cellulosome assemblies therein. From the 13 scaffoldins present in *C. clariflavum* genome, 11 were identified, and a variety of enzymes from different glycoside hydrolase and carbohydrate esterase families were identified, including glycoside hydrolase families GH48, GH9, GH5, GH30, GH11 and GH10. The expression level of the cellulosomal proteins varied as a function of the carbon source used for cultivation of the bacterium. In addition, the catalytic activity of each cellulosome was examined on different cellulosic substrates, xylan and switchgrass. The cellulosome isolated from the microcrystalline cellulose-containing medium was the most active of all the cellulosomes that were tested. The results suggest that the expression of the cellulosome proteins is regulated by the type of substrate in the growth medium. Moreover, both cell-free and cell-bound cellulosome complexes were produced which can together degrade the substrate in a synergistic manner. These observations are compatible with our proposed model of cellulosome assemblies based on in-vitro cohesin-dockerin interactions studies in this bacterium.

Biography

Lior Artzi is a direct-track PhD student at the Weizmann Institute of Science in Rehovot Israel. Her work focuses on the Gram-positive, cellulolytic, thermophilic bacterium, *Clostridium clariflavum*, which produces the most intricate cellulosomal system yet described. This year, she has presented an invited lecture at an international conference in the Dead Sea and is scheduled to present her work at a Gordon Research Conference and at International Congress and Expo on Biofuels & Bioenergy this summer.

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Comparison of myxobacterial diversity in sand from Kiritimati Island and German compost

Kathrin I Mohr

Helmholtz Centre for Infection Research, Germany

Myxobacteria harbor an enormous potential for new bioactive secondary metabolites and are at the focus of natural product research in our group since more than 30 years. Within this time more than 100 new substances and about 600 derivatives have been isolated from these fascinating bacteria. New groups of *myxobacteria* turned out to be particularly promising candidates for the discovery of unknown metabolites. Therefore the isolation of hitherto undescribed *myxobacteria* is of high importance. To examine our cultivation success with extended standard methods, the diversity of *myxobacteria* present in sand from Kiritimati Island and German compost was evaluated by both cultivation-based and -independent methods. Phylogenetic analyses of cultured and uncultured 16S rRNA gene sequences revealed a big potential of undescribed *myxobacteria* in both sampling sites which were detected by clone bank analyses but not by cultivation. A total of 79 *myxobacteria*-related sequences were identified from clones of the libraries from these two samples which grouped into 12 operational taxonomic units (OTUs) on basis of 99 % sequence similarity. Cultivation of exclusively bacteriolytic *myxobacteria* revealed 42 strains from the genera *Myxococcus*, *Coralloccoccus*, *Archangium*, and *Polyangium*, whereby the genera *Myxococcus* and *Coralloccoccus* were represented by both approaches. But even in this well studied genera, as well as in the suborders *Sorangiiineae* and *Nannocystineae*, a considerable number of clones were assigned to, if any, uncultivated organisms. However, high deficits are demonstrated in the cultivation of the remaining myxobacterial diversity. Especially clades which are exclusively represented by clones are of high interest with regard the cultivation of new bioactive secondary metabolite-producers.

Biography

Kathrin I Mohr studied biology at the TU Braunschweig. During her Postdoctoral time she investigated the "Biodiversity of algae and cyanobacteria in calcifying biofilms" and "In soil crusts from Namibia and South Africa" at the University of Göttingen. Since 2009 she is working as a Scientist at the Helmholtz Centre for Infection Research, department Microbial Drugs, Braunschweig. Her main focus is set on the isolation of *myxobacteria* and their screen and enhancement of production of new and known secondary metabolites. She is author and co-author of about 30 papers in reputed journals.

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Targeted microbial diversity in cyclic low energy ammonium removal system determined by 454 pyrosequencing and quantitative PCR

Hui-Ping Chuang¹, Ben van den Akker², Irina Mouilleron³, Mike Burch², Liang-Ming Whang¹ and Tsair-Fuh Lin¹

¹National Cheng Kung University, Taiwan

²South Australian Water Corporation, Australia

³Allwater Alliance, Australia

Cyclic Low Energy Ammonium Removal (Cleargreen) System is one type of the energy-saving de-ammonification system. A pilot scale Cleargreen system, with a feeding rate of 6.9 m³/day effluent water from a secondary wastewater treatment, has been tested in South Australia for 2 years. During a 450-day operation, the system gave a high ammonia removal of 80-85% with nitrite up to 150 mg N/L in the effluent. During the operation, 30 samples were taken for analysis of 454 pyrosequencing and quantitative PCR (qPCR). Analysis of 454 pyrosequencing showed that four nitrogen-related groups, family *Nitrosomonadaceae*, *Nitrospiraceae*, *Brocadiaceae* and *Rhodobacteraceae*, were detected, although all of them were below 4% of total bacterial population. Furthermore, the decrease of *Comamonadaceae* population was observed with the increasing of NH₄⁺-N removal; while *Brocadiaceae* population was found to increase as NH₄⁺-N removal was higher than 50%. qPCR results indicated that *Nitrospira* and other denitrifying groups containing *nirS* gene dominated in the system with the abundance of 5×10¹⁰ cell/mg VSS. Two ammonia-oxidizing bacteria and archaea groups, *Nitrobacter* and anammox were detected at 10³ - 10⁶ cell/mg VSS. Ammonia-oxidizing archaea increased with increasing NH₄⁺-N removal when NH₄⁺-N oxidation ratio was below 50%, while anammox bacteria group positively related with the removal of ammonia and total nitrogen removal. For microbial diversity of targeted nitrogen-related microbes, results from both 454 pyrosequencing and qPCR methods indicated that anammox bacteria could be enriched in the Cleargreen system, accompanying with the vicissitudes of other nitrogen-related microbes.

Biography

Hui-Ping Chuang has completed her PhD from Nagaoka University of Technology, Japan, and Postdoctoral studies from Hiroshima University, Japan and National Cheng Kung University, Taiwan. She is a Postdoctoral fellow in the Global Water Quality Research Center, NCKU, Taiwan. She has published 8 papers in reputed journals, and is specialized in the fields of biological treatment systems and improvement & application of molecular tools.

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Microbiology, biochemical changes and biogas production during composition of oil palm empty fruit bunch

Alfred Y Itah and Chinyere C Anagoba
University of Uyo, Nigeria

The microbiological, biochemical changes and biogas production during composition of Oil Palm Empty Fruit Bunch (OPEFB) for 42 weeks were studied using standard analytical procedures. The nitrogen, phosphorus, potassium, carbon-nitrogen ratio, heavy metals and proximate composition were also assayed. The results revealed abundance and heterogeneity in genera and species of heterotrophic bacteria and fungi which included *Micrococcus luteus*, *Klebsiella aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Absidia repes*, *Aspergillus niger*, *Aspergillus glaucus*, *Fusarium oxysporium*, *Mucor haemalis*, *Helminthosporium sativum*, *Saccharomyces uvarum* and *Candida pseudotropicalis*. Mean aerobic and anaerobic bacterial densities ranged from 2.9×10^5 to 5.0×10^5 cfu/g and 2.7×10^5 to 4.7×10^5 cfu/g respectively while fungal densities ranged from 3.3×10^5 to 7.4×10^5 cfu/g. Successional studies revealed primary colonizers of the compost comprised both bacteria (29.6%) and fungi (66.7%) with a pH range of 7.8 to 8.5. The results also revealed high levels of heavy metals ranging from 8.78 to 0.19 mg/I for iron, 4.80 to 0.48 mg/I for sodium, 2.79 to 0.08 mg/I for calcium, 2.53 to 0.40 mg/I for zinc, 2.41 to 0.07 mg/I for cadmium, 2.23 to 0.20 mg/I for lead and 1.89 to 0.22 mg/I for copper. The high level of Nitrogen, Phosphorous and Potassium (NPK) ranged from 1.62 to 0.10 mg/I, 11.33 to 0.17 mg/I and 8.66 to 0.11 mg/l respectively while proximate compositional studies showed varying levels of carbohydrate (76.53% to 23.94%), protein (10.15% to 0.68%), lipids (0.54% to 0.48%), ash (8.00% to 90%), fiber (4.78% to 31.00%), moisture (63.00% to 63.55%) and organic matter (92.00% to 56.10%) respectively, with a positive correlation ($p < 0.05$) in ash and fiber content over time. Anaerobic digestion of 2,750g of the OPEFB yielded biogas in the range of 0.035 m³ to 0.035 m³. The results underscore the use of OPEFB as organic fertilizer and suggest additional value as a good source of renewable energy rather than waste in developing countries.

Biography

Alfred Y Itah did his BSc (Hons.) in Microbiology (1983), Calabar, Nigeria and PhD (1987) at Graduate School Board and Senate of the University of Calabar following his excellent performance in MSc course work examinations. He worked as a Professor of Environmental and Public Health Microbiology (2004); Head, Department of Microbiology (2001-2006). He was elected as Dean, Faculty of Science (2008-2010) and re-elected as Dean (UNOPPOSED, 2010-2012). He is a member of 10 learned societies including the Nigerian Society for Microbiology and American Board of Research Advisors. He has more than 51 scientific publications in reputable national and international journals with high impact factor. He is a Consultant Environmental and Public Health Microbiologist (Since 1998) and Litigation Expert Witness on crude oil pollution matters (Since 2000). He has attended more than 22 scientific conferences and is the Editorial Board Member and Editor-in-Chief to some reputable journals in Nigeria.

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Evaluation of mucuna beans flour fermented with *Lactobacillus plantarum* as a probiotic food

Anthony Okhonlaye Ojokoh¹ and Adedayo Michael Oyetayo²

¹Federal University of Technology, Nigeria

²Rufus Giwa Polytechnic, Nigeria

Mucuna beans flour fermented with *Lactobacillus plantarum* was evaluated *in vitro* and *in vivo* for probiotic activities in this investigation. *L. plantarum* used were isolated from 'ogi' made from sorghum thereafter, it was screened for growth and survival in the mucuna beans flour. At the end of 72 hour fermentation at 37°C, the *L. plantarum* showed appreciable growth (8.83×10^6 cfu/g). After storage for 14 days at refrigeration ($4 \pm 20^\circ\text{C}$) and room temperature ($25 \pm 20^\circ\text{C}$), there was a considerable increase in the *Lactobacillus* found in the products stored at room temperature (13.67×10^6 cfu/g) compared to the one stored at refrigeration temperature (8.47×10^5 cfu/g). There was a steady increase in the total titratable acidity and temperature with concomitant reduction in the pH of samples during the fermentation period. The proximate analysis showed that there was an increase in the protein and moisture contents with decrease in carbohydrates, fats, fibre and ash contents of the fermented samples compared to the unfermented sample. Under varying pH range, *L. plantarum* showed high growth and survival at pH 2 to 3. Supplementing the diet of albino rats infected with *E. coli* and *Shigella dysenteriae* with fermented products reduces significantly ($p \leq 0.05$) the numbers of these pathogens and other enteric bacteria while the number of the *Lactobacilli* increased considerably. Furthermore, the body weight of the rats fed the fermented product was significantly ($p \leq 0.05$) higher than the control group. Also, the haematological analysis showed that the rats infected with the pathogens and later fed with the fermented mucuna beans flour recovered fully since their values were well within the permissible limit and were not significantly ($p \leq 0.05$) different from the control group. In all, the rats fed with the product fermented with *L. plantarum* showed good recovery compared to the control. Conclusively, these results suggest that mucuna beans flour fermented with *L. plantarum* could be used as an ideal probiotic food.

Biography

A.O. Ojokoh is an Associate Professor in the department of Microbiology of the Federal University of Technology, Akure, Nigeria. His current research interests include Food Microbiology and Extrusion and Fermentation Technology. He has published several papers in learned journals and academic conferences. He has visited the Institute of Food Processing, Chinese Academy of Agricultural Sciences, Beijing, China a number of times for research. He has been involved in several important research projects. He is a Member of The Society of Industrial Microbiology (SIM), USA, Nigerian Society of Microbiology (NSM), Biotechnology Society of Nigeria (BSN) and Association of Industrial Microbiologists of Nigeria (AIMN). He has supervised several Masters and Ph.D theses in Food and Industrial Microbiology.

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Aspergillus section *Flavi*, morphologic, clinical aspects, and extrolite production

Parvin Dehghan

Isfahan University of Medical Sciences, Iran

Despite intensive investigation, the taxonomy of *Aspergillus* is still highly complex. Recent data indicate that several of the species of this genus cannot be distinguished based on morphological or molecular methods, alone. *Aspergillus* section *Flavi* includes more than 25 species and this number is likely to increase significantly in the near future because of increasing application of the phylogenetic species concept based on DNA sequence data rather than on visible morphological characters. *A. oryzae* and *A. sojae* species have been used for centuries to make traditional foods and are generally regarded as safe. The data support the concept that they are derived (domesticated) from the naturally occurring *A. flavus* and *A. parasiticus* through adaptation in food industry fermentation. These two latter species produce the potent carcinogen aflatoxin and show many phenotypic similarities with the non-aflatoxigenic species. The source of *A. parasiticus* is soil and it has not been isolated from infections. *A. flavus* is the major agent responsible for fungal sinusitis, keratitis and onychomycosis in tropical and subtropical areas and surpasses *A. fumigatus* (belongs to section *Fumigati*) a common etiologic agent of aspergillosis. The genomic size of *A. flavus* is bigger than the *A. fumigatus* and that is believed the latter has lost some parts of its genome during the lifetimes. Identification of the organisms are more complex and a combination techniques including morphological characterization, physiological behaviors and molecular methods or an ITS based sequencing strategy are necessary to identify them.

Biography

Parvin Dehghan has completed her MS and the PhD in Medical mycology from Tehran University of Medical Sciences, in Iran. She is the Director of Mycology & Parasitology Department in Isfahan University of Medical Sciences. She has published more than 18 papers in reputed journals in English and Persian.

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Antitumor metabolites from *Streptomyces* sp. KML-2 isolated from Khewra salt mines, Pakistan

Usman Aftab¹, Zafar Qureshi², David L Zechel³ and Imran Sajid¹

¹University of the Punjab, Pakistan

²Veterinary Research Institute, Pakistan

³Queens University, Canada

A rare bioactive *Streptomyces* strain designated as *Streptomyces* sp. KLM-2 was isolated from the Khewra salt mines, (Punjab) Pakistan. On the basis of morphological, microscopic, biochemical and physiological, characterization and by 16S rRNA gene sequencing the isolate was identified as a close member of *Streptomyces griseus* (100% similarity with *S. griseus*, Gene Bank Accession No. NR-074787). In preliminary screening, the crude extract obtained from the culture broth of this strain showed high cytotoxic activity against larvae of *Artemia salinaria* and exhibited 84% larval mortality. The same cytotoxic/antitumor behavior was observed when the crude extract was screened against three cell lines by MTT assay. The isolate exhibited significant growth inhibition of the proliferating tumorous cells with the IC₅₀ values of 12.17 µg/ml, 47.88 µg/ml and 56.12 µg/ml against HeLa, MD-BK and Vero cell lines, respectively. Based on the potent cytotoxic and antitumor activities the isolate was investigated by cultivation upto 20 liters, and subsequent solvent extraction, through an efficient Diaion HP-20 bead extraction technique and purification of the metabolites by manual column chromatography. The preparative screening yielded two pure compounds including Chromomycin SA and 1-(1H-indol-3-yl)propane- 1,2,3-triol. The results indicate that the isolate *Streptomyces* sp. KLM2 is a potent producer of the antitumor metabolites and can be exploited for the commercial production of these compounds. Further, the Khewra salt mines are a unique and untapped ecological niche and the screening of diverse microbial strains from this source can yield highly useful antitumor compounds.

Biography

Imran Sajid completed his PhD in 2009 from University of the Punjab, Lahore, Pakistan. He has worked as guest scientist at the Institute of Organic and Biomolecular Chemistry University of Göttingen, Germany and at the Department of Chemistry University of Turku, Finland. Currently he is working as Assistant Professor, at the department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan. He is working with actinomycetes diversity of Pakistan for bioactive natural products discovery and has published about 20 research papers in reputed journals.

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Effect of Equine Herpesvirus type 1 (EHV-1) infection on different components of the extracellular matrix of nasal mucosa epithelial cells

Hossein Bannazadeh Baghi

¹Tabriz University of Medical Sciences, Iran

²Ghent University, Belgium

The mucosal surfaces are important sites of entry for a majority of microorganism, and viruses in particular. Equine Herpesvirus type 1 (EHV-1) is an example of an invasive virus of the airway mucosa. An essential prerequisite for an effective host attack of the virus is to breach the epithelial cell layer and the underlying Basement Membrane (BM) barrier. In our research, nasal mucosa explants were inoculated with EHV-1 and then double immunofluorescence staining was performed to detect viral antigen positive cells as well as integrin alpha 6, laminin, collagen IV and collagen VII. The breadth of these extracellular matrix proteins was measured in Regions Of Interest (ROI) at a magnification of 200X. ROI were defined beneath non-infected and infected regions. In infected regions, the percentage of ROI were significantly decreased for integrin alpha 6 after 24 hours and 48 hours of inoculation. However, infection did not alter the percentages for laminin and collagen IV. For collagen VII, an increase in the percentage could be observed underneath EHV-1-infected plaques only at 48 hours of inoculation. In conclusion, the results revealed a substantial impact of EHV-1 infection on integrin alpha 6 and collagen VII, two important components of the extracellular matrix, which are normally associated with the basement membrane and may play a role in virus penetration to underlying tissues.

Biography

Hossein Bannazadeh Baghi is the holder of a PhD awarded to him by the Department of Virology, Parasitology and Immunology at Ghent University, Belgium. He completed his BSc and his MSc with honors and received National Awards in his home country, Iran, for being the top student in the field of Virology. Currently, he is working as an Assistant Professor in the Department of Microbiology at Tabriz University of Medical Sciences, Tabriz, Iran.

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Lactococcus lactis: A Bi-Functional Starter-Probiotic

Fatemeh Nejati¹ and Tobias A Oelschlaeger²

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Works on discovery of novel probiotic candidates are under running. Commercially, having access to a probiotic that already has industrial functionality in addition to good viability during of food processing and shelf life of product is always advantages. Few studies have been reported regarding probiotic properties of *Streptococcus thermophilus* strains although this species hugely used as starter culture in the production of yogurt and other dairy products. In this study, 12 isolates of *S. thermophilus*, that were previously isolated from home-made dairy products, were evaluated with regard to resistance to artificial gastric (pH 2.5 containing pepsin) and intestinal (pH 8.0 containing bile and pancreatin) juices, adherence ability to Caco-2 and HT29-MTX-E12 cell lines, hydrophobicity, resistance to antibiotics, and epithelial barrier function (transepithelial electrical resistance (TER) measurement). Although it has been generally assumed that *S. thermophilus* strains are not resistant to stresses induces in the GIT, the results of this study revealed that susceptibility of almost all of the tested strains to simulated gastric and intestine conditions was significantly lower than for probiotic control strain *L. rhamnosus* GG under both simulated gastric and intestinal conditions. Regarding to adherence efficiency to human gut epithelium cell lines, the results showed 7 and 6 out of 12 isolates exhibited significantly superior adherence to Caco-2 and HT29-MTX-E12 than control probiotic *L. rhamnosus* GG, respectively. TER measurement showed that 3 strains were able to protect Caco-2's tight junction. Although further investigations are necessary, our results identified some of the *S. thermophilus* strains as probiotic candidates worth further analysis.

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New challenge: Reduction of aflatoxin M1 residues in cow s milk by MilBond Dietary Hydrated Sodium Calcium Aluminosilicate (HSCAS) and its effect on milk composition

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This study was aimed to evaluate the effect of Milbond (HSCAS) on aflatoxin M1 in artificially contaminated cow's milk. Chemisorption compounds used in this experiment were MilBond, hydrated sodium calcium aluminosilicate (HSCAS). Raw cow milk were artificially exposed to aflatoxin M1 in a concentration of 100 ppb) with addition of Nilbond at 0.5, 1, 2 and 3 % at room temperature for 30 minutes. Aflatoxin M1 was decreased more than 95% by HSCAS at 2%. Milk composition consist of protein, fat, lactose, solid non fat and total solid were affected by addition of some adsorbents were not significantly affected (p 0.05). Tthis method did not involve degrading the toxin, milk may be free from toxin degradation products and is safe for consumption. In addition, the added material may be easily separated from milk after the substance adsorbs the toxin. Thus, this method should be developed by further researches for determining effects of these compounds on functional properties of milk. The ability of hydrated sodium calcium aluminosilicate to prevent or reduce the level of aflatoxin MI residues in milk is critically needed. This finding has important implications, because milk is ultimately consumed by humans and animals, and the reduction of aflatoxin contamination in the milk could have an important impact on their health.

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Methyltetrahydrofolate reductase and its relationship with vitamin B-12 and *Helicobacter pylori* infection

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Background: Genetic polymorphisms of Methyltetrahydrofolate reductase (MTHFR) have significant roles in developing diseases including *Helicobacter pylori* infection. This association may be mediated through vitamin B-12 deficiency. The aim of this study is to determine any relationship between (c.677C>T) mutation of MTHFR gene, vitamin B-12 deficiency and *H. pylori* infection among thrombophilic patients.

Methods: A cross sectional study was designed for 130 patients with pulmonary embolism (PE), deep venous thrombosis (DVT) and recurrent abortion from AL Hussein medical city (Amman, Jordan). Laboratory investigations were carried out for vitamin B-12 measurement, *H. pylori* infection (IgG and IgA) and MTHFR (c.677C>T) gene polymorphisms.

Results: This study showed that the frequency of vitamin B-12 deficiency among thrombophilic patients was 15%, 81% were chronically infected, while 38% were acutely infected with *H. pylori*. The frequency of MTHFR (c.677C>T) gene polymorphisms: wild type 41%, homozygous 14% and heterozygous 45%. There is a significant relationship between *H. pylori* chronic infection and MTHFR (c.677C>T) gene polymorphism among wild type, homozygous and heterozygous patients. All thrombophilic patients with homozygous MTHFR (c.677C >T) were chronically infected with *H. pylori*. No statistical significant relationship between MTHFR (c.677C>T) gene polymorphism and vitamin B-12 level and no statistical significant relationship was observed between the concentration of vitamin B-12 and *H. pylori* infection.

Conclusion: A significant relationship between chronic infection with *H. pylori* and MTHFR (c.677C>T) gene polymorphism. All thrombophilic patients with homozygous MTHFR (c.677C >T) were chronically infected with *H. pylori*.

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New way to develop mixture of lacticleavens and powder of cardoon flowers (*Cynara cardunculus*) to produce yoghurt: Approach to immobilization

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The principal objective of this study is to develop a combination between lactic leavens (*Lactobacillus thermophilus*) and the powder of cardoon flowers (*Cynara cardunculus*) and their application on yoghurt. The coagulation of milk was optimized by using the two coagulant agents (with a fresh and immobilized state). The results obtained reveal that a quantity of 0.3 g of the powder of cardoon flowers has a speed of very interesting coagulation (2.55 min) in comparison with the use of the mixture optimized M2 (75% of powder of cardoon flowers and lactic leavens 25%) and the optimized quantity of the leavens (0.1 g) with respectively speeds of coagulation (3.6 min and 22.58 min). The immobilization of the various coagulant agents' improves the speed of milk coagulation. Indeed, a quantity of 6 g of the beads prepared from the powder of cardoon flowers shows a very fast speed (1.06 min) in comparison with the same quantity of the beads prepared starting from the mixture M2 (3.71 min) and the immobilized leavens (73 min). The beads prepared starting from the powder of cardoon flowers and the mixture M2 can completely substitute the immobilized lactic leavens according to the matrix of similarity (similarity of 70%). Moreover, the beads containing the powder of cardoon flowers improve on the one hand, the speed of coagulation of the yoghurt (one hour and 15 min) in comparison with yoghurt prepared at basis of the immobilized leavens (four hours and 30 min) and on the other hand, the rheological properties were ameliorated (smooth structure and the absence of syneresis phenomenon).

Biography

BENAHMED DJILALI adiba has completed his PhD at the age of 35 years from Algeria University of Boumerdes. He is teacher in the University of Mouloud Mammeri of Tizi-Ouzou.

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Bioactivity of olive leaf (*Olea europaea L.*) extracts and its phenolic compounds on a biofilm-forming pathogen

Wern Chern Chai, Heather Rickard and Rietie Venter
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Antibiotic resistance has been increasing drastically over the years despite efforts against unnecessary use of antibiotics. The ability of cells to form drug resistant biofilms, a complex architecture of cells encased in an extracellular polymeric matrix, is one of the many reasons for the failure of antibacterial treatment. A classic example is the opportunistic pathogen *Pseudomonas aeruginosa* which forms biofilms on medical devices and living tissues, which is intrinsically resistant against a wide range of antibiotics. *P. aeruginosa* release virulence factors such as pyocyanin, pyochelin and pyoverdine which contributes to tissue damage. We previously showed that olive leaf extract has anti-microbial activity against Gram-positive microorganisms, including that of MRSA. The important secoiridoids found in olive leaf such as oleuropein, hydroxytyrosol and verbascoside could play a role in the anti-microbial activity of olive leaf extracts. Therefore, this study aimed to examine the effect of olive leaf extracts and its phenolic compounds on planktonic cell growth, biofilm formation and excretion of cellular virulence factors of *P. aeruginosa*. The effect of the extracts and its phenolic compounds on bacterial motility, which is an indication of virulence, was also investigated. Given the growing concerns of antibiotic resistance, it is imperative that new therapies are developed. While the discovery of antibiotics have been considered a wonder of the century; the real wonder are the extraordinary genetic capacities these microorganisms has. Hence this study may suggest that harnessing of plant-derived agent for use as alternative therapy to promote interventions by addressing the crisis of biofilm-induced antibiotic resistance.

Biography

Wern Chern Chai has completed his Bachelor of Pharmacy (Hons) at the University of South Australia. He is currently practicing as a Pharmacist (provisional registration) in Adelaide. This research, submitted to the University of South Australia for the BPharm (Hons) award was supervised by two highly regarded microbiologists, Dr. Heather Rickard and Dr. Rietie Venter. His research interests include clinical and applied microbiology, complementary medicines and its microbiological use, pharmacology and quality use of medicines. Under the provision of a scholarship, he is currently undertaking an external research at the University of South Australia and Royal Adelaide Hospital looking at quality use of medicines in patients on statins (drugs used for management of high cholesterol levels).

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In vitro determination of baicalein and chitosan action on *Candida parapsilosis*, *Candida krusei* and *Trichosporon cutaneum* biofilm

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Biofilms are highly organized matrix-enclosed microbial communities irreversibly attached to a surface. The phenotype and gene transcription of cells in these communities is changed and they are capable to contaminate medical instruments and industrial devices or induce most serious problem – initiation of serious biofilm-associated human infections. The possible solution is to stop the biofilm formation by inhibition of microbial adhesion on the surface of such devices or to eradicate a pre-formed biofilm. In this study, we propose the option of biofilm treatment by natural substances as an alternative solution. The selected natural substances were flavonoid baicalein and polysaccharide chitosan. Polyene antibiotic amphotericin B, in medicine typically used drug often ineffective for biofilm-associated infections treatment, was used as the control. The representatives of opportunistic pathogenic yeasts were *Candida parapsilosis*, *Candida krusei* and *Trichosporon cutaneum*. These yeast strains were cultivated in 96-wells polystyrene microtiter plates and the colonized area of the well bottom was measured using a Cellavista device. We confirmed the insensitivity of amphotericin B in almost all cases, contrary to efficiency of baicalein and chitosan in significant decreasing of the colonized area in the wells. Baicalein exhibits high effectivity especially in reduction of pre-formed biofilm biomass. On the other hand, chitosan is primarily effective in microbial adhesion inhibition. Our results suggest that the application of natural substances could be a promising way for biofilm infections treatment.

Biography

Kvasnicková E is a PhD student at the University of Chemical Technology, Prague, Faculty of Food and Biochemical Technology, Department of Biotechnology. She is an author or co-author of 1 paper in peer-reviewed international journal (impact 0.604), and 6 papers in conference proceedings.

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Expression of human neonatal Fc-receptor (FcRn) in *Escherichia coli*: A novel strategy

Woei Kean Ng, Theam Soon Lim and Ngit Shin Lai
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Neonatal Fc-receptor plays an important role in maintaining the serum half-life of antibodies. This unique function had been explored in various studies in order to improve the pharmacokinetics of human immunoglobulin G (hIgG) in vivo. FcRn is composed of a α -chain which non-covalently associates with a β -chain, named β -2-microglobulin (β 2m). Studies have shown that the α -chain contains several interaction sites to the Fc segment of IgG, while β 2m is important for the proper folding of FcRn. Genetic expression of FcRn has been conducted in many eukaryotic tissues, ranging from mammalian tissue to yeast, and also prokaryotic organism. Study designed by Andersen *et al.* had shown the production of functional FcRn in bacteria. However, protein refolding step is required to ensure the native activity of FcRn. In this study, we have demonstrated a novel expression strategy by using bacterial system, which produces the functional α -chain of FcRn. Expression vector that carries the cDNA of α -chain, was transformed into expression host, Rosetta-Gami 2. The bacterial culture was grown at 22°C for 16 hours after induction in a modify growth medium. The α -chain was expressed as soluble supernatant after sonication and centrifugation. The results of ELISA have indicated the native affinity of the α -chain towards hIgG and also retained its unique pH-dependent binding to the antibody. Our study proposed that the binding of FcRn to IgG may remain active in the absence of its β -chain. Further study will be conducted to confirm this finding.

Biography

Woei Kean Ng currently is a 3rd year PhD student in University Sains Malaysia. His research mainly focuses on the study of FcRn in the application of diagnostic. He has also conducted study on development of diagnostic test to identify the antibiotic resistance bacteria in tertiary hospital.

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Molecular characterization of Extended Spectrum Beta Lactamases (ESBLs) producing clinical isolates of *Klebsiella pneumoniae* among Karachi population, Pakistan

Uzma Nadeem

NED University of Engineering and Technology, Pakistan

Objective: Resistance to antibiotics by Extended Spectrum Beta-lactamases (ESBLs) producing clinically significant bacterial strains has continuously been emerging and is a great threat to therapeutics. SHV and TEM derived ESBLs producing *Enterobacteriaceae* have been reported throughout the world but there is a limited data available for the molecular characterization of these enzymes in Pakistan.

Materials & Methods: A total of 214 clinical samples were collected from Liaquat National Hospital, a tertiary care hospital in Karachi, Pakistan, out of which 125 were males and 89 were females. Sample source included pus, blood, tissue swabs, urine, stool, sputum and wound. Differential identification of clinical bacterial isolates was done using a series of biochemical methods including MR-test, VP-test, Indole test, Citrate test and Motility test. Susceptibility and resistance against cefotaxime and ceftazidime antibiotics for *K. pneumoniae* isolates were detected using AST disk diffusion method and MICs were obtained using agar dilution method. Molecular characterization included plasmid extraction, ESBL screening as recommended in CLSI document, PCR amplification and DNA sequencing.

Results: The most common infection sites constituted were urological (39.23%; n=51) followed by blood infection (21.53%; 28). A total of (60.7%; n=130) isolates were found positive for *K. pneumoniae* by biochemical tests. Prevalence of *K. pneumoniae* was observed more in males (68.4%; n=89) than females (31.6%; n=41). MICs profile by agar dilution method showed that ceftazidime was (62.9%) susceptible and (37.0%) resistant to clinical isolates of *K. pneumoniae*. Susceptibility rate of cefotaxime was 48.8%. The most ineffective antibiotic found was cefpodoxime with a high resistance rate (97.7%). Through ESBL screening, a total of (45.3%; n=59) isolates were determined as ESBL positives which were then subjected to PCR assays with specific *bla*SHV primers. The result of PCR showed (11.8%; n=07) *K. pneumoniae* isolates producing SHV derived ESBL. BLAST of all nucleotide sequences retrieved from DNA sequencing showed a significant identity >90% to *bla*SHV-158 type ESBL.

Conclusion: We have reported the first case study of SHV-derived beta lactamase *bla*SHV-158 (57.1%; n=4) producing *K. pneumoniae* isolates among Karachi population, Pakistan.

Biography

Uzma Nadeem has completed her Masters in Biomedical Engineering from NED University of Engineering and Technology, Karachi, Pakistan. Since May, 2012, she has been working as a Laboratory Engineer in NED University of Engineering and Technology, one of the premier oldest institutions in Pakistan for teaching and producing Engineering graduates.

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