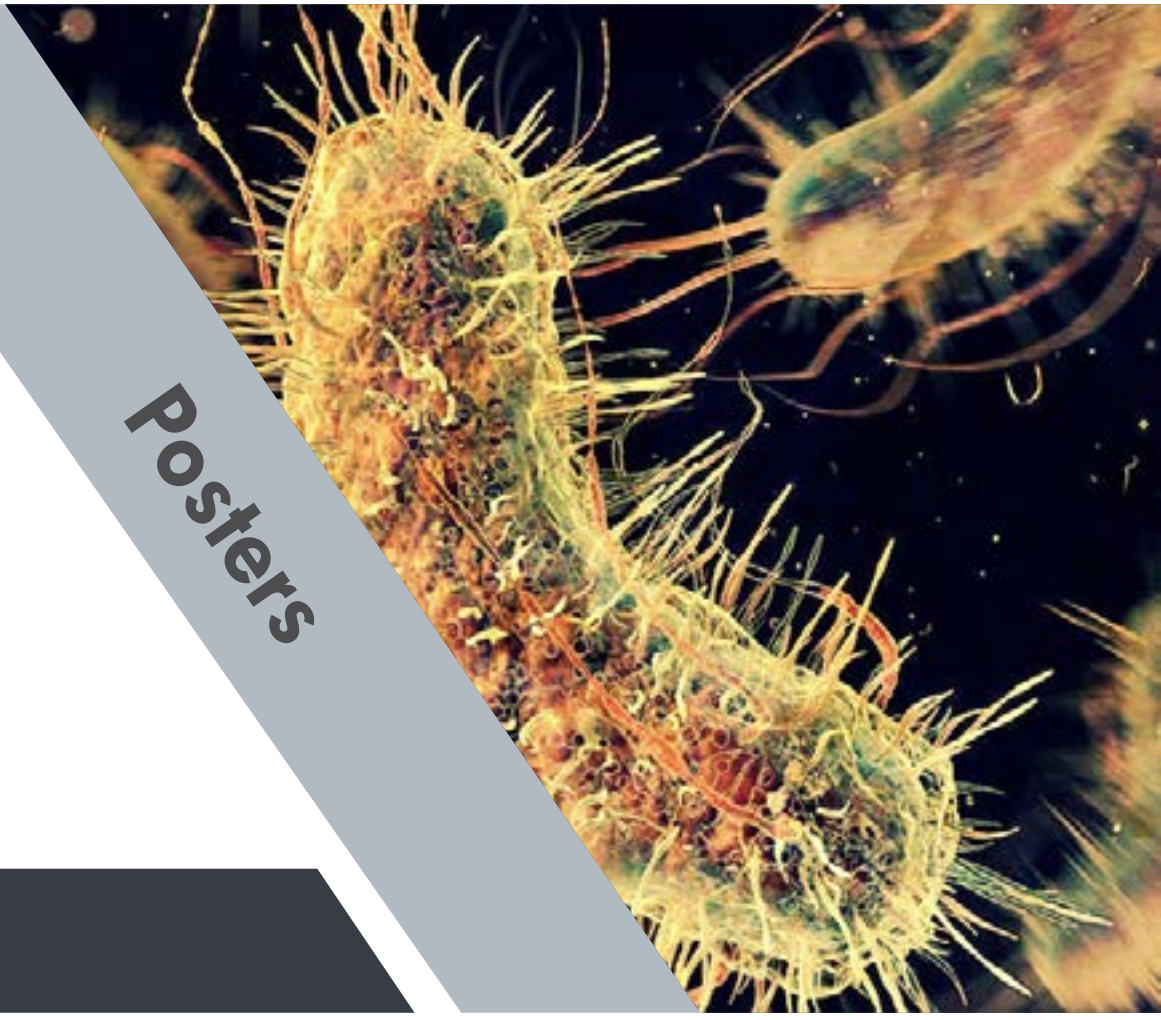
7th World Congress on

MICROBIOLOGY

November 28-29, 2016 Valencia, Spain

Posters






Microbiology 2016

Tamar Shamatava et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Distribution of microbes-antagonists in tomato rhizosphere in Georgia

Tamar Shamatava, Guliko Dvali, Leila Zviadadze and **Naili Lomtadze**
Georgia Technical University, Georgia

Tomato culture has important place among the vegetables in Georgia. A variety of microorganisms is widely distributed in its rhizosphere, including pathogenic agents, the appearance and development of specific types of which largely depends on the climate and soil conditions. Our research goal was to isolate microorganisms in the tomato rhizosphere, to study their antagonistic interaction to choose strong antagonists to be applied against pathogenic microorganisms in the tomato culture. We have studied the tomato rhizosphere microbial flora according to the plant vegetation phases; 120 cultures were isolated, the antagonistic action of these cultures was tested by the diffusion and streak inoculation methods. The result showed that the strong antagonist strains are: N6, N20 and N30, which have inhibited the growth of microbes, by 25-30%, 20-15% and 13-15% respectively. For the identification of cultures we have studied the morphological, physiological and cultural features of antagonist strains. Bacillus sp. strain N6 and Actinomyces strain N20 and strain N30 have been identified. In conclusion, we have isolated a strong antagonist Bacillus sp., strain N6, which has inhibited (25-30%) the growth of microorganisms extracted from tomato rhizosphere.

Biography

Tamar Shamatava is currently a Doctoral student of St. Andrew the First Called Georgian University of the Patriarchate of Georgia. She is the Researcher Scientist at Technical University, Biotechnology Center and Scientific-Research Center. She has published more than 15 papers in reputed journals and has great experiences in Agriculture and Biotechnology sphere.

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Notes:

Yong Bong Kim et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Comparative analysis of *Middle East respiratory syndrome coronavirus* subunit vaccine candidates in mice

Yong Bong Kim, Sehyun Kim, Yeondong Cho, Hee-Jung Lee, Ki Hoon Park and **Hanul Choi**
Konkuk University, South Korea

Since *Middle East respiratory syndrome coronavirus* (MERS-CoV) emerged in 2012, MERS-CoV has spread from Middle East to Europe, America and Asia. South Korea also affected by MERS-CoV and it has been a serious threat to public health. Until today there is no available vaccine for MERS-CoV. Therefore, it is required to develop a vaccine against the MERS-CoV. It is well known that MERS-CoV spike protein (S protein) has an important role in the host cell entrance and that makes many researchers working on the subunit vaccine development using S protein. Especially receptor-binding domain (RBD) at the S protein is usually considered for making recombinant antigen proteins. Jiang et al. reported that RBD is a critical neutralizing domain and the recombinant RBD protein induced strong immune responses and neutralizing antibodies in mouse models. However, other region of MERS-CoV S protein has not been studied well relatively. Here, our group selected several parts of MERS-CoV S protein according to some properties, such as antigenicity and hydrophilicity and then we studied to compare an immunodominance of them. Six partial regions from MERS-CoV S gene were cloned into pASK-IBA7 plus vector. Then they were transformed into *E. coli* Rosetta strain and each clone was produced to soluble recombinant proteins and purified by strep-tag affinity chromatography. Each purified recombinant protein was confirmed by Western blot assay using sera from MERS patient. Immunogenicity of these proteins was characterized through animal experiment.

Biography

Young Bong Kim has completed his PhD from Sogang University in Korea and Postdoctoral studies from NIAID, NIH, USA. He is the Director of Institute of Global Infectious Disease Control at Konkuk University. He has published more than 60 papers in reputed journals.

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Sher Ali, J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Research work on antimicrobial activity of honey against specific microbes

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The study was carried out in Hazara University, Mansehra, Pakistan in 2011 on the antimicrobial activity of honey against specific microbes which include like *E. coli, Salmonella, Staphylococcus aureus, Enterococcus faecalis* and *Candida albican*. During the study the 37 honey samples were collected from different district of Hazara division and Malakand division like Mansehra Swat and Dir were selected for the samples collection. For the samples collection process 170 indoor and outdoor patients were visited. The microorganisms were isolated from the various samples collected from the patients having the confirmed infection which were further processed in the microbiology laboratory by using nutrient agar incubated on 37°C for 24 hours. During the research work agar well plate technique were used to examine the maximum zone of inhibition on Muller Hinton agar against all the specified organisms. Result showed that *E. coli* showed 66 mm, *Salmonella Typhi* 62 mm, *Enterococcus faecalis* 60 mm, *Candida albican* 50 mm and *Staphylococcus aureus* 38 mm. From the study it is concluded that honey is used against different diseases and infections like wound infection, diarrhea, dehydration, paralysis, enterococcus faecalis, chest infection, jaundice, tuberculosis and Urinary tract infections.

Biography

Sher Ali has completed his BSc in Microbiology from the Faculty of Health Sciences, Hazara University, Mansehra, Pakistan in 2011. After completion of his degree, he has joined the SRSP (Sarhad Rural Support Program) as a Health Promotion and Research Officer.

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Notes:

Wittwer Geraldine et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Study of marine actinobacteria diversity from X region de Los Lagos and evaluation of genes PKS/ NRPS as markers of biological activity

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[2]Universidad Tecnica Federico Santa Maria, Chile

Marine bacteria of the phylum actinobacteria have shown to exhibit the ability to produce a wide range of drugs with multiple biological activities such as antimicrobial, antitumor, antiviral, antiparasitic, insecticides and others. These bacteria are widely distributed in marine environments, but its greatest diversity is found in sediments and sponges. PCR primers targeted production of secondary metabolites as Non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) is usual used as molecular markers of metabolic and biotechnological potential screening of these bacteria. In the present study we isolated 2400 strains of sponge and sediment samples, from Region de Los Lagos in Chile. The 16S rRNA gene sequencing showed that nearly 20% of these strains belong to phylum actinobacteria, compound of 22 different genera. The most representative genera found in the samples was *Streptomyces* spp., *Rhodococcus* spp., *Nocardiopsis* sp. and *Arthrobacter* spp. To determine the biotechnological potential of these strains one screening was performed, to detect the presence of PKSI, PKSII, PKSIII and NRPS genes. Result indicates that 80% of the strains had the presence of one of these genes. And about 30% of the strains obtained had genes two or more of the synthetic routes discussed. These results demonstrate that marine actinobacterias have broad potential for finding natural compounds with biological activity and sediment and sponges Los Lagos Region has a wide diversity of marine Actinobacterias.

Biography

Geraldine Wittwer is a Dr(c) in biotechnology of UTFSM and PUCV from Chile. She has 9 years' experience working in enviromental marine Microbiology. Biochemistry from Austral University.

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Notes:

Tine Descamps et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Transposon-mutagenesis based identification of virulence factors of *Paenibacillus larvae*

Tine Descamps, Lina De Smet, Paul De Vos and Dirk C de Graaf
Ghent University, Belgium

Paenibacillus larvae, is an etiological agent of American Foulbrood, a deadly disease of the European Honey Bee (*Apis mellifera*). American Foulbrood is the most important bacterial honey bee disease but relatively little is known of its virulence. In recent years, however, some virulence factors have been identified. In our research, the goal is to identify virulence factors of the pathogen in an unbiased way. This is accomplished by EZ-Tn5 transposon mutagenesis. Using the EZ-Tn5 transposome complex (Epibio), a library of random knock-out transformants was created. The virulence of these transformants was tested using infection assays. Spores were fed to 1st instar honey bee larvae, which were further reared *in vitro*. Of the 158 transformants, only 93 were able to sporulate. Preliminary tests with these 93 transformants rendered 15 with atypical virulence compared with the wild type. These 15 transformants were fully tested using three independent infection groups with 30 larvae each. Statistical test confirmed that 7 transformants had a mortality rate that was significantly lower than the wild type. Identification of the interrupted genes was done using rescue cloning. The genomic DNA was sheered by three restriction enzymes, blunt ended by T4 DNA polymerase and circulized by T4 DNA ligase. Since the transposon carried *R6Kyori* and kanamycin resistance marker, selection of plasmids carrying the transposon could be done by cloning into pir E. coli. In a final step, the regions flanking the transposon were sequenced to identify the knocked out gene.

Biography

Tine Descamps has completed her Master's degree in Biochemistry and Biotechnology in 2013 and is currently pursuing PhD in the Laboratory for Molecular Entomology and Bee Pathology (L-MEB) at Ghent University, Belgium.

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Daniel Oliver Connor, J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Identification of immunogenic proteins of human pathogenic bacteria utilizing phage display

Daniel Oliver Connor
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Recent evolutionary development of antibiotic resistances of many human pathogens leads to an inevitable necessity to discover new ways in diagnostics and treatment of those pathogenic bacteria. Unfortunately, knowledge of suitable antigens and promising protein candidates is not provided for many pathogens. Therefore, the aim of this study was to identify novel immunogenic proteins of three different human pathogenic bacteria: *Borrelia burgdorferi*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Genomic phage display libraries of all three pathogens were constructed with 106-107 individual clones and screened for the identification of immunogenic proteins. Subsequently, corresponding full length proteins were expressed and their immunogenic character verified by ELISA. 21 potentially immunogenic proteins were identified for *N. gonorrhoeae* wherefrom six proteins were described as immunogenic for the first time. The determined immunogenic proteins of *B. burgdorferi* and *N. meningitidis* had been mostly described in literature but could be verified in this work. Additionally, these results showed that the identification of immunogenic proteins utilizing phage display is feasible for human pathogenic bacteria and a fast and straightforward approach. The identified proteins will be further examined for linear epitopes to identify the immunodominant regions. Furthermore, ongoing studies include the development of recombinant antibodies against the identified proteins. These antibodies will then be used for further characterization of the identified immunogenic proteins and to investigate their potential as suitable candidates including diagnostics.

Biography

Daniel Oliver Connor has recently submitted his PhD thesis at the University of Potsdam including two first author peer reviewed publications. He has completed his BSc at the Hochschule Furtwangen University and MSc at the Westfälische Wilhelms-Universität Münster. He has contributed his knowledge and scientific experience at Merckle Biotech, NMI Tübingen and the Fraunhofer IZI-BB (formerly IBMT) in Potsdam.

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Notes:

Ahmed Ali Mohammed, J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Does hydatid cyst fluid from *Echinococcus granulosus* cysts have any effect on cells involved in fibrosis in cystic echinococcosis?

Ahmed Ali Mohammed, Jeremy Allen and **Michael T Rogan**
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Cystic echinococcosis is characterized by the presence of slow growing hydatid cysts, usually in the liver or lungs. Survival of the parasite is based on an interaction of the host immune system and a range of parasite immune-evasive strategies. Fibrosis in the tissues surrounding the cysts can be seen as a host protective response isolating the parasite and restricting its growth or from another perspective fibrosis may be protective for the parasite by providing a barrier to more effective immunological responses. In this study the adenocarcinomic human alveolar basal epithelial cell line (A549) was used as model system. This cell line can be involved in fibrosis as cells can transform into mesenchymal cells and differentiate later to fibroblasts and/or myofibroblasts which can ultimately secrete collagen. Cells were initially cultured *in vitro* in RPMI-1640 medium containing 1-10% hydatid cyst fluid (HCF). The possible effect of the parasite extracts on cell migration was investigated using a wound healing assay. The ability of HCF components to modify cell surface markers of mesenchymal transition was also investigated by fluorescence microscopy. Results showed that there was a dose-dependent increase in cell growth in the presence of cyst fluid after 5 days of culture. The migratory response of cells was also enhanced by the presence of HCF. Both the enhanced growth and migratory activity were still evident when the HCF had been boiled indicating that the components responsible were thermostable. Semi-purified extracts of a major HCF component, antigen B showed a similar high stimulatory effect similar to that of HCF. The fluorescence microscopy showed a significant expression in the fibronectin and E-cadherin cell markers in cells treated with HCF. These results indicate that components within HCF have a stimulatory effect in the possible enhancement of fibrosis.

Biography

Ahmed Ali Mohammed has completed his MSc in Immunoparasitology from the University of Baghdad, College of Science and currently pursuing his PhD in the University of Salford, School of Environment and Life Sciences-Biomedical Research Centre. He has been working as a formal Staff Member (Assistant Professor) in the Branch of Clinical Laboratory Sciences in the College of Pharmacy Al-Mustansiriyah University in Iraq. He was granted a patent in 2002 and has 11 published papers in local Iraqi reputed journals. He has authored two curricular books and one practical guide booklet in Medical Parasitology. He has also served as a Referee in three valued journals one in Iraq and two international journals.

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Lena Danckert, J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Infection-like approach to identify immunogenic proteins of *Salmonella* enteritidis

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Salmonella enteritidis is a human food-borne pathogen and one of the most frequently detected serovars of *Salmonella*. Around 100000 cases of salmonellosis are reported in the European Union annually. After ingestion, the first step of infection is within the small intestine. The pathogen invades the host and leads to an inflammation within the intestinal epithelium from which the intruder may disseminate systemically. Bacteria are fast changing microorganisms with the ability to adapt their metabolism in response to environmental variations. Hence in this study, *Salmonella* enteritidis is examined under infection-like conditions to reveal the RNA, which is a snapshot of the bacterial environment. The aim of this study is to identify new immunogenic and virulence-associated proteins of *Salmonella* enteritidis. Therefore, the bacterial transcriptome is analyzed through RNA sequencing and cDNA library screenings. The infection-like approach is based on the invasion of *Salmonella* enteritidis in the human intestinal CaCo-2 cell line and the subsequent addition of gentamicin. Infection-like conditions may favor the number of immunorelevant proteins during immunoscreenings. That implies an advanced method to identify immunogenic proteins. Moreover, using RNA-seq, virulence-associated factors can be identified by gene expression profiles, thus furthering the understanding of the underlying pathogenicity of *Salmonella*, in general and of *Salmonella* enteritidis, in particular.

Biography

Lena Danckert has completed her MSc at the University of Potsdam and pursuing PhD since in 2013. She is currently working in the Molecular Biology Department of the Fraunhofer Institute for Cell Therapy and Immunology at the branch Bioanalytics and Bioprocesses in Potsdam, Germany.

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Notes:

Jovanka Lukic et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Spent culture supernatant (SCS) from *Lactobacillus sucicola* BGLMM7 inhibits proliferation of tumor cardiac myocytes

Jovanka Lukic, Miroslav Dinic, Giulia Ruozi, Nemanja Mirkovic, Jelena Djokic, Mauro Giacca, Ivana Strahinic and **Jelena Begovic**
University of Belgrade, Serbia

Statement of the Problem: *Lactobacilli* have been shown to reduce proliferation of many cancer cell types, including liver, cervival and intestinal epithelial cells. However, their role in muscle tumor proliferation has not been described yet.

Aim: The aim of this research was to test the effect of spent culture supernatant (SCS) of *Lactobacillus sucicola* BGLMM7 on proliferation of HL-1 cells, which are immortalized mouse tumor cardiac myocytes.

Methodology: Propidium iodide staining was used to analyze cell cycle by flow cytometry, while immunofluorescence was used to visualize the expression of mitotic markers, phospho histone H3 (Ser10) and cyclin dependent kinase 1 (cdk1) as well as Beclin1, which is known as tumor suppressor and its expression is decreased in many tumors. Additionally, level of Cyclin B1 (CCNB1), which is required for mitosis, was tested by Western blot.

Findings: Our results showed arrest of HL-1 cells in G2/M phase of cell cycle after treatment with BGLMM7 SCS for 18 hours. This was followed by a decrease of the expression of phospho H3 (Ser10) and CCNB1 expression and allocation of cdk1 from nucleus to cytoplasm. Additionally, treatment with BGLMM7 SCS increased the number of Becn1-positive dots in cytoplasm.

Conclusion & Significance: This study showed a potential of BGLMM7 SCS to block proliferation of cardiac muscle tumor cells and this effect could potentially be associated with Becn1 induction. Our current research is oriented towards estimation of the effects of BGLMM7 SCS on proliferation of myoblasts using mouse C2C12 cell line, in order to test the selectivity of the extract towards tumor cell lines.

Biography

Jovanka Lukic has completed her PhD from the University of Belgrade, Serbia and continued her Postdoctoral studies in the IMGGE. She is actively involved either as a participant or a leader in international projects with the aim of exploring the effect of probiotics as well as postbiotics on immune response, physiology and pathogen elimination both *in vitro* using cell lines and *in vivo* in fish and in rats as model systems.

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Notes:

Maria Soledad Gutierrez et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Sterols and carotenoids overproduction by expressing the transcriptional activation domain of Sre1 (Sre1N) in the carotenogenic yeast *Xanthophyllomyces dendrorhous*

Maria Soledad Gutierrez, Melissa Gomez, Ana Maria Gonzalez, Carla Garate, Dionisia Sepulveda, Marcelo Baeza, Victor Cifuentes and Jennifer Alcaino
Universidad de Chile, Chile

The yeast Xanthophyllomyces dendrorhous is one of the few known natural sources of the carotenoid astaxanthin. Mutant strains incapable of producing ergosterol, the main sterol in yeasts, overproduce carotenoids and sterols, having increased transcript levels of several genes controlling both pathways. Considering that the synthesis of carotenoids and sterols share the same precursors that derive from the mevalonate pathway, the main goal of this work was to study the mechanism that regulates the biosynthesis of both type of metabolites in *X. dendrorhous*. Sterol Regulatory Element Binding Proteins (SREBPs) are a family of membrane-bound transcription factors that activate the transcription of target genes depending on sterol and oxygen levels. These proteins have been recently identified in fungi and named as Sre1. Under low oxygen or ergosterol levels, Sre1 is proteolytically cleaved by Stp1 releasing the N-terminal activation domain (Sre1N) that activates gene expression at the nucleus. Our recent studies indicate that X. dendrorhous has an orthologous sterol regulated SREBP activation pathway regulating sterol and carotenoid biosynthesis as production of both types of metabolites is affected in sre1 and stp1 mutant strains. In this study, we analyzed the effect of the Sre1N constitutive expression in *X. dendrorhous*. Strains CBS 6938 (wild-type), CBS.sre1- and CBS.SRE1N were included. Strain CBS. SRE1N was obtained by replacing through homologous recombination, the endogenous SRE1 gene by a module designed to express just Sre1N and an antibiotic resistance marker. Strains were cultured with constant agitation in YM medium at 22 °C and samples were taken to extract carotenoids and sterols to evaluate their content (measured at 465 or 280 nm, respectively) and composition (analyzed by RP-HPLC). SRE1N expression increased sterol and carotenoid production, suggesting that Sre1 is responsible for the carotenoid and ergosterol overproducing phenotype in mutants unable to produce ergosterol.

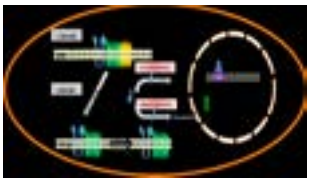

Figure: SREBP Pathway: The Sre1 (in yeast) transcriptional activator is synthesized as an inactive precursor that is bound to the endoplasmic reticulum (ER) membrane through two transmembrane helices.

Biography

Maria Soledad Gutierrez was graduated in Molecular Biotechnology Engineering from University of Chile in 2014, studying the alternative electron donor in P450s systems of the carotenogenic yeast *Xanthophyllomyces dendrorhous*.

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Notes:

Maria Soledad Gutierrez et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
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Sterols and carotenoids overproduction by expressing the transcriptional activation domain of Sre1 (Sre1N) in the carotenogenic yeast *Xanthophyllomyces dendrorhous*

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Biography

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Notes:

Rana Abdulrahim Alaeq et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Bacterial contamination of used manual toothbrushes and effects of toothpastes on isolated potential pathogens

Rana Abdulrahim Alaeq and **Milton Wainwright**
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Toothbrushes play an essential role in oral hygiene and are commonly found in community and hospital settings. The toothbrushes may act as a reservoir for potential pathogens transferred from the oral cavity and from the bathroom environment. The purpose of this study is to determine the bacterial contamination of used toothbrushes and determine the antibacterial effect of toothpastes. Scanning electron microscopy (SEM) was used to visualize biofilms on toothbrush bristles. 50 used toothbrushes obtained from volunteers were analyzed bacteriologically using standard microbiological techniques. Bacteria present on all toothbrushes heads were cultured to determine the presence of bacteria and scanned by SEM. The antibacterial effect of toothpastes was determined using seven types of commercial toothpastes and chlorhexidine toothpaste by inoculation bacteria on the toothpaste plates. The result showed that all the toothbrushes were contaminated with the following bacteria: *Roseomonas mucosa, Stenotrophomonas maltophilia, Pseudomonas aeruginosa, Leclercia adecarboxylata, Enterobacter asburiae, Candidatus Roseomonas massiliae, Pseudomonas parafulva, Bacillus licheniformis, Pseudomonas aeruginosa, Agrobacterium larrymoorei, Pantoea septica, Stenotrophomomnas rhizophila, Citrobacter freundii* and *Pseudomonas frederiksbergensis*. The bristle surfaces, being rough, provided ample sites for trapping organisms. Examination of a brush revealed a biofilm on the brush head. The biofilm seen on the surface of the head to be composed of a compacted mixed community of microorganisms, including cocci, bacilli and filamentous organisms, together with cellular and debris. The toothpaste used proved antibacterial and inhibited bacterial growth, based mainly in the activity of fluoride which is widely used as an effective anticaries agent. In conclusion the isolated organisms are potentially pathogenic, particularly in relation to immunocompromised patients. The appropriate rinsing and drying of the toothbrushes before storage will however, likely reduce the incidence of these bacteria and the health risk associated with these pathogens.

Biography

Rana Abdulrahim Alaeq is currently a PhD student in Department of Molecular Biology and Biotechnology, University of Sheffield, UK, under the supervision by Prof. (Hon. Cardiff) Milton Wainwright. In 2004, he was awarded Master of Microbiology, Faculty of Science in Taibah University and Master of Medical Microbiology, Faculty of Biology, Medicine and Health in University of Manchester in 2013. He has worked as a Teacher Assistant in Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Taibah University and also cooperated in the educational laboratories of the Department of Medical Laboratory Technology like medical microbiology, medical parasitology and medical virology.

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Notes:

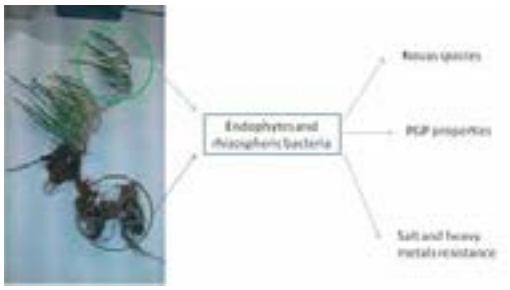
Salvadora Navarro-Torre et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Isolation and characterization of endophytic and rhizospheric bacteria of *Arthrocnemum* in the Odiel marshes and the heavy metals effects in their PGP properties

Salvadora Navarro-Torre, E Mateos-Naranjo, M A Caviedes, E Pajuelo and **I D Rodriguez-Llorente**
Universidad de Sevilla, Spain

Arthrocnemum macrostachyum is a halophyte plant Cd-hyper accumulator and hyper tolerant and it could be used as phytoremediation tool. This plant grows naturally in Odiel marshes, a polluted ecosystem with high levels of heavy metals. For this reason, the aim of this work was study the bacteria associated to this plant and see the effects of heavy metals over them. A total of 48 bacteria were isolated (18 from the rhizosphere and 30 endophytes) and all of them were able to grow in presence of heavy metals and salt. At least one of the PGP properties was present in the isolated bacteria and there were several strains that showed high values of these properties. The heavy metals presence affected to the PGP production by the bacteria both negatively and positively. On the other hand, a high number of isolated bacteria could be novas species. The results of this work suggest that the isolated bacteria could promote the plant growth even in presence of heavy metals and the set of plant and bacteria could be an interesting tool for the phytoremediation.

Recent Publications



1. Navarro-Torre S, Mateos-Naranjo E, Caviedes MA, Pajuelo E, Rodríguez-Llorente ID (2016). Isolation of plant-growth promoting and metal resistant cultivable bacteria from Arthrocnemum macrostachyum in the Odiel marshes with potential use in phytoremediation. Marine Pollution Bulletin, 110:133-142. doi: 10.1016/j.marpolbul.2016.06.070

Biography

Salvadora Navarro-Torre is currently pursuing her PhD in the Department of Microbiology and Parasitology in Faculty of Pharmacy, University of Seville, Spain. Her work is about phytoremediation of heavy metals from polluted soils like the Odiel marshes (Huelva).

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Notes:

Sahar Saad Shar et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Biomineralization of platinum by microorganisms

Sahar Saad Shar, Frank Reith and **Andrew S Ball**
RMIT University, Australia

The biogeochemical cycling and mobility of platinum (Pt) in the environment have only recently been investigated. In particular, to date the interactions of microorganisms with Pt remain largely unknown. Understanding microbial interactions with platinum will lead to greater understanding of the biogeochemical cycling of Pt which will be crucial in determining biochemical pathways of Pt biomineralization. This study aimed to address this gap in our knowledge by assessing the differences in uptake of Pt complexes between the heavy metal-resistant bacteria *Cupriavidus metallidurans* and non-heavy metal resistant bacteria *Escherichia coli*. Sand columns inoculated with the bacteria and containing Pt were used to assess the interactions between bacteria and Pt. Results during the 126 day experiment provide evidence of bacteria playing an important role in platinum biomineralization. Scanning electron microscopy observed the formation of platinum nanoparticles by both bacteria. These platinum nanoparticles were found to be similar to those naturally occurring. This work highlights the potential role of microorganisms in the biogeochemical cycling of Pt.

Biography

Sahar Saad Shar is a PhD candidate in the School of Science, Royal Melbourne Institute of Technology (RMIT University) working under the supervision of Andrew Ball, Director of the RMIT Centre for Environmental Sustainability and Remediation. Her research focuses on the determination of gold and platinum mobility in the environment with a focus on determining the roles of microbes. She holds a Master's degree in Biotechnology from Flinders University in Adelaide. She also holds a Bachelor's degree from Baha University Saudi Arabia.

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November 28-29, 2016 Valencia, Spain

Analysis of triatomine midgut bacterial diversity, insect vectors of Chagas disease by next generation sequencing methods (NGS)

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Chagas disease affects more than 6 million people in Latin America, it is a parasitic disease caused by the protozoan *Trypanosoma cruzi*, which is transmitted mainly by bloodsucking insects of the subfamily Triatominae; the study on microbial communities that inhabit the insect gut is important to understand their role in the parasite transmission and development. The aim of this work was to evaluate the gut bacterial composition of two triatomine species from Colombia, using high-throughput sequencing technologies. The insects were collected from housing peridomestic area at Vichada department and wildlife habitat at Magdalena department then they were identified by conventional taxonomy as: *Triatoma maculata* and *Rhodnius pallescens* and their gut were dissected under aseptic conditions in order to obtain total DNA. After DNA quality confirmation, the sequencing of V4 region from 16S rRNA gene was carried out using the Illumina platform MiSeq. The reads thrown were edited and paired obtaining a size of 250 bp. These sequences were analyzed with the RDP-Classifier software and it showed that 13 bacterial genus are present in both species, being *Burkholderia, Gordonia, Lactococcus* and *Ralstonia*, the most abundant genus. Furthermore, representative genuses of each species were found. Williamsia and Kocuria were the most common in *R. pallescens* and the genus *Curvibacter, Dietzia* and *Pelomonas* were only observed in T. *maculata.* This is the first study of microbiota associated with these triatomine species using massive sequencing methods. Some of the genus found in this research, have been reported in previous studies of other species of Chagas disease insect vectors, which may suggest a close association between microbiota and host.

Biography

Luisa Maria Montoya Porras is a Biological Engineer of the National University of Colombia, studying Master of Science in Biotechnology. She has worked in the research group Microbiodiversity and Bioprospection for four years in microbial diversity of insect crop pests and insect vector of tropical disease. Currently she is a young Researcher with the "call 706" of the Administrative Department of Science, Technology and Innovation of Colombia (COLCIENCIAS) for the second consecutive year with the project: Analysis of triatomine midgut bacterial diversity, insect vectors of Chagas disease by next generation sequencing methods (NGS).
Luisa Maria Montoya Porras is a Biological Engineer of the National University of Colombia, studying Master of Science in Biotechnology. She has worked in the research group Microbiodiversity and Bioprospection for four years in microbial diversity of insect crop pests and insect vector of tropical disease. Currently she is a young Researcher with the "call 706" of the Administrative Department of Science, Technology and Innovation of Colombia (COLCIENCIAS) for the second consecutive year with the project: Analysis of triatomine midgut bacterial diversity, insect vectors of Chagas disease by next generation sequencing methods (NGS).

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Notes:

Karina Paredes Paliz et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Design of a bacterial inoculants for phytoremediation of heavy metals in contaminated estuaries

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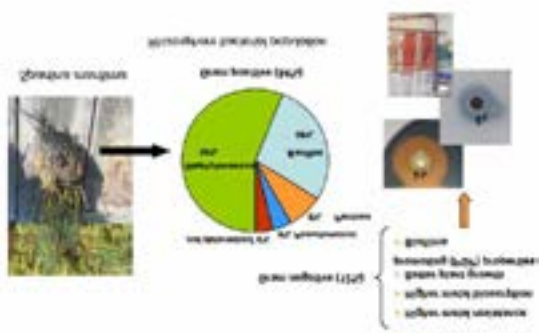
The aim of our work was the isolation and characterization of bacteria from the rhizosphere of *Spartina maritima* in the metal contaminated Odiel estuary (Huelva, SW Spain). From 25 strains, 84% were identified as Gram-positive, particularly *Staphylococcus* and *Bacillus*. Gram-negative bacteria were represented by *Pantoea* and *Salmonella*. Salt and heavy metal tolerance, metal bioabsorption, plant growth promoting (PGP) properties and biofilm formation were investigated in the bacterial collection. Despite the higher abundance of Gram-positive bacteria, Gram-negative isolates displayed higher tolerance toward metalloids (As, Cu, Zn and Pb) and greater metal biosorption, as deduced from ICP-OES and SEM-EDX analyses. Besides, they exhibited better PGP properties, which were retained in the presence of metals and the ability to form biofilms. Gram-negative strains *Pantoea agglomerans* RSO6 and RSO7, together with Gram-positive *Bacillus aryabhattai* RSO25, were selected for a bacterial consortium aimed to inoculate *S. maritima* plants in metal polluted estuaries for phytoremediation purposes.



Biography

Karina Paredes Paliz is a Young Researcher from Ecuador who has completed her degree in Biology. She is currently pursuing her PhD in Molecular Biology and Biomedicine in the Department of Microbiology and Parasitology of the Faculty of Pharmacy, University of Seville, Spain. Her research focuses on environmental biotechnology, specifically the bioremediation of heavy metals with the use of bacteria and plants. Several of her works focus in the phytoremediation of estuaries contaminated near to the River Odiel, province of Huelva-Spain.

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Notes:

Roshan Kumar et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Comparative genomic analysis reveals environment and habitat specific gene diversity within genus *Novosphingobium*

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The genus *Novosphingobium* is comprised of metabolically versatile bacteria within the family *sphingomonadaceae*. *Novosphingobium* species have been isolated from a wide range of ecological habitats and possess diverse physiological and biotechnological importance. In this study genomic attributes, phylogenetic relationships, the pan-genome and core genome content of 27 *Novosphingobium* strains were compared and analyzed. The study revealed a high level of variation in the genome size, coding potential and %GC content, suggesting the role of geographical location in shaping the genome of bacteria as they have different source of isolation. Interestingly, the phylogenetic analysis revealed that the impact of habitat on phylogeny was masked by overall genomic repertoire. The core genome and pan-genome analysis revealed the overall genomic trend of genus *Novosphingobium*. Thereafter, the strains were categorized based on their ecological habitats into four groups i.e., rhizosphere, contaminated soil, marine and freshwater. Out of 27 *Novosphingobium* strains, only 19 strains fall in this category. The habitat-based study revealed different modes of sulfur acquisition and metabolism across the four habitats with the presence of alkane sulfonate (ssuABCD) assimilation pathway in all the rhizospheric isolates. We also find that the genes/pathway for ectoine biosynthesis was present beyond the marine habitat, suggesting its relevance beyond the marine habitat. Further, the phage mediated acquisition appears to play a prominent role in adaptability of the members in their respective habitats. We also find that these *Novosphingobium* stains codes for numerous mono- and dioxygenases, responsible for their ability to metabolize several aromatic compounds. The current study provides the genetic basis for understanding their adaptability into their habitats and their vast potential to degrade a variety of aromatic compounds.

Biography

Roshan Kumar has completed his PhD from University of Delhi, India. His work mostly comprised of comparative genomics, taxonomical studies of strains isolated from stressed niches such as hexachlorocyclohexane contaminated dumpsites. He has published more than 10 papers in reputed journals and has been serving as an Assistant Professor at the University of Delhi, India.

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Martina Janouskova et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Synthesis and degradation of Ms1 in *Mycobacterium smegmatis*

Martina Janouskova, Michaela Sikova, JiriPospisil, Petra Palenikova, Jarmila Hnilicova and **Libor Krasny**
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Adaptation of microorganisms is necessary for their survival in changing environment. In this process, regulatory roles are played by small non-coding RNAs (sRNAs). Ms1 is an abundant sRNA (rivaling in amounts those of rRNA) found in *Mycobacterium smegmatis* and it has homologs in all mycobacteria including *Mycobacterium turberculosis*. Ms1 forms a complex with the RNA polymerase (RNAP) core and it is a pleiotropic regulator of gene expression, enhancing survival of the cell under various types of stress. Ms1 is highly expressed and stable in stationary phase and it is rapidly degraded when the cell is shifted into nutrient-rich medium. The accumulation of Ms1 in the cell depends both on its synthesis and degradation but the specific mechanisms involved are unknown. Here, we identify and characterize the Ms1 promoter, the dynamics of Ms1 expression and reveal the presence of a transcription factor involved in regulation of its expression. Further, we identify an RNase, Polynucleotide phosphorylase (PNPase) to interact with Ms1. With recombinant PNPase, we demonstrate that it is able to degrade Ms1 *in vitro* and identify Ms1 secondary structures that affect its stability. RNAseq data show that PNPase is expressed ~10x more in exponential than in stationary phase, inversely correlating with the accumulation dynamics of Ms1. In summary, we provide a comprehensive characterization of how the intracellular level of Ms1 is controlled, paving the way to potential future designs altering its expression in the case of pathogenic species.

Biography

Martina Janouskova is currently a PhD student of Charles University in Prague. She has recently published her first article. She works in the Department of Microbial Genetics and Gene Expression at the Institute of Microbiology, Czech Academy of Sciences in Prague.

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November 28-29, 2016 Valencia, Spain

Effect of toxicity of monocyclic aromatic hydrocarbons, BTX in insolated strains of Laguna Mecoacan, Paraiso, Tabasco, Mexico

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The monocyclic aromatic hydrocarbons are unsaturated organic compounds formed by one or more planes of covalently bonded carbon, which possess the properties of benzene ring and comprise 30% of fuels like gasoline and diesel rings. In addition, compounds are considered high risk to be carcinogenic in high concentrations and prolonged exposure times. Mecoacan lagoon is an aquifer site of great importance for economic and social development of the municipality of Paraiso, Tabasco in Mexico. This lagoon has been affected since the 70's by the oil industry because of its proximity to the Maritime Terminal "Dos Bocas" which is dedicated to the exploration and production of crude hydrocarbon. The loss of the ecological balance of the lagoon Mecoacan is due to increased industrial activity and the existence of emission sources by incomplete combustion of gasoline and diesel, releasing various concentrations of monocyclic aromatic hydrocarbons to the environment. For this reason, we want to find bacteria that tolerate high concentrations of compounds BTX, exceeding harmful concentrations recorded, in order to be usable in the future as bioremediation strategy, so that an analysis of bacterial tolerance was performed by test agar diffusion. Our results show tolerant strains 22 monocyclic aromatic hydrocarbons; their phenotypic identification shows seven different species belonging to the genera of *Burkholderia, Pseudomonas, Sphingomonas, Rhizobium* and *Vibrio*. These species are of great biotechnological and medical interest because they relate to the presence of xenobiotic compounds, organic matter and are opportunistic pathogens that cause respiratory disease.

Biography

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Notes:

Golbarg Malekhoseini, J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Simultaneous identification of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by duplex PCR assay

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Mycoplasma gallisepticum and *Mycoplasma synoviae* have recognized as common respiratory pathogens especially in chickens causing lots of economical losses in poultry industries. The aim of this study was to develop and validate duplex Polymerase Chain Reaction (PCR) for simultaneous detection of *M. gallisepticum* and *M. synoviae*. A total of 50 samples from tracheas, lungs and air sacs were taken from commercial broiler chicken farms in Iran. The samples were cultured in PPLO broth supplemented for *M. gallisepticum* and *M. synoviae* isolation and bacteria DNA were extracted by phenol/chloroform extraction method. The conserved region of *16S rRNA* gene was applied for the detection of *Mycoplasma* genus in 163 bp fragment and *M. gallisepticum* in 183 bp fragment and vlhA gene was also employed for detection of *M. synoviae* in 350 bp fragment. Hence, duplex PCR amplified the conserved region of *16S rRNA* and *vlhA* genes which were then applied for detection of *M. gallisepticum* and *M. synoviae*. 20 samples in *Mycoplasma* genus and 7 samples in *M. gallisepticum* and *M. synoviae* were positive in the single PCR whereas in 3 samples *M. gallisepticum* and *M. synoviae* were simultaneously positive in the duplex PCR method. The results showed that duplex PCR was successful to simultaneous identification of *M. gallisepticum* and *M. synoviae* and suggested that duplex PCR is more rapid and inexpensive method than the single PCR for detection of *M. gallisepticum* and *M. synoviae*.

Biography

Golbarg Malekhoseini has completed her MSc in 2011 from University of Qom, Pakistan. Currently, she is an Assistant Professor at Islamic Azad University of Arak and also working as a Manager of Quality Control in Ice Factory of Arak.

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Felipe Hernandes Coutinho, J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Marine viruses discovered through metagenomics shed light on viral strategies throughout the ocean

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Marine viruses are key drivers of host diversity, population dynamics and biogeochemical cycling, contributing to the daily flux of billions of tons of organic matter. Yet much of their biodiversity remains uncharacterized, impairing the study of viral communities through metagenomics and consequently our understanding of their interactions with microbes and their environment. By combining data from 78 previously published marine viral metagenomes we obtained a dataset of 27346 marine virome contigs which include 44 complete (circular) genomes. Genome clustering revealed that these are the first members of previously uncharacterized lineages. Furthermore, they outnumber all currently known phage genomes in marine habitats. Computational host prediction was performed including a newly developed and highly accurate method based on co-occurrence associations, revealing that many of the novel viruses infect numerically dominant members of the marine microbiome such as Cyanobacteria and Proteobacteria. A negative association was observed between host abundance and virus-to-host ratio, supporting the recently proposed Piggyback-the-Winner model of reduced phage lysis at higher host densities. Analyzing abundance patterns of both the new and previously known viruses throughout the oceans revealed strategies taken by marine viral communities to adapt between photic/aphotic, warm/cold and winter/summer regimes according to targeted hosts and diversity of auxiliary metabolic genes. Our results chart an important part of the marine viral sequence space and provide new handles to understand the interactions of these viruses with their hosts and the abiotic conditions of their environment.

Biography

Felipe Hernandes Coutinho is currently a PhD candidate at Universidade Federal do Rio de Janeiro, Brazil. He researches on microbial and viral communities from diverse marine habitats with a focus on discovering new taxa, characterizing the factors that shape the composition of the marine microbiome and describing ecological interactions between biological entities and their environment.

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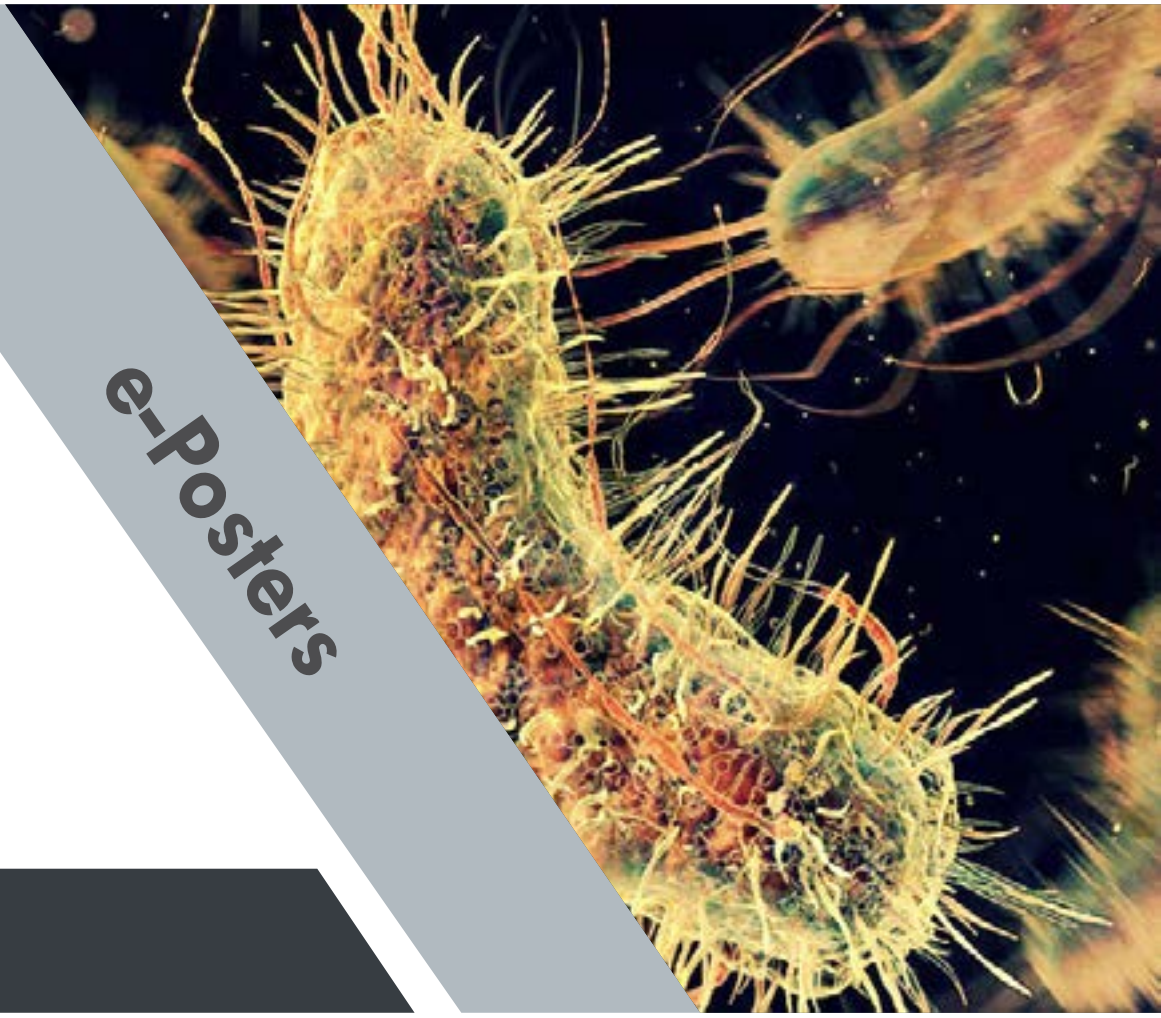




Microbiology 2016

Sahar Sultan Essa et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Immunomodulatory effects of pegylated interferon-α and Ribavirin on Th1 and Th2 cytokine responses to chronically infected hepatitis C patients

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Introduction: *Hepatitis C virus* (HCV) is a life threatening human pathogen. Chronicity of the disease leads to cirrhosis, hepatocellular carcinoma and end-stage liver disease. Clearance of the virus is characterized by a vigorous, persistent T-cell response. Standard treatment involves a combination of pegylated interferon-α (PEG-IFN) and ribavirin, Mechanisms for the observed synergistic effects of the two drugs are still not well understood, but in addition to direct antiviral mechanisms, immunomodulatory effects of both drugs seem to be important with a possible shift from Th2 to Th1 cytokine profiles in successfully treated patients.

Aim & Method: This study will determine Th1 and Th2 cytokine responses to infection with the major HCV genotypes, before, during and after treatment with PEG-IFN and ribavirin. The proliferative response of peripheral blood mononuclear cells to mitogen will be assessed by measuring uptake of radioactive thymidine and the production of Th1 type and Th2 type cytokines by ELISA.

Results: We have inducted a total of 36 patients (27 responders and 9 non-responders). There were a significant increase in the levels of Th1 type cytokines IFNγ, IL-17A and IL-17F between responders and non-responders. IL-4 and IL-6 anti-inflammatory Th2 cytokines is produced at significantly higher levels in non-responders. The ratios involving IFNγ and IL-4 showed interesting differences. At baseline measurement, IFN/IL-4 and IFN/IL-10 ratios were 18 and 10 fold higher in responders. Likewise, at the end of the treatment, IFN/IL-4, IFN/IL-6 and IFN/IL-10 ratios were 90, 80 and 50 fold respectively higher in responders as compared to non-responders.

Conclusion: Our data suggests that, Th1 biased reactivity and poor Th2 response state appears to be associated with drug effectiveness. In other words, we suggest that the host cytokine profile can either dampen or aid the immune viral response to recent and future drug therapy

Biography

Sahar Sultan Essa is an Assistant Professor at the Department of Microbiology, Faculty of Medicine in Kuwait University, Kuwait.

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Notes:

Tea Koiava et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

First description of CTX-M group 1 producing *Klebsiella pneumonia* in an acute care hospital in Adjara, Georgia

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Background & Aim: In the previous years, CTX-M extended spectrum β-lactamases (ESBLs) have emerged worldwide and have replaced classical TEM and SHV-type ESBLs in many countries. CTX-M-15 is currently the most frequent, with a pandemic distribution, and the rapid spread is facilitated by incorporation of resistance genes in mobile genetic elements. This successful ESBL in Gram negative bacteria is closely associated with nosocomial environments and as an intestinal colonized, particularly in old and dependent patients. Little is known about the CTX-M ESBLs among *Klebsiella pneumonia* in Adjara. Our goal is the detection and characterization of ESBLs among *Klebsiella pneumonia* isolates from patients in two different hospitals in Adjara.

Material & Methods: Susceptibility profile and identification of the infection of *Klebsiella pneumonia* (n=23) isolates collected from different hospital services (2013-2015) were performed by disc diffusion methods according to the CLSI guidelines and API 20E, respectively. ESBL producers were detected and/or confirmed by the double disk synergy test using oximino-β-lactamic antibiotics with and without clavulanic acid. Genes of families' bla_{TEM}, bla_{OXA}, bla_{SHV} and bla_{CTX}-M group 1 were investigated by PCR. Sequencing was performed using group specific primers for CTX-M group 1.

Results: Fourteen *Klebsiella pneumonia* producing CTX-M group 1 ESBL infection isolates were detected in different biological samples, namely in sputum (n=8), urine (n=5) and abdominal fluid (n=1), collected in different hospital services. The infection isolates showed an extended resistance profile to aminoglycosides, fluoroquinolones and tetracycline. CTX-M group 1 ESBL isolates showed specific amplification for bla_{TEM}, bla_{OXA} and bla_{SHV} families.

Conclusion: This is the first report of CTX-M group 1 in infection *Klebsiella pneumonia* isolates in Adjara. This situation might represent the spread of these multidrug resistant Gram negative in acute care hospital in Adjara. The implementation and/or reinforcement of infection control measures, active antibiotic resistance surveillance and colonization screening of high risk patients is important in order to limit the dissemination of CTX-M ESBL producing *Klebsiella pneumonia* in health care institutions and to the people of Georgia. Colonization screening in elderly and/or dependent patients, upon admission at different health care institutions and their evaluation before discharge are extremely important to prevent the spread of cycle of multidrug resistant *Klebsiella pneumonia* in various healthcare facilities.

Biography

Tea Koiava was graduated from Batumi Shota Rustaveli State University in 2007 with a Master's degree in Genetics. Since 2007, she has been working at Batumi Shota Rustaveli State University as a Chief Specialist of the Department. She is actively engaged in medical/educational and many other kinds of measures taking place at university and leading training courses in Biology as well. She is also engaged in scientific activities of the department. She is the author of five scientific papers and currently pursuing her PhD in Biology Educational Program, specializing in Microbiology.

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November 28-29, 2016 Valencia, Spain

Deep sequencing of subgingival microbiota according to subclinical atherosclerosis in Korean adults: Pilot study

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Subgingival microorganisms could be associated with cardiovascular disease and no study has analyzed the subgingival microbiota according to subclinical atherosclerosis (SA). This study aims to investigate subgingival microbiota related to SA by next generation sequencing (NGS) among Korean adults. From the Yangpyeong cardiovascular cohort, 15 SA cases 15 controls (mean age of 65 years), matched for age, sex and smoking history by 1:1 ratio, recruited in this cross sectional study. SA was assessed by carotid intima-media thickness. SA was defined as Carotid intima-media thickness ≥0.754. Gingival crevicular fluid (subgingival plaque) was sampled from the gingival sulcus of the tooth with the most severe alveolar bone loss. For NGS, 16S rRNA genes from bacteria in subgingival plaque were pyrosequenced. Mann-Whitney test and Chi-square test were performed to assess the association between percentage amount and prevalence of microorganisms and SA, respectively. A total of 926 operational taxon units (OTUs) were pyrosequenced. In terms of percentage amount of OTU, 19 species showed difference between cases and controls. Among them, five species such as EU335295, *Capnocytophaga leadbetteri,* Oral014, EU150278 and AF385506 were higher in SA. In terms of prevalence of OUT, five species showed difference between cases and controls and Oral014 was high in SA. Five subgingival microorganisms, especially Oral014_s, were associated with SA. Further main studies are needed to rectify our results.

Biography

Hyun-Duck Kim has obtained his Doctor of Dental Surgery and PhD in Public Health Dentistry from Seoul National University School of Dentistry. He has worked as a Visiting Scientist in the Department of Oral health Policy and Epidemiology, Harvard School of Dental Medicine from 1998-1999 and 2006-2007 and in the Department of Periodontology, UNC in 2000. Currently, he is a tenure-track Professor in the Department of Preventive and Social Dentistry, SNU SOD. He has serviced as a Vice-Dean during 2010-2011 and the Chairman of the Department of Preventive and Social Dentistry during 2012-2013. He has published and presented more than 200 topics, papers and books.

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Philips Akinwole et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Consumption of terrestrial dissolved organic carbon in microbial mesocosm

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Dissolved organic carbon (DOC) is the largest organic carbon pool in lotic systems. Current paradigms describing terrestrial DOC in streams depict DOC as both an important carbon and energy source for microorganisms and containing large amounts of chemical and biological refractory humic substances. To better evaluate the reliance of stream microorganisms on terrestrial DOC, we produced ^{13}C-labeled DOC by leaching composted ^{13}C-labelled tulip poplar leaves and twigs in soil columns for 3 months and then leaching the soil with water. This process yields ^{13}C-labeled DOC with size and liability fractions approximating stream water DOC. To determine the microbial groups actively using stream water DOC we incubated streambed sediments in recirculating mesocosm chambers amended with ^{13}C-labeled DOC and examined ^{13}C incorporation into microbial phospholipid fatty acids. Prokaryotes comprised 61% of the mesocosm microbial community and consisted of aerobic, facultative anaerobic and anaerobic bacteria while microeukaryotes comprised the remaining 39%. Comparison by principal component analysis of the microbial communities in stream sediments and stream sediments incubated with or without ^{13}C-labeled humic DOC showed our mesocosm-based experimental design was sufficiently robust to investigate the use of ^{13}DOC by sediment microbial communities. After 48 hours of incubation, phospholipid fatty acids i15:0, 16:0, 16:1w9, 18:1w9c, 18:1w7c (aerobic/facultative anaerobic bacterial biomarkers) and 20:4w6, 20:5w3 (microeukaryotic biomarkers) showed increased abundance of ^{13}C. This suggests that the hetero organotrophic bacteria actively utilized the ^{13}DOC and that microeukaryotic predators consumed those bacteria. These findings indicate that DOC, although generally considered refractory and poorly utilized by microbiota, substantially contributes to the energy and carbon flow in aquatic ecosystems.

Biography

Philips Akinwole is a Senior Researcher at the Department of Biological Sciences, University of Alabama, USA.

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Young Ah Kim et al., J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Community associated extended spectrum β lactamase producing *Escherichia* coli infection in Korea

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Background: The community associated infections due to extended spectrum β lactamase (ESBL) producing *Escherichia coli* has been well known clinical problems. Serogroup O25-multilocus sequence typing (MLST) sequence type ST131 *E. coli* has been known as a major clone for worldwide spread because of its multidrug resistances and higher virulence traits.

Methods: The recent extent and significance of community associated infections caused by ESBL producing E. coli were evaluated by a prospective observational study. We collected non-duplicated *E. coli* isolates, isolated from consecutive, sequentially encountered patients with community onset episodes (either outpatients or within 48 hours of admission) between March and April 2016 in two community hospital in Gyeonggi province, Korea. Sites of acquisition of the organisms (community associated or healthcare associated), antimicrobial susceptibility and PCR of O25/O16 genes to screen global epidemic ST131were evaluated.

Results: Of 213 patients infected or colonized with *E. coli* as outpatients or within 48 hours of hospitalization, 119 (55.9%) had community associated infection (65.5% of which represented urinary tract infection), while the remainder had healthcare associated infection. Of the community associated infections, 26.9% (32/119) were caused by the globally epidemic ST131 strain (25/119 for O25-131 and 7/119 for O16-ST131 respectively). ESBL production was confirmed by phenotypic methods in 24.4% (29/119) of the community associated infections.

Conclusions: A considerable portion of community onset, ESBL producing *E. coli* infections now occur among patients in Gyeonggi Province, Korea. Increase of ST131 *E. coli* infections in community without healthcare associated risk factors could be worrisome public health threats.

Biography

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November 28-29, 2016 Valencia, Spain

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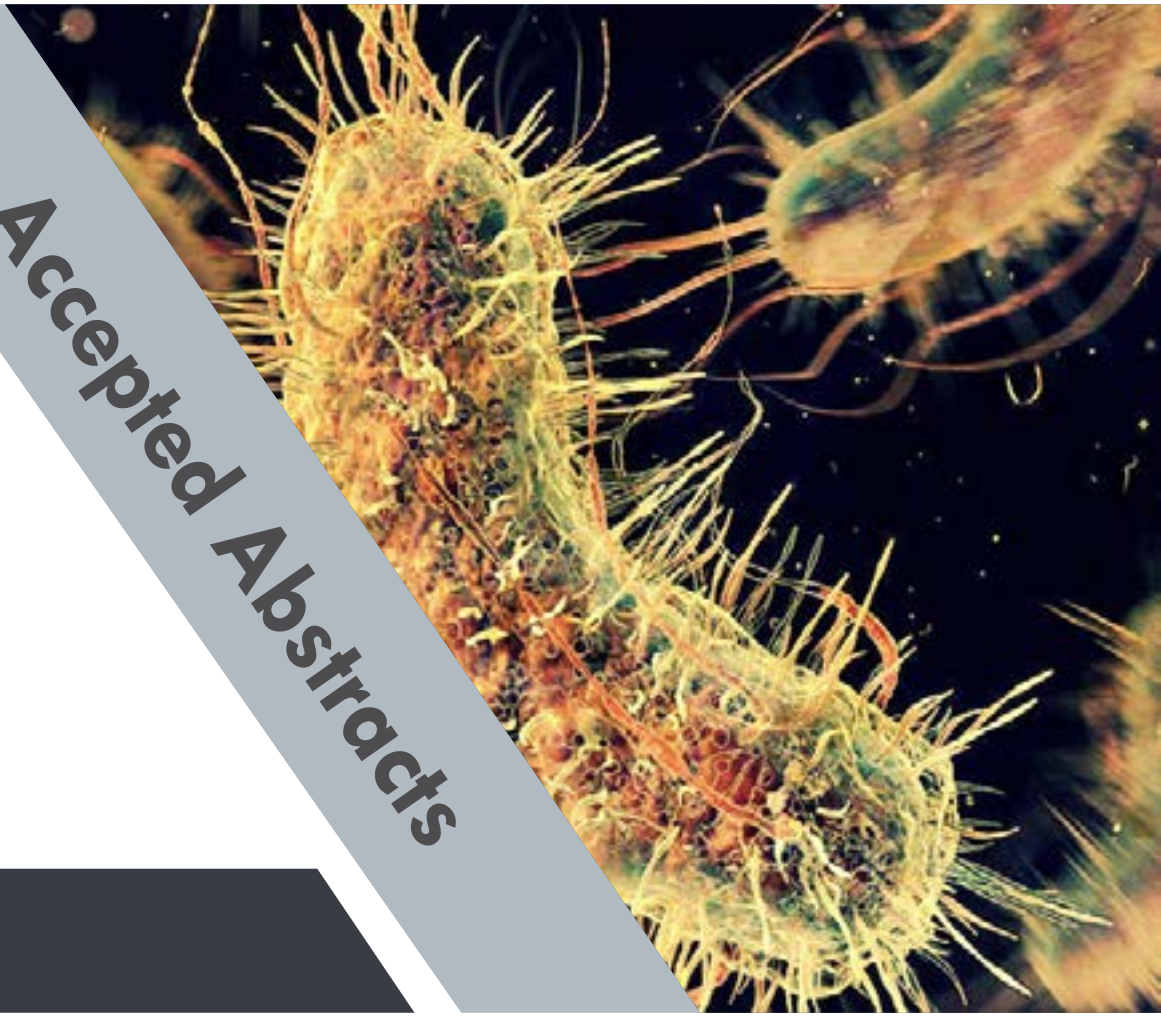




Microbiology 2016

J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Hepatitis C virus (HCV) genotype 1b is associated with a higher hepatocellular carcinoma incidence in patients with underlying HCV cirrhosis

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Background: Hepatocellular carcinoma (HCC) is one of the most common causes of morbidity worldwide, accounting for about 7% of all cancers and over 80% of primary liver cancer. A recent estimate indicates that it is the fifth and the seventh most common cancer in males and females respectively with approximately one million deaths per year, especially in developing countries and represents one of the major dreaded complication of chronic liver disease, frequently associated with compensated cirrhosis. It has been reported, in fact, that approximately 3-4% of HCV chronically infected patients with underlying cirrhosis will develop HCC on average 30 years after infection.

Aim: The purpose of this study was to clarify the role of hepatitis C virus genotype 1b in HCC development.

Methods: 152 consecutive cases of HCC, fulfilling the criteria from the Barcelona 2000 EASL conference and 147 patients with other tumors as control group were included in the study. Serum of each patient was evaluated for serology of HCV, viral load estimation and genotyping.

Results: 80.9% of HCC patients had positive anti-HCV significantly greater than the control group (39.4%; $p<0.0001$) with a risk of progression to HCC 6 times higher. Significantly higher rate of anti-HCV seropositivity was shown among male patients with HCC (90.5%) than among females (59.6%; $p<0.0001$). Males anti-HCV positive were significantly showed to have about 6 and half times risk of progression to HCC (OR=6.45; 95% C.I=2.6-15.4) than females. Furthermore, anti-HCV rate increased steadily with the age, ranging from 5.7% in patients with less of 60 years to 94.3% in patients with over 60 years ($p<0.001$). On the contrary, this pattern was not recorded among the control group, suggesting that anti-HCV positive older patients have a risk of progression to HCC almost 13 times higher than the control group (OR=12,8, 95% C.I=5.2-31.4) HCV RNA rate was significantly higher (83.7%) among HCC patients than in the control group (44.2%, $p<0.0001$) and the most prevalent genotype was 1b (68.0% in HCC vs. 26.3 in the controls $p<0.001$) with a risk of progression almost 6 times greater than patients infected by other genotypes.

Conclusion: HCV genotype 1b is associated with a statistically higher risk of developing HCC if compared to other genotypes. A prospective study with larger number of samples will be needed to confirm our results.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Misinsertion and mispair extension by human immunodeficiency virus type 1 reverse transcriptase (HIV-1RT) as a mechanism of development of mutations in the viral DNA

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The unique properties of human immunodeficiency virus type 1 reverse trancriptase (HIV-1 RT) include its high propensity for misinsertion and misincorporation of deoxyribonucleotide triphosphate (dNTP) in the growing chain's 3' terminus during proviral cDNA synthesis. It was envisaged that the interaction of the side chain of K154 in HIV-1RT with the penultimate nucleotide of the template may be crucial in determination of fidelity of proviral DNA synthesis. This hypothesis was tested by steady-state kinetic studies using wild-type HIV-1 RT and five K154 mutants. These mutants contained replacement of positively charged side chain of lysine with two amino acids' hydrophobic and two amino acids' negatively charged side chains. In one of the mutants, the positive charge of Lysine was retained but the side chain was enlarged by one carbon atom while replacing it with arginine. The results indicated variations in their respective activities, extent of formation of binary and ternary complexes as well as in misinsertion and mispair extension of nucleotides in the growing chain of DNA. All of these mutants when tested for their response to 3TC, an approved antiHIV-RT agent, displayed significant resistance to this nucleotide analog when compared to wild type enzyme. The error prone DNA synthesis by HIV-1RT and the development of the antiHIV-1RT drugs resistance may be explained in the light of the three dimensional crystal structure of the enzyme.

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Valorization of microalgal biomass

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The use of microalgae for high value applications such as food ingredients, feed proteins, cosmetics, pharma and nutraceuticals represents a promising way of increasing the cost competitiveness and diminishing the pressure on land resources. The laboratory of Phycobiotechnology of the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova has developed a series of innovative technologies for the cultivation of microalgae, separation of the chemical components present in biomass and their valorization into high added value applications. Based on an ecological approach, these technologies concern the production of antioxidants and pigments (β-carotene, astaxanthin, phycocyanin, phycoerythrin), proteins and essential amino acids, polysaccharides, lipids and unsaturated fatty acids, bio components and bio additives, etc., using new strains of *Arthrospira (Spirulina) platensis, Nostoc linckia, Dunaliella salina, Haematococcus pluvialis* and *Porphyridium cruentum*. The studies include optimization of the cultivation, separation, extraction and purification process and evaluation of the bioactive properties in view of their application. Moreover, our recent studies have focused on the use of microalgae cultures to synthesize nanoparticles as an alternative to chemical approach.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

A proposal for the unification of two strains of cyanobacteria genus *Nostoc* to the same species

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Studies of cyanobacteria are important to the global scientific community because of their ecological and applied importance. Amongst the different cyanobacterial orders, the *Nostocales* and *Stigonematales* orders are especially important as they fix atmospheric nitrogen and thus contribute to the fertility of agricultural soils worldwide. However, in spite of their ecological importance and environmental concerns, their identification and taxonomy is still problematic and confusing, often being based on current morphological and physiological studies, which generates confusing classification systems based on plastic characters that vary with the environmental and cultural conditions. The present research aimed to investigate through a polyphasic approach, the differences in morphological and genotypic features of two cyanobacteria strains isolated from paddy fields of Iran, belonging to the family *Nostocaceae* (subsection IV. I). Based on the description of the morphology provided by Desikachary (1959), the two strains were identified as *Nostoc ellipsosporum* and *Nostoc muscorum*. Challenges arose when the two *Nostoc* strains could not be discriminated by 16S rRNA and ITS genes sequencing. The results of sequencing of the cloned bacterial 16S rRNA fragment strongly indicated that the current morphological classification of the two Nostoc species is invalid. Moreover, phylogenetic study of these two *Nostoc* strains has demonstrated that genetic relationships are in conflict with the morphological classification. Besides, after doing DNA-DNA re-association experiments, we concluded that the two Nostoc strains investigated might possibly be united into one species.

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Plant parasitic weed endophytic bacteria triangle

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Phelipanche and *Orobanche* species (broomrapes) are holoparasitic plants that connect to the vascular systems of their hosts, allowing the transfer of various substances including a possible exchange of endophytic bacteria that inhabit the internal tissues of both plants. To shed light on the microbial aspects of the parasitic interaction between *Phelipanche aegyptiaca* and its host, tomato, we characterized the endophytic composition in both plants before and after attachment using mass sequencing analysis. Endophyte communities of the parasitic weed were significantly different from that of the non-parasitized tomato root but no significant differences were observed between the parasite and its host, parasitized tomato root, suggesting bacterial exchange between these two plants. In addition to molecular analysis, isolation of endophytic bacteria from the parasitic weed-host plant system enabled to examine whether these isolates can affect the dynamics of host-parasite interaction. Endophytic bacteria isolates were examined for their ability to secrete substances that may affect the dynamics of this system and indeed, a few isolates inhibit the growth of the parasitic weed. The current study focuses on the bacterial aspect of host-parasite interaction and highlights the potential of exploiting alternative environmentally friendly approaches for parasitic weed control.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Evaluation of *Bacillus subtilis* metabolites as plant growth promoters in *Solanum lycopersicum*

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Soil microorganisms called plant growth-promoting rhizobacteria (PGPR) have beneficial effect in plants since they are able to produce plant hormones such as auxins, gibberellin, cytokinins, antibiotics and other compounds. *Bacillus subtilis* is categorized as a PGPR has antifungal activity and is auxin and siderophore producer. In the present research were evaluated fermentation broths of *Bacillus subtilis* by colorimetric and chromatographic techniques to determinate the production of plant growth-promoting metabolites and was evaluated the effect of the broths in *Solanum lycopersicum* plants and seeds. Results showed that fermentation broths of *Bacillus subtilis* presented auxin precursors, AIA, siderophores and jasmonic acid and they have effect in the germination time of seeds, plants length, root length and other agronomic parameters evaluated. BS8 broth presented the biggest concentration of 3-indolilacetonitrile and jasmonic acid (352.64±19.37 and 54.48 µg per mL), BS14 broth the highest concentration of AIA (147.80±3.03 µg/mL) and BSN broth of triptamine (605.54±39.60 µg/mL). All broths were positives for siderophores production. BS8 broth increases the germination rate of seeds. In plants length the best treatment was BS8 broth, then BS14 broth and finally BSN broth. Since every *Bacillus subtilis* has different concentration of the metabolites evaluated, the effect of each broth was different in the plants and seeds; this way is concluded that the effect of metabolites depends of the concentration and also of the combination with other plant growth-promoting metabolites.

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Biological activities of lanthanide (III) nitrate complexes with N-(2-hydroxynaphthalen-1-yl) methylene) nicotinohydrazide Schiff base

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The field of coordination chemistry has registered a phenomenal growth during last few decades. It is well known that precious metals have been used for medicinal purposes for at least 3500 years. At that time, precious metals were believed to benefit health because of their rarity but research has now well established the link between medicinal properties of inorganic drugs and specific biological properties. The current study was designed to explain the synthesis and characterization of the lanthanide (III) nitrate complexes with N-(2-hydroxynaphthalen-1-yl) methylene) nicotinohydrazide Schiff base and to evaluate the antibacterial and the antioxidant activities of the Schiff base and its lanthanide ion complexes. Antimicrobial activity of the lanthanide (III) nitrate complexes with N-(2-hydroxynaphthalen-1-yl) methylene) nicotinohydrazide Schiff base was estimated by minimum inhibitory concentration (MIC, µg/mL) using a micro-broth dilution method for different clinical isolates such as Escherichia coli and Enterococcus faecalis. Our present study has shown that moderate antimicrobial activity exists against both ligand and its complexes. There was no significant difference between Gram-positive and Gram-negative bacteria towards the tested ligand and its complexes. The results obtained herein indicate that the ligand and its complexes have a considerable antibacterial activity.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Isolation and virtual screening of antimicrobial prodigiosin pigment from oxalotrophic *Serratia marcescens* OX_R strain

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Prodigiosin is a multifaceted secondary metabolite produced by *Serratia* spp. having great potential as a pharmaceutical agent. In the present study we demonstrate that oxalate supplementation in peptone glycerol production media increased organoleptic characters and yield of prodigiosin pigment extracted from oxalotrophic *Serratia marcescens* OX_R isolated from Indian bat guano sample. The pigment was demonstrated in vitro as an antibacterial agent against common opportunistic skin surface pathogen *Staphylococcus aureus* NCIM 5021 strain as killing activity by agar well diffusion method. The docking analysis and pharmacophore modeling indicated that the probable mechanism of action of the prodigiosin was against *Staphylococcus aureus* DNA gyrase protein. The pigment was also found to efficiently dye both cotton and latex polymer. In summary, we describe here an oxalotrophic *Serratia marcescens* which may serve as a potent and economical resource of prodigiosin which owing to its dyeing and anti-bacterial activities finds future avenues to be developed as dressing material for nosocomial subjects or burn victim patients.

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Quinolone resistant molecular mechanisms in *Escherichia coli* isolated from University Hospital in Egypt

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Fluorpquinolone resistant *E. coli* (FQREC) is an important cause of many infectious diseases worldwide. Our goal is to detect the prevalence of FQREC in Egypt in addition to unrevealing the most important molecular mechanisms of FQ resistance. Forty E. coli isolates were tested for their ciprofloxacin MIC by E-test and for mutations in genes coding for DNA Gyrase (gyrA & gyrB), Topoisomerase IV *(parC & parE)* and transcriptional regulators of AcrAB efflux pump; *soxR, soxS, marR* and *acrR* using Polymerase Chain Reaction (PCR) and sequencing. The contribution of PMQR to resistance was based on PCR detection of *qnrA, qnrB, qnrS, qnrD, aac(6')-lb-cr*, and qepA determinants. All FQREC isolate had at least double mutations within *gyrA* plus a third mutation in *parC*. Some FQR isolates also manifested an additional mutation either in the *parC* or in parE. Regarding the *AcrAB* pump regulators, stop mutations both in *soxR* and *acrR* were recorded in addition to silent mutations in *marA*. Interestingly the A12S mutation in *soxS* discovered in canine *E. coli* isolates have been found for the first time in 2 of human isolates of this study. Three of the FQSEC showed decreased susceptibility to ciprofloxacin, one of them harbor a single *gyrA* mutation and another one showed a stop codon in *soxR*. *qnrS* and *aac(6'lb)* were the most prominent plasmid found in 13 and 14 FQREC isolates respectively. *qnrA*. *qnrB* and *qepA* were found in fewer isolates. Interestingly, one FQREC isolate harbor all the five plasmids together. Two of the FQSEC isolates with reduced susceptibility contain PMQR.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Lactobacillus plantarum and EGCG in a synbox: An effective intervention for alcohol induced endotoxin mediated liver disease

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Alcohol abuse can cause substantial liver insufficiencies leading to significant mortality worldwide, regardless of the available therapeutic options. Recently, we have evaluated the use of alternative agents like Epigallocatechin gallate (EGCG) and *L. plantarum*, to control ALD. The prebiotic property of EGCG for enhancing the growth of *L. plantarum* was evaluated. Based on this property a coupled formulation consisting of both EGCG and *L. plantarum* was developed by encapsulating these agents in Ca-alginate beads to achieve enhanced biological effects. The formulation was extensively characterized and evaluated for its enhanced in vivo efficiency. Effect of EGCG to enhance the growth of *L. plantarum* was significantly better than inulin. Combination beads lowered serum transaminases and blood alcohol levels. Alcohol fed rats elicited raised plasma endotoxin levels, attributable to the disrupted intestinal permeability, confirmed by lactulose-mannitol test using HPTLC. Transcription studies for TLR-4 receptor mediated signaling molecules (MyD88, CD14, MD2) revealed modulation in the expression of these molecules, resulting in the down-regulation of NF-κB in rats treated with combination beads. Expression of pro-inflammatory cytokines i.e., TNF-α and IL-12β/40 subunit, COX-2 in addition to the levels of antioxidants, oxidants and micronuclei formation also assumed normal levels. Histo-architecture depicted normal liver and intestine in rats treated with co-encapsulated beads whereas severely distorted histology was observed for respective tissues in alcoholic rats. Thus, the formulation of *L. plantarum* with EGCG in a synbox can be a promising therapeutic option ensuring enhanced bioperformance against ALD. To the best of our knowledge, these findings are being reported for the first time.

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Study of bacteriophages as indicators of the microbiological quality of water

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The current methods to detect enteric viruses in the water source system are very complicated and not efficient economically. In the U.S. EPA, the detection of bacteriophage is a good alternative among the trace methods of using microorganisms to detect feces pollution. Amongst the diverse bacteriophages, DNA somatic coliphage and RNA male-specific phage that infect *E. coli* and other coliforms possess similar size and structural characteristics to enteric viruses. Therefore, bacteriophages are attractive candidates as indicators of enteric viruses in the surface waters and serve as simple water quality assessment tools to diagnose human health risks. In this study, we evaluated the potential of bacteriophages as an indicator of the microbiological quality of water. Water samples were collected at two representative study sites: Mulgum of Nakdong River and the Hoidong reservoir which supplies water to Busan City. DNA somatic phages were detected in 75% samples of Mulgum and 70% samples of Hoidong, while RNA male specific phages ranged from 22-0 PFU/10 L in Mulgum and 25 PFU/10 L in Hoidong. Enteroviruses were detected in 6 cases of Mulgum region and in 5 cases in Hoidong. *Noroviruses* were not detected in any of the samples. Both somatic phages and male specific phage were detected in the *Enterovirus* positive samples. In conclusion, detection of an index organism like bacteriophage before pathogenic bacteria analysis could be a major tool to predict the presence of enteric viruses.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Theme and variations in autotransporter adhesins

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Many persistent and chronic bacterial infections are associated with the formation of aggregates and biofilms that are difficult to treat, including respiratory and urinary tract infections (UTIs), infections on medical devices and infections of the ear, gums and heart. Thus, an increased understanding of the mechanisms employed by bacteria to form biofilms is essential for the development of strategies to combat these persistent and intrinsically resistant communities. One mechanism of bacterial aggregation and biofilm formation involves the expression of self-associating surface located autotransporter (AT) proteins. Our work focuses on investigating the structural diversity of AT proteins to understand their mechanism of action. We have recently elucidated the structure of Antigen 43 (Ag43), an AT protein from uropathogenic *E. coli* (UPEC) that self associates forming bacterial aggregates and biofilms. Our studies have shown how Ag43's L-shaped structure drives the formation of cell aggregates via a molecular Velcro-like mechanism. Furthermore, our recent studies on other AT proteins from *E. coli* pathotypes show unexpected structural diversity among this family of proteins, which results in different virulence functions. For example UpaB shares low sequence and structural similarity with Ag43, does not self associate to form bacterial aggregates but binds extracellular matrix proteins (e.g., fibronectin) and increases bladder colonization.

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Design and construction of 1,2,4-butanetriol-producing pathway in *Escherichia coli*

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1,2,4-butanetriol (BT) is an important non-natural chemical with a variety of industrial applications. Here we constructed a prototype strain for BT production from xylose by assembling a four-step synthetic pathway and disrupting the competing pathways in *E. coli.* By fine-tuning of the pathway enzymes expression level, the potential bottlenecks was identified and the BT production was increased by 4.3 fold, achieved a final titer of 1.58 g/L after 72 hours. Furthermore, we designed a novel six-step biosynthetic pathway for BT from malate for the purpose of using glucose as a cheap substrate. Following tests of several combinations of enzymes for the pathway, a five-enzymes-catalyzing-six-step pathway was constructed in *E. coli.* By assembling these enzymes, BT was detected in the fermentation broth upon addition of malate, proving BT can be biosynthesized from malate. As well, BT was detected in the fermentation using glucose as the sole carbon source, suggesting that such novel BT biosynthetic pathway has created the possibility for the production of BT from the cheaper substrate glucose.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Prevalence of tinea capitis and corporis in Benghazi

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Across sectional prospective study was carried out over a period of one year from (April 2008-March 2009), depending on prestructured questionnaire, study was carried out on a total of two hundred patients with different age, sex and with clinically suspected cases with tinea capitis and corporis. Specimens were obtained from skin scales of the lesion. Hair specimens were collected by plucking the hair with forceps. The aim of this study to identify the etiological agents involved in these infections. Out of 200 patients who presented with suspected superficial fungal and to determine prevalence of tinea capitis and tinea corporis in Benghazi population. Infected, 113 (56.5%) were male and 87 (43.5%) were female. Out of these, 117 children (65 male and 52 female) were provisionally diagnosed with tinea capitis and corporis. The youngest patient was a 5 months old infant, whereas the eldest patient a 71 year old man. Greater number of positive cases of dermatophytes is seen in children under the age of 15 year. Tinea capitis was predominant in 31 (57.4%) children, while tinea corporis were (14.8%) children. 125 (62.5%), were found to be positive by direct microscopic examination only, while 50 (25%) by culture only and 45 (22.5%) positive by both techniques. In addition 36 (18%) patients give positive family history of dermatophytosis, 9 patients of them were positive culture while 55 (27.5%) patients had history of contact with animals 16 of them were positive culture. Also17 (8.5%) were foreign patients, of these 8 were soudanense. In this study, the most common sites where dermatophytes in Tinea corporis isolated were the neck and back. Also we observed that, *T. violaceum* was the most common dermatophyte isolated 13 (24%) (mainly among children under age of 15 years). *T. soudanense* 9 (16%) was the second common isolated, followed by *T. schoenleinii* 8 (14.8%), other dermatophytes in descending order, were *M. canis* 5 (9.3%), *T. mentagrophytes* 4 (7.4%), M. *ferrugineum* 3 (5.5%), *T. rubrum* 3 (5.5%), *T. tonsurans* 2 (3.7%), *M. nanum* 2 (3.7%), *T. yaoundi* 1 (1.8%), *T. terrestre* 1 (1.8%), *T. verrucosum* 1 (1.8%), *M. audouinii* 1 (1.8%) and 1 (1.8%) were unidentified. Culture the isolates were a mixed of dermatophytes, in 2 cases of tinea capitis the culture revealed in mixed of *T. violaceum* and T. mentagrophytes, while 2 cases of tinea corporis; *T. tonsurans* and *T. schoenleinii* where the culture revealed a growth of *T. rubrum* and *M. nanum*. The infection was found to occur more frequently in males (29 cases than in females (25 cases). In the present study, grey patch was the predominant type of tinea capitis 32 (16%), black dot 2 (1%) and kerion 2 (1%) was the least common types.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Transcription factor c-Rel plays a role in driving experimental acute and chronic ileitis in rats

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Statement of the Problem: Genetic and environmental factors, including the commensal microbiota, have a crucial role in the development of inflammatory bowel disease (IBD). IBD is also associated with chromosome 2p16, which contains REL, which encodes c-Rel, a subunit of NF-κB. Aberrant activation of the transcription factor NF-κB is associated with acute and chronic intestinal inflammation in rats and plays a key role in cytokine gene regulation, in patients with IBD. c-Rel is required also within cells of the innate immune system for the activation of T cell-dependent as well as innate mechanisms of mucosal inflammation.

Aim: The purpose of this study is to investigate the expression of the NF-κB subunit c-Rel in the development of acute and chronic ileitis in rats.

Methodology & Theoretical Orientation: Male Wistar rats weighing 200-250 g were housed in standard wire-mesh bottom cages at constant temperature of 25 °C and 12/12 hours light/dark cycles. Acute ileitis was induced in fed rats (n=10) by one subcutaneous injections of indomethacin, an inhibitor of the cyclooxygenase pathway of arachidonic acid (15 mg/kg). Chronic ileitis was induced by two subcutaneous injections of indomethacin (10 mg/kg in 5% freshly prepared $NaHCO_3$ at 37 °C) were administered 24 hours apart. Expression of c-Rel mRNA was determined by real-time reverse-transcription polymerase chain reaction performed using a CFX96™ Real-Time PCR Detection Systems (Bio-Rad Laboratories, Inc., USA). GAPDH was used as endogenous control to normalize gene expression data and a relative quantitation value. All statistical analyses were performed using STATISTICA 6.0 software. Results are expressed as mean values±SEM.

Findings: The expression of c-Rel was assessed in ileum. Greater expression of c-Rel predominated during chronic ileitis in rats compared to control group (4.8±0.7, $P<0.003$). Consistent with the pronounced expression during chronic disease, the level of c-Rel expression was also elevated in rats with acute ileitis (4.1±1.1, $P<0.02$).

Conclusion & Significance: In summary, these results suggest that the expression of c-Rel in ileum is essential for initiating intestinal inflammation and may advance our understanding of IBD pathogenesis and that targeting NF-κBc-Rel can be used as a novel molecular approach for the treatment of patients with IBD.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

The role of flagellar proteins in epidemic PCR-ribotype 027 (B1/NAP1) *Clostridium difficile* virulence

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Clostridium difficile is a major cause of healthcare-associated infection and inflicts a considerable financial burden on healthcare systems worldwide. Disease symptoms range from self-limiting diarrhea to fatal *pseudomembranous colitis*. Whilst *C. difficile* has two major virulence factors, toxin A and B, it is generally accepted that other virulence components of the bacterium contribute to disease. *C. difficile* colonizes the gut of humans and animals and hence the processes of adherence and colonization are essential for disease onset. Bacteria within biofilms are protected from multiple stresses including immune responses and antimicrobial agents. Increased antibiotic resistance and chronic recurrent infections have been attributed to the ability of bacterial pathogens to form biofilms. While biofilms have been well studied for several gut pathogens, little is known about biofilm formation by anaerobic gut species. We have limited understanding of how the causative bacterium *C. difficile* colonizes the host or how it can resist antibiotics and persist within the gut. While persistent infections have been previously linked to biofilm-formation by pathogens, biofilm development by *C. difficile* has not been characterized. Our work demonstrates the ability of this anaerobic pathogen to form complex biofilms, the involvement of important clostridial pathways in biofilm development and perhaps a connection between formation of spores which are believed to mediate persistence and biofilm formation. Importantly, we show that bacterial sensitivity to antibiotics is reduced in clostridial biofilms. Biofilm formation may be a mechanism employed by *C. difficile* to survive in hostile environments such as the human gut. Here we tested this hypothesis by comparing flagellated parental strains to strains in which flagella genes were inactivated using ClosTron technology. Our focus was on a UK-outbreak, PCR-ribotype 027 (B1/NAP1) strain, R20291. We compared the flagellated wild-type to a mutant with a paralyzed flagellum and also to mutants (*fliC, fliD and flgE*) that no longer produce flagella in vitro and in vivo. Our results with R20291 provide the first strong evidence that by disabling the motor of the flagellum, the structural components of the flagellum rather than active motility, is needed for adherence and colonization of the intestinal epithelium during infection. The R20291 flagellar mutants adhered less than the parental strain in cell adherence in vitro model. Finally we demonstrated that in strain R20291, flagella do play a role in colonization and adherence and that there are striking differences between *C. difficile* strains. In addition, we also demonstrate that clinical *C. difficile* hyper virulent strain R20291, form structured biofilms in vitro with R20291 accumulating substantially more biofilm. Microscopic analyses show multiple layers of bacteria encased in a proteinaceous biofilm matrix. Employing isogenic mutants, we show that virulence associated proteins, cwp84 and a putative quorum sensing regulator, *luxS* are all required for maximal biofilm formation by *C. difficile*. Interestingly, a mutant in *spo0A*, a transcription factor that controls spore formation was defective for biofilm formation indicating a possible link between sporulation and biofilm formation. Furthermore, we demonstrate that bacteria in clostridial biofilms are more resistant to high concentrations of vancomycin, a drug commonly used for treatment of CDI. Biofilm formation by *C. difficile* is a complex multifactorial process and may be a crucial mechanism for clostridial persistence in the host.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

Non-induction of resistance amongst *Salmonella Typhi* strain passaged through sub lethal dose of Beri honey

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Introduction & Aim: Resistance to conventional anti-typhoid drugs is well documented and the recent emergence of fluoroquinolone resistance has made it very difficult and expensive to treat typhoid fever. As the therapeutic approach becomes even more limited, it is crucial to probe into non-conventional modalities. In this perspective, honey is a promising nominee for skirmishing antimicrobial resistance. Antimicrobial activity of honey is well established against variety of bacteria. It contains a broad range of antibacterial compounds that act synergistically at multiple sites, thus possibly making bacterial resistance unlikely. In this study, the ability of honey to resist the occurrence of resistance under conditions that induce resistance in antibiotics was analyzed.

Methods: Minimum inhibitory concentrations (MICs) of two antibacterials, Ciprofloxacin and Beri honey were determined against *Salmonella Typhi* using broth dilution technique. The isolates thus obtained were then exposed and passaged through serially increasing the sub lethal concentrations of the two in test tubes, till the concentrations reached the original minimum inhibitory concentrations. After successive passages, the MICs of both bacterial inhibitors against *Salmonella Typhi* were determined again using broth dilution technique.

Results: After exposure to the sub lethal dose, the MIC of Ciprofloxacin against *Salmonella Typhi* rose up to eight times. The minimum inhibitory concentration of honey remained unchanged from its original value even after exposure to sub lethal doses.

Conclusion: The emergence of extensive resistance to antibiotics has arguably occurred due to their misuse and overuse but also as a natural phenomenon. The results in this study show that sub lethal concentrations of the extraordinary natural antimicrobial agent, Beri honey, do not favor the development of resistant bacterial phenotype. Here we demonstrate that *Salmonella Typhi* did not develop resistance against honey, as it did against Ciprofloxacin. Further exploration of the molecular and cellular basis of this behavior is the next line of research.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

The *pPOX2* and *pLIP2* regulation in response to carbon source in the yeast *Yarrowia lipolytica*

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The non-conventional yeast *Yarrowia lipolytica* has been extensively used to produce recombinant proteins for various biotechnological applications. Actually, more than 110 proteins from human, plants and bacterial systems have been successfully expressed and produced in Y*arrowia lipolytica*. However, understanding the regulation of the promoters used to drive heterologous gene expression is a key parameter in the development of an efficient process. In this study, the regulation of the promoter of acycl-CoA oxidase gene 2 (*pPOX2*) and of the extracellular lipase 2 (*pLIP2*) were considered in regard to the medium composition and to the carbon source used (glucose, glycerol, oleic acid). Induction levels of promoters were measured using a reporter system based on a red fluorescent protein (DsRed). Specific fluorescence measurement revealed that *pLIP2* is more strongly induced than *pPOX2*, especially in complex medium. Interestingly, higher levels of induction were obtained when a combination of glucose and oleic acid was used as carbon source compared to an oleic acid based medium. In order to define the optimal ratio of glucose/oleic acid to be used, several ratios of carbon sources have been tested for their induction potential. A high induction level of *pLIP2* was obtained when oleic acid fraction in the culture medium was in the range of 0.6-0.9 cmol. Interestingly, relative fluorescence was increased slightly in this range by a factor of 18% compared to the use of 100% oleic acid. This result suggests that glucose can be considered as the most promising co-substrate to enhance recombinant protein production under *pLIP2*. Nonetheless, glycerol can replace partially oleic acid to express heterologous protein under *pPOX2*. Thus, the use of glycerol permits to lower the process cost but it also opens new perspectives for glycerol based microbiological processes. In conclusion, this work provides alternative strategies to enhance heterologous protein production in *Yarrowia lipolytica* which increase its interest as a promising recombinant expression system.

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J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

A novel approach to antibiotics and antifungals: Testing the effectiveness of *Azadirachta indica* extracts

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Azadirachta indica (neem) extracts have proven themselves to be a promising tool because they are natural and do not cause the harmful side effects of most artificial substances. Preliminary research has shown that certain natural substances can be used without the fear of a new resistant strain developing. Current treatments are plagued by artificial substances that can have harmful side effects to the body and may not be effective for multiple uses. Thus, this project aims to determine the effectiveness of natural substances as antibacterial and antifungal. Early research suggested that the neem oil would be the most effective extract because it would envelop the bacteria and fungi. Cultures of bacteria, specifically *Staphylococcus epidermidis* and *Serratia marcescens* and cultures of fungi, specifically *Aspergillus niger* and *Saccharomyces cerevisiae*, were cultured and placed in separate plates. Zones of inhibitions were created using neem leaf extract, neem soap, neem oil, a water control and antibacterial soap control disks. The diameters of the zones where growth has stopped were compared using statistical significance tests to see if any of the natural extracts were more effective than the controls. The zones that were significantly different from the controls' zones were compared amongst each other to see if one extract was more effective than the others. This analysis has shown that the natural substances are extremely effective and significantly stronger than antibiotic and antifungal substances and the artificial substances in the soap. The remainder of the plate was then considered to be the pool of potential resistant strands. Thus repetitions were completed with each of the treatments. Since the growth was still inhibited without resistance, it became apparent that the neem extracts could have many practical purposes in treatments of infections. Given that only a few trials were completed, the experiment would have to be completed with more trials to prove the consistent effectiveness.

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Optimization of fermentation conditions for extracellular production of the antineoplastic enzyme, L-asparaginase by novel actinomycete *Nocardiopsis synnemasporogenes* sp. nov. NEAE-85

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The optimization of different fermentation conditions for L-asparaginase production by *Nocardiopsis sp*. NEAE-85 and its validation using Plackett-Burman experimental design and response surface methodology was carried out. 15 nutritional variables (temperature, pH, incubation time, inoculum size, inoculum age, agitation speed, dextrose, starch, L-asparagine, KNO_3, yeast extract, K_2HPO_4, $MgSO_4.7H_2O$, NaCl and $FeSO_4.7H_2O$) were screened using Plackett-Burman experimental design. The most positive significant independent variables affecting enzyme production (inoculum age, dextrose and L-asparagine) were further optimized by the central composite face-centered design-response surface methodology. An overall about 3 and a half-fold increase in L-asparaginase production was achieved in the optimized medium as compared with the un-optimized basal medium. As a result, a medium of the following formula is the optimum for producing an extracellular L-asparaginase in the culture filtrate of *Nocardiopsis synnemasporogenes* sp. nov., NEAE-85: Dextrose 4 g, starch 20 g, L-asparagine 10 g, KNO_3 1 g, yeast extract 1 g, K_2HPO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, NaCl 0.5 g, $FeSO_4.7H_2O$ 0.01 g, pH 7, temperature 37 °C, agitation speed 100 rpm/min, inoculum size 4% ,v/v, inoculum age 24 h and fermentation period 5 days.

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