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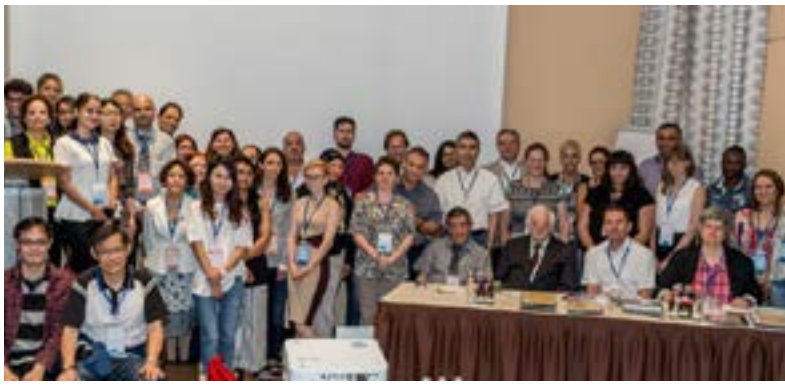

Proceedings of
7th Annual Summit on

# Microbiology: Education, R&D and Market

September 28-29, 2018 | San Antonio, USA

## Posters Presentations

Manoj Pastey, Clin Microbiol 2018, Volume: 7
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## Interaction of the human respiratory *Syncytial virus* matrix protein with cellular adaptor protein complex 3 plays a critical role in trafficking

**Manoj Pastey**
Oregon State University, USA

Human Respiratory Syncytial Virus (HRSV) is a leading cause of bronchopneumonia in infants and the elderly. To date, knowledge of viral and host protein interactions within HRSV is limited and are critical areas of research. Here, we show that HRSV Matrix (M) protein interacts with the cellular adaptor protein complex 3 specifically via its medium subunit (AP-3Mu3A). This novel protein-protein interaction was first detected via yeast-two hybrid screen and was further confirmed in a mammalian system by immunofluorescence colocalization and co-immunoprecipitation. This novel interaction is further substantiated by the presence of a known tyrosine-based adaptor protein MU subunit sorting signal sequence, YXXΦ: where Φ is a bulky hydrophobic residue, which is conserved across the related RSV M proteins. Analysis of point-mutated HRSV M derivatives indicated that AP-3Mu3A- mediated trafficking is contingent on the presence of the tyrosine residue within the YXXL sorting sequence at amino acids 197-200 of the M protein. AP-3Mu3A is up regulated at 24 hours post-infection in infected cells versus mock-infected HEp2 cells. Together, our data suggests that the AP-3 complex plays a critical role in the trafficking of HRSV proteins specifically matrix in epithelial cells. The results of this study add new insights and targets that may lead to the development of potential antivirals and attenuating mutations suitable for candidate vaccines in the future.

### Biography

Manoj Pastey is a Diplomate ACVM and presently working as an Associate Professor and is the head of Molecular Diagnostic Laboratory, Department of Biomedical Sciences in College of Veterinary Medicine, Oregon State University, Corvallis, USA.

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# The production and application of bioflocculants and their nanoparticles in dairy wastewater treatment

**Adeleke R Oluwakamiye** and **Bukola C Adebayo-Tayo**
University of Ibadan, Nigeria

One of the most pervasive and challenging problems faced by dairy industries is the availability of clean water, reclamation of wastewater and its discharge. This challenge requires modern biotechnological and the fast-growing nanotechnological approaches as robust and newest methods of treating and purifying water at lower cost with less energy in production industries, while at the same time minimizing the use of chemical flocculants and the deleterious health and environmental effects. Bioflocculants, and its silver and magnetic nanoparticles were produced and applied in dairy wastewater treatment. The flocculating activity of all the isolates ranged from 12.14 - 85.39% in which *Bacillus subtilis* B2 had the highest flocculating efficiency (85.40%). The best three with high flocculating efficiencies were selected for further studies and production of nanoparticles. They were *Bacillus subtilis* B2 (85.39 %), *Fusarium* sp. F6. (81.30%) and *Bacillus licheniformis* B5 (70.88 %). The application of the bioflocculant nanoparticles brought about a reduction in BOD, COD, TSS, TDS, pH, Salinity, Conductivity and turbidity with percentage reduction ranging from 1.11% - 44.17% for BOD, 16.12 – 71.44% for COD, 7.61 – 83.70 % for TSS, 2.02% – 74.94% for TDS, 4.8 – 6.2 for pH, 2.38% - 85.20% for salinity, 15.25% - 85.69% for conductivity and for turbidity 2.56% - 85.09%. Metal content reduction ranged from 2.91% - 71.46% for Fe, 6.15% - 95.38% for Cu and 12.57% - 97.96% for Zn. Fourier transform infrared spectroscopy revealed the carboxyl (COH) and hydroxyl (OH) group that gave rise to reduced and stable nanoparticle bioflocculants. Scanning electron micrograph showed their crystalline fluffy structures, dendritic nature in different shapes and sizes.

## Biography

Rachael is a first year PhD student in the Biotechnology and Industrial Microbiology Program, University of Ibadan, Nigeria. Where she is working to proffer solution to the seemingly unending problem faced by industries in the reuse and discharge of wastewater. She is interested in employing biotechnological and nanotechnological approach in mitigating and recycling wastewater discharge from factories/ industries.

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# *In vitro* induction of bacterial resistance to Ceftazidime-avibactam and investigation of the resistance mechanisms

**Yosra Modafer, Fur-Chi Chen** and **Xiaofei**
Tennessee State University, USA

Ceftazidime-avibactam antibiotic agent is a compound of third-generation cephalosporin ceftazidime and a novel non-β-lactam β-lactamase inhibitor avibactam. Avibactam was approved for use in the United States in 2015. It is an active inhibitor of class A, class C, and some class D enzymes. A few clinical studies have been conducted to study the overall effect of avibactam against carbapenem-resistant Enterobacteriaceae (CRE) that produce *Klebsiella* pneumoniae carbapenemase (KPC). To date, no studies on the antimicrobial activity of the combination against Salmonella species have been published. In this study, we looked at the impact of adding avibactam to ceftazidime to treat ceftazidime-resistance *Salmonella. Senftenberg* (that produce TEM and or OXA). The primary goal of this examination was to determine if ceftazidime-avibactam can induce resistance in bacteria after long-term exposure to the combination. We employed a selection method of the combination and avibactam alone for *S. Senftenberg*. We monitored bacterial resistance, characterized the stability and cross-resistance. The combination was very effective against the *S. Senftenberg*, the addition of avibactam resulted in a significant increase in ceftazidime activity, with MICs generally reduced from 512 to 4 μg/ml. S. Seftenberg evolved resistance to the combination and to avibactam alone under long-term selection pressure with continuously increasing concentrations of drugs. Cross-resistance of the induced strains to other antimicrobial agents (ampicillin and ciprofloxacin) was observed. Our results indicated that resistance to the combination could be formed at 5-fold (higher than 1/2 the MIC). Highly resistant bacteria at 10-fold was isolated for further analysis. Our undergoing aim is to investigate the potential role of beta-lactamase and other enzymes in resistance mechanism using RNA sequencing. Most importantly, our preliminary results raise serious attention concerning the long-term risks correlated with the development and clinical use of ceftazidime-avibactam.

## Biography

Yosra Modafer is a PhD student at Tennessee State University. She has her expertise in studying resistance mechanisms to the antibiotic. She also has her master research in investigating the effect of Miswak (which is a natural wood stick has been used many years ago in Asia and Africa to clean oral teeth and believed to have antimicrobial activity) on the oral microbes using saliva samples from 18 volunteers (in 2015).

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# Investigating the role of arginine phosphorylation in the regulation of Clp protease-mediated degradation of cellular proteins in *Mycobacterium tuberculosis*

**Emmanuel Ogbonna**
University of Delaware, USA

*Mycobacterium tuberculosis* remains a leading cause of mortality worldwide. Increasing antibiotic resistance associated with the bacterium makes it critical to study potential targets for development of novel therapeutics. The Clp protease system which comprises of a peptidase barrel (ClpP) and an unfoldase (ClpC1 or ClpX) has been studied extensively in a host of other bacteria and is essential for protein homeostasis and viability in mycobacteria. This work proposes to describe the relationship between arginine phosphorylation tagging (as carried out by a specific arginine kinase McsB which is well characterized in *Bacillus subtilis*) and the biochemical activity of the Clp protease system in *Mycobacterium tuberculosis*. To study the arginine phosphorylation mechanics further, *in vitro* reconstruction of the McsB-ClpC1P1P2 system was done to test the direct correlation between arginine phosphorylation tag and substrate recognition by ClpC1 and its eventual degradation by the ClpP1P2 peptidase. Epitope-tagged active-site mutants of ClpP1 and ClpP2 would be utilized in an *in vivo* substrate-trapping analysis to reveal arginine phosphorylated ClpC1P1P2 substrates within the degradome to be generated upon appropriate affinity-based pull-down and quantitative mass spectrometry. More specifically, an arginine phosphatase trap (YwlE C7A mutant) has been constructed and would be used for identification of arginine-phosphorylated substrate proteins and interaction partners in lysate from *Mycobacterium tuberculosis* or its non-pathogenic close relative *M. smegmatis*. In totality, the proposal hypothesizes that arginine phosphorylation plays a key role in marking protein substrates for Clp-mediated proteolysis in *Mycobacterium tuberculosis*.

## Biography

Emmanuel Ogbonna is a PhD student at the University of Delaware. He works in the Schmitz Lab in the Department of Biological Sciences, which is a protein biochemistry and structural biology laboratory that studies the caseinolytic proteases (Clp proteases) in Mycobacterium tuberculosis(Mtb), with the aim of understanding how the component proteins work, and how they interact with specific substrates. He had previously worked on identifying substrates for the ClpXP1P2 system in Mtb, but now studies how some of these substrates might be specifically tagged on arginine residues prior to degradation by the Clp proteasome-like machinery.

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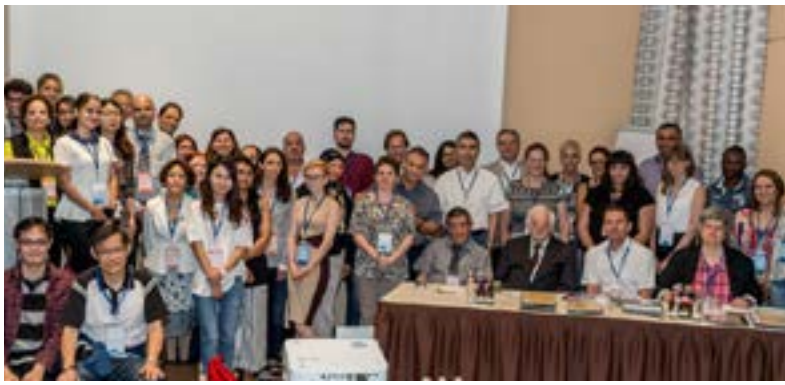

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# Role of microbiology in nursing

**Anju Dhir**
Shivalik Institute of Nursing, India

Knowledge of microbiology helps a nurse in every field of health care. Nurses should have known about the mode of spread of infection. This knowledge would help a nurse to look for specific control of the spread of infection. Knowledge of medical microbiology would help them to understand the difference between the causative organism of disease and patient's normal flora. A nurse must know procedures used to create and maintain a sterile field in the hospitals based on the knowledge of microbiology. The principles of asepsis are also based on microbiology. The proper disposal of biomedical waste is equally important and knowledge of microbiology helps in this field also. The nurse must recognize the importance of the proper collection of specimens to be sent for bacteriological examination to obtain accurate results. One of the most important things is hand washing which helps in reducing surgical infections and transmission of diseases in hospitals. Nurses also play an important role in immunization to control threats of various diseases. She/he follows not only aseptic techniques but also uses sterile equipment while looking after such patients. It is the duty of a nurse to ensure that the atmosphere of the operation theatre is free of microorganisms. The nurse can play a role while the female needs antenatal care, help during delivery or after giving birth for six weeks called as puerperium. A nurse must have sound knowledge of the sterilization methods and controls of sterilization so that good quality could be maintained while providing nursing care.

## Biography

Anju Dhir will complete her PhD from Maharaj Vinayak Global University, Amer, Jaipur in 2018. She is teaching in a nursing institute at Shimla, Himachal Pradesh, India, which is an institution for nursing students from graduate to postgraduate levels. She has published more than 12 papers in reputed journals, one e-book on 'Indian Common Krait' and an offline book on Microbiology for degree students of nursing.

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# Pathogen prevalence comparison between cystic fibrosis patients with and without cystic fibrosis-related diabetes

**David Chattin** and **Mirna Ayshoa Al Gabara**
National Jewish Health, USA

Chronic respiratory tract infection leading to respiratory failure is the major cause of morbidity and mortality for patients with cystic fibrosis (CF). Pathogens causing infective exacerbations must be treated appropriately to minimize lung function attrition. Two distinct patient populations were compared to identify trends in recognized pathogens isolated from lung secretions: CF patients with a diagnosis of CF-related diabetes (CFRD) and CF patients without CFRD. Electronic medical records from 2008-2017 were scrutinized, and 4,157 bacterial isolates from 5,324 cultures performed on 88 patients with CFRD were compared to 17,766 isolates from 23,831 cultures from 722 patients without CFRD. Identification of microorganisms was performed using standard clinical microbiology techniques in accordance with guidelines published by the Cystic Fibrosis Foundation in a medical laboratory accredited by the College of American Pathologists. Patients with CFRD had a 7% higher probability of having an organism recognized as a respiratory pathogen isolated than patients without CFRD, but CFRD patients had nearly twice the chance of being infected with *Burkholderia cepacia*, the organism often attributed to end-stage CF disease (growth in 4.3% of cultures from CFRD patients vs. growth in 2.2% of cultures from non-CFRD patients). The findings from this study raise the question of whether or not the CFRD disease state impacts the probability of a patient becoming infected with *B. cepacia* specifically, and what, if any, are the mechanisms of that process. One possible explanation for these results is the correlation between increasing age and higher prevalence of diabetes and the established evidence that age is usually higher when CF patients become infected with *B. cepacia*. Due to the impact, a diagnosis of *B. cepacia* infection has on the CF patient, any factors which impede or promote the growth of that organism will have clinical significance.

## Biography

David Chattin is working as a Research Scientist in Microbiology Laboratory, National Jewish Health, and Denver.

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## We should search the origin of life on the earth in microbes and viruses

**Anindya Das**
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Origin of life on our earth is a mystery. Nobody exactly knows when the first life appeared on the earth. But it is sure that the basic constituents of life like Carbon, Hydrogen, Oxygen, Nitrogen, and Phosphorous combined in a proportionate way to create first life on earth. We know that for any chemical reaction an optimum temperature, pressure, catalyst & reagents are required which if present in suitable proportion, the reaction can occur & the desired product is formed. Probably the origin of life started in the first production of a purine, pyrimidine rings, amino acids & nucleic acid chains & the first life on earth is prokaryotic microbes & viruses. So it is clear that we should search the origin of life in viruses & microbes which are the smallest living particles. Microbes and viruses are the living specks of dust & probably it was formed even when the earth was hot. Archaebacteria, thermophiles, methanogens are the proof that living organisms can even survive in high temperature, acidic pH & absence of oxygen. These microbes originated long before the origin of higher plants, as for the production of fertile land suitable for growth of plants, microbes are required. Inorganic substances, water & microbes for many years formed the layer of fertile land on the surface of the earth. Structure of viruses which are lacking the cytoplasm & definite cellular structure but containing nucleic acid core & protein covering are the proof that nucleic acid strands were first produced but as they cannot survive independently, in presence of water they created the desired products to form cytoplasm & biological membrane to create a primitive cell. To make it logically more comprehensible the origin of life can be divided into several steps discussed below –

Step I – Formation of basic structural elements or building blocks of life like purine & pyrimidine rings, amino acids, glucose, phosphate energy bonds etc.

Step II – Formation of more complex structural forms by chain elongation of basic structural molecules.

Step III – This is the most vital or crucial step where systematic assembling of all these structural elements lead to a structural unit with functional autonomy where all the biochemical reactions can occur automatically, repeatedly in an organized way, making it an autonomic functional unit capable of biochemical synthesis, degradation (metabolism), energy production & self-duplication (reproduction).

### Biography

Anindya Das is a Clinical Microbiologist & Assistant Professor of Microbiology, KPC Medical College & Hospital, Kolkata, India. He is a trusted, patient focused and experienced Microbiologist with a history of serving patients by successfully diagnosing and helping in managing their illnesses and diseases, having rich experience of five years as a Microbiologist.

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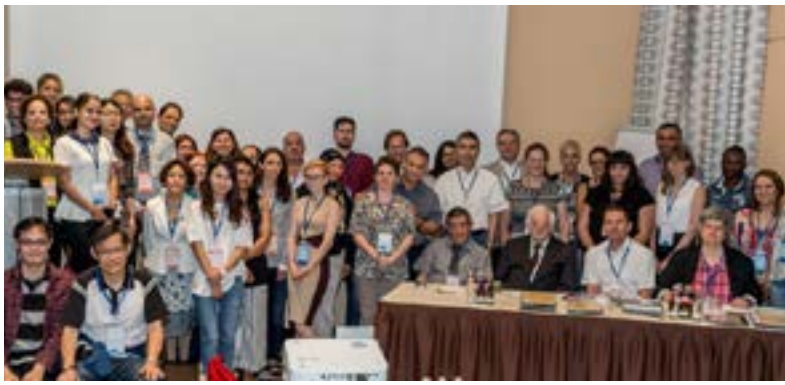

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# Accepted Abstracts

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## Molecular mechanisms governing heme regulation of Jumonji C domain-containing proteins in yeasts

**Li Zhang**
The University of Texas, USA

Heme, iron protoporphyrin IX, is a crucial metallonutrient and a major source of iron for living organisms ranging from bacteria to humans. In humans, 95% of functional iron is in the form of heme. Heme is a central molecule in oxygen metabolism and utilization. It serves as a prosthetic group or cofactor for many proteins and enzymes involved in oxygen utilization and metabolism. The utilization of heme as an iron source strongly influences the virulence of most pathogenic bacteria and some pathogenic fungi. For example, *Candida albicans* secrets a hemolytic factor and uses heme and hemoglobin as an iron source. *Cryptococcus neoformans* can subsist on solely heme- and hemoglobin-sourced iron. Further, *Histoplasma capsulatum* can only utilize iron in the form of heme. Consequently, disrupting heme uptake may be a viable approach to inhibit fungal infection. Additionally, understanding how heme acts to control various cellular processes should provide novel insights into how pathogenic fungi can be suppressed. Particularly, our lab has extensively investigated the molecular mechanisms underlying heme regulation of two yeast regulators, the heme activator protein Hap1 and the heme activator protein Hap1 and multi-functional regulator Gis1. Gis1 is a yeast orthologue of the KDM4/JMJD2 subfamily of proteins containing a Jumonji C (JmjC) domain, which functions as an α-ketoglutarate (AKG) and Fe(II)-dependent histone demethylase. Heme directly binds to Gis1 and promotes transcriptional and demethylase activities of Gis1. The molecular mechanism by which heme promotes Gis1 activities will be discussed.

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# Zero to hero: When there are no *Escherichia coli* showing on a comprehensive stool analysis, to thriving gut health and reversing symptoms

**Raewyn Ma**
Griffith University, Australia

Expected and beneficial bacteria found normally in the gut were totally absent. A comprehensive stool analysis revealed no growth for *Escherichia coli* and low numbers of other expected bacteria under beneficial flora. However, an overgrowth of pathogens was present under Dysbiotic flora. Treatment is given of multiple antibiotics, along with natural herbs which were unsuccessful to resolve symptoms of the overgrowth of pathogens and as well as later finding a parasite. The female patient continued with symptoms previously experienced including the elimination of a bowel movement happening once every 9 days. This continued to cause a stressful situation to this female's wellbeing. The long-term effects of the long absence of elimination cause the buildup of toxins, damage to the intestinal lining and in her case, leaky gut syndrome had occurred where this leads to inflammation forming. Inflammation was of high levels and arthritis pain was evident. This person contacted me and took one of my programs, looking at all areas of a healthy lifestyle, medications, chemicals exposed to, diet and dealing with stress. Adjusting her diet made a significant impact on her health and constipation was improved in 2 days. It's not only consuming whole foods and eliminating processed foods but knowing the right foods for your gut and microbiome. Probiotics were also introduced and made a significant difference as well. The 2 probiotic strains were *Lactobacillus rhamnosus* LGG® and *Bifidobacterium* (BB-12®) strains. The benefits of taking Probiotics support a healthy digestive system and maintaining healthy gut flora. Feed your gut well and symptoms will be improved to being reversed.

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# Decolorization of textile Azo-metal complex dyes by a bacterium isolated from of ceramic industry

**Ferruh Asci**[1], **Gulderen Uysal Akkus**[2] and **Safiye Elif Korcan**[3]
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Synthetic dyes are widely used in textile. İndustry. Bacteria can achieve a higher degree of degradation and even complete mineralization of dyes under optimum conditions. A research was executed where eighteen textile effluent adapted bacterial isolates belonging to the genera, *Bacillus, Acinetobacter, Staphylococcus, Legionella* and *Pseudomonas* were investigated for the potential of textile effluent adapted bacteria in decolorizing it. The present study was aimed to isolate a bacterial strain capable of decolorizing Acid Blue 193, Acid Red 88 and Acid Yellow 42 dye commonly used in textile industries. Isolation of dye decolorizing bacteria was carried out from mud and waste samples of ceramic industry. A total of 16 bacterial isolates were tested for the screening of dyes tested. Bacterial culture growth as a pure culture was streaked out onto plates of azo dye. Screening for resistance to dyes was carried out by using nutrient agar medium containing 0.15g/L. Seven bacterium was found to be resistant against two of dyes.

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# Antibiotic resistance: A global threat

**Vichal Rastogi**
Sharda University, India

**Statement of the problem:** Antimicrobial resistance is an under-appreciated threat to public health in nations around the globe. With globalization booming, it is important to understand international patterns of resistance. The effectiveness of many antibiotics is decreasing due to its extensive use for over a decade or two. This has led to an increase in the number of bacterial strains acquiring resistance to these antibiotics. When pathogenic microorganisms can multiply beyond some critical mass in the face of invading antimicrobials, treatment outcome is compromised; this phenomenon is referred to as antimicrobial resistance (AMR).

**Methodology & Theoretical Orientations:** The retrospective study was carried out in a teaching hospital, Greater Noida to determine the prevalence of multidrug resistance in patients in relation to empirical antibiotic therapy in the hospital. Various samples (pus, urine, blood) were collected for bacterial culture and antibiotic sensitivity. Total 476 bacterial strains were taken in these studies which were isolated from ICU, surgery, obstetrics & gynecology and orthopedics. The 56 bacterial isolates were found to be resistant to multiple drugs. The 29 (51.78%) of resistant bacteria were prevalent in ICU, 12(21.42%) in Gynaecology,10(17.85%) in Surgery, 05(8.92%) in Orthopedics and were studied for their antibiotic sensitivity pattern. The highest numbers of resistant bacteria were Staphylococcus aureus i.e. 15(26.78%) cases followed by 13(23.21%) of *Pseudomonas* sp., 10(17.85%) of *Proteus vulgaris*, 09(16.07%) of *Escherichia coli*, 08(14.28%) of *Klebsiella* sp. and 01(1.78%) of *Citrobacter* sp. All the bacterial strains were resistant to common antibiotics like Penicillin, Amoxicillin, Doxycycline & Cotrimoxazole and some were even resistant to Imipenem. Therefore we have outlined the nature of the antimicrobial resistance problem as an important health and cost issue for the national and international community.

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## Comparative metabolomics and proteomics between *Saccharomyces cerevisiae* hybrids after adaptive evolution in the lignocellulosic substrate for bioethanol production

**Thalita Peixoto Basso**
University of Sao Paulo, Brazil

In the search for alternative sources to fossil fuels, biofuels, such as bioethanol, are shown highly efficient. However, due to ethical issues regarding the use of foods such as sugar and corn in ethanol production, research has been carried out in search of by-products, such as lignocellulosic residues, which can be used in the production of bioethanol. Sugarcane bagasse is a promising by-product that can be used for this purpose, to be available in the plant, does not require transportation expenses, relatively be abundant and cheap. Nevertheless, lignocellulosic hydrolyzate from bagasse is constituted of non-naturally metabolizable sugars by *Saccharomyces cerevisiae* (employed microorganism for ethanol production), and the presence of inhibitors against microorganisms responsible for fermentation, as hydroxymethylfurfural, furfural and acetic acid formed during acid pretreatment of bagasse. The challenge of this work was to circumvent these difficulties through the use of *S. cerevisiae* hybrids tolerant to inhibitors found in the substrate for second-generation ethanol (2GE) production in Brazil. Such hybrids had been obtained in previous work by massal and direct crossings of mutagenized *S. cerevisiae* followed by adaptive evolution. These hybrids were genetically engineered with the cassette X123 containing the three genes responsible for xylose metabolism (xylose reductase, xylitol dehydrogenase, and xylulokinase), and then were followed by adaptive evolution (in YPX and hydrolysate media) in search of an optimal strain for pentose and hexose simultaneous fermentation. Therefore, the objective was to obtain strains with the potential of industrial use in the production of 2GE from sugarcane bagasse. The evolved strain was compared with the original by evaluating their physiological and technological traits. The proteomic and metabolomic analysis was performed in order to better understand the metabolic basis of any improvement observed.

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## Microbial degradation of glyphosate: Integrating first order rate in the estimation of process kinetics

**Amechi S Nwankwegu** and **Yiping Li**
Hohai University, PR China

In the present study, experimental data fit of first-order kinetic model ($LnC_o/C_n$) was evaluated in the estimation of half-life (t½) and degradation constant (k) of glyphosate using four microbial species *including Aspergillus niger* (KC796387), *Serratia* marcescens (AJ233431), *Micrococcus* luteus (AJ536198), and *Fusarium proliferatum* (JF740779) in single inoculation and consortium. The degradation potentials of different inoculated systems were studied and compared with control (no microbial seeding). Initial glyphosate concentration of 64,281mgml$^{-1}$ was the same in all studied microcosms at zero hours and this was respectively reduced to 7070.90 mgml$^{-1}$ by *Aspergillus*, 9,642.20 mgml$^{-1}$ by *Serratia*, 10,927.80 mgml$^{-1}$ by *Micrococcus*, 12,856.60 mgml$^{-1}$ by *Fusarium* and as low as 1,285 mgml$^{-1}$ by the consortium after four weeks incubation. High residual glyphosate concentration of 34,068.90 mgml-1 was reported in the control at the same time and this indicated slow degradation scenario. The result showed that the microbial system involving the consortium displayed an effective rate of glyphosate biodegradation thus obtained high removal efficiency (%RE= 98%) at the end of the study. *Aspergillus* microcosm (89%) had the highest removal efficiency among the single inoculations followed by *Serratia* microcosm (%RE = 85%) then *Micrococcus* and *Fusarium* microcosms 83% and 80% respectively. Poor removal efficacy (47%) was reported in the control. Experimental data adequately fitted into the first order kinetic model and supported the optimal glyphosate utilization in the microbial consortium amended system (t½= 0.70 d, k= 0.45 ± 0.1d$^{-1}$, $R^2$ = 0.96). Model interaction with experimental data in this system generated a curve with absolute linearity than the other systems. However, all the microcosms demonstrated significant glyphosate utilization except the control (t½=4.3d, k=0.16±0.2d$^{-1}$, $R^2$=0.54).Therefore, use of high cell density (consortium) effectively metabolized glyphosate thus can be recommended for pilot scale study in glyphosate removal from the environment.

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# Molecular characterization of cotton infecting *Begomovirus(es)* and DNA satellites associated with cotton leaf curl disease in Pakistan

**Malik Nawaz Shuja**[1,2] and **Muhammad Tahir**[1]
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Cotton leaf curl disease (CLCuD) has been a considerable encumbrance to the cotton production worldwide including Pakistan. The disease has been caused by a complex of *Begomoviruses* in association with cotton leaf curl Multan *alpha*/*beta-satellite*. Previously, we reported a new strain of cotton infecting *Begomovirus* from District Layyah, a probable recombinant of Cotton leaf curl *Kokhran virus* and Cotton leaf curl *Multan virus* – the two predominant species attributed to CLCuD in Pakistan. In this study, symptomatic cotton samples were collected from the cotton belt in Punjab province. Total nucleic acid from leaf tissues was extracted and subjected to PCR. Thirty full-length genomic components were cloned and sequenced, corresponding to *Begomovirus* and associated DNA *alpha*-and *beta-satellite*. Further, a partial dimeric (0.63 mer) DNA-A, (0.55 mer) beta- and (0.9 mer) *alpha*satellite clones were constructed in a binary vector and used in Agrobacterium-mediated inoculation of *Nicotiana benthamiana, N. tabacum, Lycopersicon esculentum, Cucurbita* and *Cucumis sativus*. Leaf curling, deformation and yellowing like symptoms were observed on inoculated plants. Following PCR amplification, the viral DNA, as well as sub-genomic components, were detected in newly emerging non-inoculated leaves. Both molecular and biological studies indicate that this new isolate is a virulent strain of CLCuD and has the potential to aggravate symptomology.

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## Diagnose fungal infectivities of catheters: What are the appropriate techniques to choose

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The nosocomial infections is a real public health problem due to its epidemiological frequency. These infections can occur at any time when using catheters. As a result of their alterations by bacteria and/or fungal microorganisms, these medical devices can be the support of biofilms and, therefore, become a potential source of infection. These alterations are ordered into three classes, named "Types of Infectivities", which may be simple contaminations/colonizations or serious infections. On the other hand, it has been described that the diagnosis of catheter-related candidemia is difficult to prove before the removal of the catheter. Whatever the clinical data is, the only way to assert catheter infection is to remove it and put it into the culture. However, bacterial infectivities were well studied using the technique of (Brun-Buisson et al., 1987); conversely, no technique was designed for yeast. It's why we aimed to adapt the bacterial technique toward the evaluation of the fungal infectivities. In order to check its reproducibility, both techniques were used. The first one consists in carrying out a culture of the sample on agar, and the results were then evaluated by an enumeration of CFU/mL. The second was based on a direct enumeration of yeasts using a hemocytometer and then, the results were reported in cells/mL. The results obtained showed that the Brun-Buisson technique better expresses the fungal contamination or colonization of catheters; however, the modified technique was well appropriate to their infections considering the reduced time for its realization. For that reason, the simultaneous use of both techniques may be the best way to provide the clinician with useful information to guide his/her practical attitude towards establishing an antifungal treatment or not.

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# Phytochemical and antibacterial properties of fermented *Chrysophyllum albidum* cotyledons on methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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The antibacterial activity of extracts from various parts of *Chrysophyllum albidum* against different virulent bacteria has been carried out due to its high usage folk medicine, but no investigation on the fermented seed. This work is aimed at determining the phytochemical and antibacterial properties of methanol extract of fermented cotyledons of *Chrysophyllum albidum* against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Pulverized *C. albidum* cotyledons were fermented aerobically and spontaneously through Solid State Fermentation (SSF), Semi-Solid State Fermentation (sSF) and Liquid State Fermentation (LSF) while the unfermented (UF) served as control. The physicochemical and phytochemical properties of the fermented cotyledons were determined quantitatively. The extracts were assayed for antibacterial properties using the broth dilution method. During fermentation, the temperature increased significantly ($p \leq 0.005$) from 25±0.00 to 39.3±0.47oC with a significant difference of 4.7±0.04 (unfermented) and 3.8±0.08 - 6.3±0.80 pH for the fermenter. The Total titratable acidity (TTA) decreases significantly as fermentation progresses. There was a significant increase in the flavonoids, Trypsin Inhibitor factors, and Phenol in comparison with the control. The other phytochemical properties (Phytic acid, Terpenoids, Tannin, Alkaloids, and Oxalate) were significantly reduced. The Minimum Inhibitory Concentration (MIC) of the methanol extracts of UF, SSF, sSF, and LSF seeds on Methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* range between 12.5-50 mg/ml. It can, therefore, be concluded that fermented cotyledons of *C. albidum* pose an inhibitory effect on methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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# Interference of *Bacillus cereus* and *Clostridium perfringens* isolation from frozen buffalo meat with 16S rRNA Sequencing of *B. cereus* isolate conducted in India

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India produces 1.49 million tons of buffalo meat contributing 45.56% of world buffalo meat production. *Bacillus cereus* and *Clostridium perfringens* both are spore formers. Hence, tolerate high temperature, high pressure, radiation etc. Consequently, germinate in favorable condition. *B. cereus* is aerobic but, *C. perfringens* anaerobic in nature. Both spoil and contaminate frozen buffalo meat product during processing in an abattoir. *B. cereus* causes a series of illness like nausea, vomiting, diarrheal syndrome, emetic syndrome, abdominal pain and produces lethal enterotoxin. *C. perfringens* attributed to protein enterotoxin, during sporulation can infect the wound, gas gangrene, intense abdominal cramps, gas, and diarrhea (nausea and vomiting are rare). Meat contaminated with *C. perfringens* leads to approximately 160,000 disease cases annually in the Netherlands. Vacuum packing contains $CO_2$ and $N^2$ to inhibit the spore formers. But as *B. cereus* is aerobic so if there is low in said gases so *B. cereus* will reappear. In this research, out of 61 frozen meat sample, 52 positive pink color colonies with lecithinase halo zone around the colony on MYP agar and 9 negatives for B. cereus. This article also reveals, among 26 samples of frozen meat all are positive for *C. perfringens* appear as yellowish - gray or black colonies with rotten smell of egg and lecithinase activity on perfringens agar supplemented with TSC or SFP undergo anaerobic condition. Microbiological risk assessment analysis of abattoir air reveals out of 25 samples, 51% are positive for B. cereus, 25 samples of water are 100% free from B. cereus, out of 25 swab samples of slaughter equipment 60% are positive for *B. cereus* and out of 25 swab samples of food handlers are negative. One isolate from positive *B. cereus* colonies isolated and subjected to 16S rRNA sequencing analysis which chromatogram and blast results identified as Bacillus spp. EC2 16S ribosomal RNA gene, partial sequence. Above all, the risk and hazards obtained more with *C. perfringens* as food spoilage contaminant, but, the association of *B. cereus* with meat also prevail equal risk for human in this study. Hence, Microbiological quality monitoring, implementation of international standards, safe production practice of meat may reduce the risk of food born disease.

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## Salmonellosis: A key foodborne disease of the worldwide purport

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Salmonellosis is a momentous worldwide public hygiene matter. Consumption of food of animal paternity such as milk, ice cream, cheese and other milk products, poultry, eggs, meat, fruits, and vegetables bearing the bacteria is thought to be the fundamental origin of human salmonellosis. There are over 2500 serotypes in which Typhimurium and Enteritidis are the guidance serotypes provoke salmonellosis worldwide. Symptoms generally occur 12 to 72 hours after taint and comprehend fever, abdominal pain, diarrhea, nausea, and sometimes vomiting. Near, 2 million individuals are infected each year in the USA. A large outbreak of salmonellosis due to a repast of contaminated ice cream occurred in the USA in 1994, affecting 224,000 persons. Pulse field gel electrophoresis is the elementary typing method for disease prevalence investigation. The sense of *Salmonella* and its evolution in pertinent to ascertain the indemnity and exorcism of foods. It accentuates that dispensation of HACCP at each stage of the food chain is pertinent in order to impede the filth of food from *Salmonella*.

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