



# 47<sup>th</sup> World Congress on Microbiology

September 10-11, 2018 | London, UK

## Posters

Microbiology 2018

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## A monster live in my kitchen!

**Jose Inaki Alava Marquinez**

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Fermentation, cultures of microorganisms, pasteurization, sterilization and many other scientific processes which have their origin in many culinary processes, that are more or less handmade. Today, where biotechnology and microbiology are part of our daily lives, we have to look at the microbiology in our kitchens. When a popular chef on TV, mixes a salad with dirty hands, we never ask any questions on whether it is right or not. These last couple years, our group has been focused on microbiology in the kitchen. Surprisingly, we have found a high quantity of undetected microorganism on, restaurant menus, cutting boards, chef's hands, clothes and accessories. Theoretically modern hygienic procedures ensure the safety of food, in the kitchen of homes and restaurants, but every day, we listen to news about food contamination illnesses. We do not understand why, but a monster is living in our kitchens.

### Biography

Dr.J. Iñaki Álava (M) – Born in Caracas (Venezuela) (1960). He obtained his first degree in Chemistry (MSc) at the University of Basque Country (Spain) in 1981. He moved in 1982 to the Research & Development at the Ramon y Cajal Hospital (Madrid -Spain), performing toxicology research granted by the Spanish Research Council. During the period of 1983 to 1986, he worked in the pharmaceutical company ALTER as a research scientist, participating in the development of new pharmaceutical products. He continued at the Cell Culture Unit in the Haematology Dpto., in the Ramon y Cajal Hospital (Madrid - Spain) for a period of 3 years. He obtained a doctorate (PhD) in Biochemistry by the University of Alcalá de Henares (Madrid) in 1989. During the period from 1988 to 1991 he was Technical Manager of the consultant Company SEIC. Since 1991, Dr. Alava is a member of the research staff of Health Unit at INASMET-Tecnalia As part of the research staff, has participated and led a relevant number of international projects in Biomaterials field and is author of more than 40 scientific papers and member of many scientific societies. Until 2011 is staff of Basque Culinary Center as Doctor Professor of Mondragon University.

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## Novel high throughput macrophage based screening assay to identify anti-Shigella drugs

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Shigella infections cause more than 200,000 deaths per year, being most of these deaths in children in low income countries. Current treatments of choice for Shigella-caused severe diarrhea and dysentery include Ciprofloxacin, Azitromycin, Pivmecillinam and Ceftriaxone; however, in the last decade the development and spread of resistance against most antibiotics poses a challenge in controlling shigellosis morbidity and mortality. Shigella is a highly virulent bacterium which colonizes the gastrointestinal epithelium by transferring from the apical to the basolateral site through M cell invasion followed by macrophage phagocytosis. Shigella induces macrophage apoptosis and is released at the basolateral site of the intestinal epithelium where it invades epithelial cells and spreads. Current phenotypic assays available for Shigella include MIC determination in its extracellular context, but a better surrogate of the infection course in order to identify differentiated hits is desirable. Here, we present the standardization and validation of a high throughput screening macrophage based assay using THP-1 (human monocytic cell line) in 1536 plate format with a luciferase based readout, which allows rapid screening of large compound collections. This methodology opens new avenues for antimicrobial drug discovery and development of novel medicines to treat shigellosis.

### Biography

Working as a GSK scientist for 26 years in different therapeutic areas: Antibacterial, antifungal, Cancer and Malaria, Collaborating with other authors in more than 25 publications in different journals. Currently part of the unit of enteric diseases where as part of its role is the development of new tools for HTS.

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### Notes:

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## Antibacterial activity of *Sepedonium* spp. strains isolated in Chile

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<sup>1</sup>Núcleo de Estudios Ambientales, Universidad Católica de Temuco, Temuco. CHILE;

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The increase in antimicrobial resistance (AMR) during the last two decades and the absence of new antibacterial products in the market has highlighted the importance of searching for compounds active against human and animal pathogenic bacteria. In Chile, this situation is no less worrisome, because many of the most resistant bacteria are present in hospitals and in the community. ARM increases morbidity/mortality, the extension of treatment as well as its total cost, where it has been seen in addition, that more than 25% of the budget of a hospital is dedicated to the cost of antibiotics alone. *Sepedonium* (Hypocreaceae, Ascomycota) infects fruiting bodies of members of the order Boletales, and specimens that produce Peptaibols active against some bacteria have been identified. In this work we studied the antagonistic activity of 28 strains of *Sepedonium* spp., recovered from 7 host species collected from different Chilean sites, against Gram-negative and positive bacteria by the agar diffusion method. *Sepedonium* spp. isolates were recovered from specimens of *Chalciporus*, *Paxillus*, *Xerocomus* and *Suillus* (introduced host species); and from *Boletus* and *Gastroboletus* (endemic host species) collected in Concepcion and St. Juana (Biobio Region), and Angol, Curacautín and Temuco (Araucania Region). Three species were molecularly identified: *S. ampullosporum*, *S. aff. chalcipori*, *S. chrysospermum*. A greater antagonistic activity against Gram-positive bacteria was observed, mainly upon both methicillin-susceptible and resistant *Staphylococcus aureus* and vancomycin-susceptible and resistant *Enterococcus* spp. with inhibition zones >11 mm. No activity was observed against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. FONDEF ID16110286.

### Biography

G. González-Rocha has completed his PhD in Biological Sciences from Universidad de Concepción, Chile in 2002. He is a Full Professor at Universidad de Concepción, Department of Microbiology, Faculty of Biological Sciences. He has published more than 70 papers in peer reviewed journals, mainly related to antibiotic resistance in pathogenic bacteria.

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### Notes:

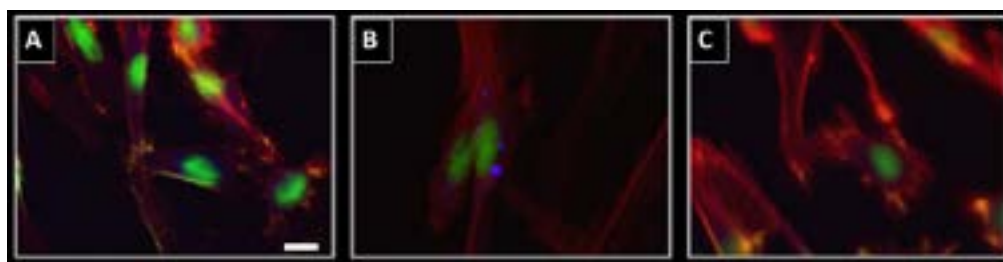
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## Effects of host cholesterol availability and distribution over the susceptibility of infection with *Piscirickettsia salmonis*

Rodrigo Pulgar Tejo, Liliana Montt, Ignacio Chávez, Madelaine Mejías, Angel Parra, Francisca Vera, Verónica Cambiazo and Dinka Mandakovic  
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**Statement of the Problem:** Chile is one of the principal salmon producers in the world, even though this industry has confronted severe economic losses due to infectious diseases in recent years (1). The most important infectious disease in the history of the Chilean salmon farming is the Salmonid Rickettsial Septicaemia (SRS), which is produced by *Piscirickettsia salmonis*, a gram negative facultative intracellular bacterium, phylogenetically related to *Legionella pneumophila* and *Coxiella burnetii* pathogens (2). These bacteria share the capacity to replicate within host membrane-bound cytoplasmic bacteria-containing vacuoles (BCVs) to facilitate their intracellular replication and survival. The formation and maintenance of the BCVs are dependent on the capacity of the bacteria to manipulate host cell vesicular trafficking pathways, which in turn, is dependent of the host cholesterol metabolism (3). The purpose of this work is to study if changes in the abundance and distribution of cholesterol in salmon cells affect the entry and/or intracellular proliferation of *Piscirickettsia salmonis*. **Methodology & Theoretical Orientation:** The alteration of biosynthesis of endogenous cholesterol in salmon cell lines was induced by using HMG-CoA reductase inhibitors (*statins drugs*) (4), while intracellular cholesterol distribution was induced by the NPC1 protein inhibitor (U18666A drug) (5), which produces cholesterol accumulation in late endosomal/lysosomal compartments (Niemann-Pick C disease phenotype). The infection capacity of *Piscirickettsia salmonis* was evaluated in vitro following the typical cytopathic effect (CPE) of cells during infection progression by IFAT, while the intracellular bacterial load was quantified by qPCR. **Findings:** The results indicated that salmon cell lines exposed to increased doses of statins and U18666A (non cytotoxic neither antibiotic concentrations) showed a decreased susceptibility to infection with *Piscirickettsia salmonis*, phenotype that can be partially reversed by mevalonate and M $\beta$ CD, respectively. **Conclusion & Significance:** The results indicated that disturbing cholesterol drugs and the modulation of the cholesterol availability in fish could be possible strategies to combat SRS.



### Recent Publications

1. Rozas M, Enriquez R. (2014). *Piscirickettsiosis* and *Piscirickettsia salmonis* in fish: a review. *Journal of fish diseases* 37, 163-188.
2. Gómez FA, Tobar JA, Henriquez V, Sola M, Altamirano C, Marshall SH. (2013). Evidence of the presence of a functional Dot/Icm type IV-B secretion system in the fish bacterial pathogen *Piscirickettsia salmonis*. *PLoS One* 8, e54934.
3. Isberg RR, O'Connor T, Heidtman M. (2009). The *Legionella pneumophila* replication vacuole: making a cozy niche inside host cells. *Nature Reviews. Microbiology*, 7(1), 13-24.

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4. Bedi O, Dhawan V, Sharma PL, Kumar P (2016). Pleiotropic effects of statins: new therapeutic targets in drug design. *Naunyn Schmiedebergs Arch Pharmacol*.
5. Schultz ML, Krus KL, Lieberman AP. 2016. Lysosome and endoplasmic reticulum quality control pathways in Niemann-Pick type C disease. *Brain Res*. 26. pii: S0006-8993(16)30166-4.

## Biography

Rodrigo Pulgar has his expertise in studying host-pathogen interactions, focusing his research in salmonid intracellular bacterial infections. Since 2016, he has been studying the susceptibility effects of salmon macrophages to *Piscirickettsia salmonis* and *Renibacterium salmoninarum* infection, specially associated to the effects of nutritional requirements and host cholesterol availability and distribution to accomplish bacterial infection. These two bacteria generate infections that cause major economic losses in the salmonid Chilean industry, and which currently have no effective prophylactic treatments. His striking recent results in cell culture have led to a promissory opportunity to apply his findings in whole fish by using new funds he have reached during 2017 and 2018.

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## Screening of carbohydrate-utilizing bacteria as a new probiotic candidate for development of Thai-pigmented rice probiotic products

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<sup>2</sup>Silpakorn University, Sanam Chandra Palace Campus, Thailand

Currently, functional food products are regarded as benefits to human health. Therefore, the development of functional dietary supplements has increased in the food industry. One of the high demand dietary supplements is probiotic products which are now generally popular with consumers. The ideal use of Thai rice cultivars as carriers for probiotics was approached to develop new multifunctional food products. The purposes of this research were to screen for carbohydrate-utilizing bacteria isolated from rice and to evaluate the preliminary probiotic properties. The isolate KPS-FR07 had an ability to utilize carbohydrates of all Thai rice cultivars. The carbohydrate-utilizing bacteria were identified as *Bacillus coagulans* on the basis of morphological and molecular analysis. This strain was tested for its antibacterial activity using agar diffusion method. It was found that antibacterial substances produced by *Bacillus coagulans* KPS-FR07 had an inhibitory effect against *Bacillus cereus*. From the result of haemolytic activity, *Bacillus coagulans* KPS-FR07 was non-haemolytic. In addition, this strain exhibited rapid growth in nutrient broth medium and the highest number of cells was at hour 36 of incubation (10.5 log<sub>10</sub> CFU/mL). Moreover, *Bacillus coagulans* KPS-FR07 also exhibited high antioxidant activity. This strain will be further studied for probiotic properties and developed a novel Thai-rice synbiotic products.

### Biography

Saran Promsai has completed his PhD from Chiang Mai University. He is a Lecturer at Kasetsart University. His work is focused on development of probiotic-supplemented rice products and functional food.

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## Structure and co-occurrence patterns in microbial communities under acute environmental stress reveal ecological factors fostering resilience

Dinka Mandakovic<sup>1</sup>, Jonathan Maldonado<sup>1</sup>, Alexis Gaete<sup>1</sup>, Claudio Latorre<sup>1,2,4</sup>, Rodrigo A Gutiérrez<sup>1,2</sup>, Alejandro Maass<sup>1</sup>, Verónica Cambiazo<sup>1</sup>, Sergio A Navarrete<sup>2,3</sup>, Damien Eveillard<sup>4</sup> and Mauricio Gonzalez<sup>1</sup>

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<sup>2</sup>Pontificia Universidad Católica de Chile, Chile

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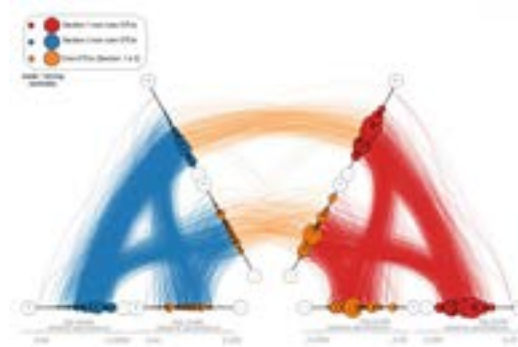
<sup>4</sup>Université de Nantes, Nantes, France

**Statement of the Problem:** Understanding the factors that modulate bacterial community assembly in natural soils is a longstanding challenge in microbial community ecology. In this work, we tried to identify some of these factors by comparing two microbial co-occurrence networks representing bacterial soil communities from two different sections of a pH, temperature and humidity gradient occurring along a western slope of the Andes in the Atacama Desert.

**Methodology & Theoretical Orientation:** A topological graph alignment of co-occurrence networks was used to determine the impact of a shift in environmental variables on OTUs taxonomic composition and their relationships.

**Findings:** We observed that a fraction of association patterns identified in the co-occurrence networks are persistent despite large environmental variation. This apparent resilience seems to be due to: (1) a proportion of OTUs that persist across the gradient and maintain similar association patterns within the community and (2) bacterial community ecological rearrangements, where an important fraction of the OTUs come to fill the ecological roles of other OTUs in the other network. Actually, potential functional features suggest a fundamental role of persistent OTUs along the soil gradient involving nitrogen fixation.

**Conclusion & Significance:** Our results allow identifying factors that induce changes in microbial assemblage configuration altering specific bacterial soil functions and interactions within the microbial communities in natural environments.



### Recent Publications

1. Diaz F P, Frugone M, Gutierrez R A and Latorre C (2016) Nitrogen cycling in an extreme hyperarid environment inferred from delta(15)N analyses of plants, soils and herbivore diet. *Scientific Reports* 6:22226.
2. Faust K and Raes J (2016) CoNet app: inference of biological association networks using Cytoscape. *F1000Research* 5:1519.



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3. Baldassano S N and Bassett D S (2016) Topological distortion and eorganized modular structure of gut microbial co-occurrence networks in inflammatory bowel disease. *Scientific Reports* 6:26087.
4. Louca S, Parfrey L W and Doebeli M (2016) Decoupling function and taxonomy in the global ocean microbiome. *Science* 353:1272–1277.
5. Lauber C L, Hamady M, Knight R and Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75:5111–5120.

## Biography

Dinka Mandakovic has her expertise in soil microbial communities' analyses and isolation of microorganisms associated to extreme environments. She has been working in studying microbial bacteria from the Atacama Desert since 2013, complementing the list of microbial taxa diversity from this extreme environment and assessing pipelines to isolate selective bacteria that could represent useful elements for biotechnological approaches. Recently, she has focused her research in the study of bacteria and fungi with potential attributes as plant growth promoting microorganisms, useful for crop cultivation under agricultural adverse conditions like drought.

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## Histological analysis of tomato roots and arbuscular mycorrhizas interaction

Joanne Alava and J.Inaki Alava

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Mycorrhizas collaborate with plant roots in many ways. But even today not all mechanism of these communication are well understood. We have try to analyze at histopathological level one of these signal, those of disease mutual protection. For signal optimization, we design a matrix of plant distribution that help transmission of protective alert signal, including control plants physically separated. All of them are cultured in orchard or isolate plot both in "ecological culture" conditions. This work try to show how structure of the tomato plants roots change, when are connected to arbuscular mycorrhizas web. The root staining technique is necessary in the work with arbuscular mycorrhizal fungi (AMF). Root branching is one of the most important factors contributing to the creation of a complex root architecture and change if tomato plants are in direct contact with fungal hyphae or not. Although lateral root initiation occurs at some distance from the primary root apical meristem, it has recently been hypothesized that xylem pole pericycle (XPP) cells become primed in the basal meristem, a zone neighboring the root apical meristem. In addition we show distribution of specific miRNA in both tissues plant roots and fungal hyphae.

### Biography

Joanne Alava has completed his professional degrees at the age of 23 years, in Anatomic Pathology and Laboratory Technician in microbiology and clinical analysis. She has done specialization courses in Forensic Criminology and Cell Culture. Now is doing her postgraduate stage in BCC.

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## *Sphingomonas turrisvirgatae* a new agar-degrading *Sphingomonas* species

P Marmo<sup>1</sup>, M M D'Andrea<sup>2</sup>, F Casu<sup>3</sup>, G Di Lallo<sup>1</sup>, L Migliore<sup>1</sup> and M C Thaller<sup>1</sup>

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<sup>2</sup>University of Sienna, Italy

<sup>3</sup>The University of Auckland, New Zealand

*Sphingomonas turrisvirgatae* (type strain MCT13<sup>T</sup>), was isolated from a drainage ditch within a disused system of constructed wetlands, flowing through uncultivated land. Its growth on agarized media is associated with clearing and pitting around the colonies, a feature so far never reported among sphingomonads. The MCT13<sup>T</sup> isolate is characterized by a quite narrow carbon sources assimilation spectrum, and agarase activity is enhanced on poor media. This trait suggests the existence of nutrients-related regulation mechanisms, and/or the possibility of associative interactions with other environmental microorganisms. The analysis of the *S. turrisvirgatae* (MCT13<sup>T</sup>) draft genome, has detected the presence of four different agarase-like enzymes encoding genes. Up to now, agarolytic activity has been more often found in marine-bacteria and has not been observed in any of the characterized or validly published *Sphingomonas* species. A BLAST search, using the amino acid sequences of the four putative agarases, showed the best alignment scores (48 to 71% identity) with three proteins of the uncharacterized *Caulobacter* sp. X isolate, where the genes order is also partially conserved. The preliminary bioinformatics analyses have also detected the presence of genes potentially useful in bioremediation or in industrial applications, rendering *Sphingomonas turrisvirgatae* MCT13<sup>T</sup>, a possible tool for both the degradation of complex carbohydrates and pollutants.

### Biography

P Marmo is a PhD student in Evolutionary Biology and Ecology, Department of Biology, University of Rome "Tor Vergata", 00133 Rome, Italy. His PhD project is centered on the study and characterization of environmental bacteria which could be potentially used either in bioremediation or in industrial applications. A second research topic is the screening and characterization of bacteriophages from environmental samples (see doi: 10.1038/s41598-017-02788-9). He teams up with both teaching and research activities performed in the Laboratory of Microbiology.

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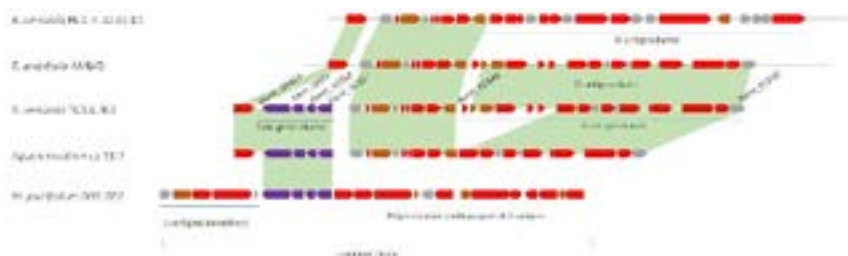
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## Evidence of horizontal gene transfer of a four-genes cluster exclusively in the sugarcane endophytic strain *Burkholderia seminalis* TC3.4.2R3

Sarina Tsui

University of Sao Paulo, Brazil

The *Burkholderia seminalis* strain TC3.4.2R3, isolated from sugarcane roots, belongs to the *Burkholderia cepacia* complex (BCC) and inhibits phytopathogenic fungi such as *Fusarium oxysporum*, *Ceratocystis paradoxa* and *Colletotrichum falcatum*. We show that antifungal production in *B. seminalis* TC3.4.2R3 could be due to the expression of a methyltransferase gene present in a unique four-gene cluster. AlienHunter software analysis revealed that this four-gene cluster has the highest score for potential sequences acquired by horizontal gene transfer. In silico comparisons revealed that the four-genes cluster had no matches with any BCC group. The GC content was not a good methodology to compare and speculate about the horizontal gene transfer since the GC content of the four-gene cluster was too similar to the average GC content of the strain TC3.4.2R3 (61-68%). Using PCR, we examined the presence of the four-gene cluster among 16 environmental *Burkholderia* species. However, no positive PCR products for the analyzed sixteen *Burkholderia* species were recovered. Thus, the methyltransferase gene present in the cluster appeared to be exclusive for TC3.4.2R3, reinforcing its possible origin by horizontal gene transfer. Horizontal gene transfer is a critical factor determining virulence, divergence, and survival in *Burkholderia*, and apparently in *B. seminalis* it occurred as an adaptative mechanism for survival. A better understanding of this four-genes cluster will provide clues about their origin and how the synthesis of antifungal compounds evolves.



### Recent Publications

- Lopes, R., Tsui, S., Gonçalves, P. J., & de Queiroz, M. V. (2018). A look into a multifunctional toolbox: endophytic *Bacillus* species provide broad and underexploited benefits for plants. *World Journal of Microbiology and Biotechnology*, 34(7), 94.
- Batista, B. D., Lacava, P. T., Ferrari, A., Teixeira-Silva, N. S., Bonatelli, M. L., Tsui, S., ... & Quecine, M. C. (2018). Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. *Microbiological research*, 206, 33-42.
- Bonatelli, M. L., Tsui, S., Marcon, J., Batista, B. D., Kitajima, E. W., Pereira, J. O., ... & Quecine, M. C. (2016). Antagonistic activity of fungi from anthracnose lesions on *Paullinia cupana* against *Colletotrichum* sp. *Journal of Plant Pathology*, 197-205.
- Quecine, M. C., Araújo, W. L., Tsui, S., Parra, J. R. P., Azevedo, J. L., & Pizzirani-Kleiner, A. A. (2014). Control of *Diatraea saccharalis* by the endophytic *Pantoea agglomerans* 33.1 expressing cry1Ac7. *Archives of microbiology*, 196(4), 227-234.
- Quecine, M. C., Araújo, W. L., Rossetto, P. B., Ferreira, A., Tsui, S., Lacava, P. T., ... & Pizzirani-Kleiner, A. A. (2012). Sugarcane growth promotion by the endophytic bacterium *Pantoea agglomerans* 33.1. *Applied and environmental microbiology*, AEM-00836.

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## **Biography**

Sarina Tsui is a Bachelor in Biology, she has got Master of Science degree in 2015, now has been doing her PhD in Microbiology since 2016 at University of Sao Paulo in Brazil. Most of the research projects have been involving Genetics, with emphasis on Molecular Genetics of Microorganisms, the main working topics: molecular interaction between beneficial bacteria and plant-host; cloning and gene expression study aiming at biological control through endophytic bacteria, molecular analysis of microorganisms diversity and also study of potential genetic tools applied as plant pathogen control.

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## Long-term active surveillance increased the imported malaria cases at point of entry (PoE), China

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Chinese Academy of Inspection and Quarantine, Beijing-100176, China

In order to implement the elimination programme, we reviewed the epidemiologic characteristics of the cases reported at PoE from 2010 to 2017, so as to provide suggestions to prevent the imported cases away from domestic spreading in China. Symptom-based surveillance was carried out on travellers at the PoE of China, those who have fever and/or from the malaria endemic areas were actively monitored by infrared temperature monitoring or medical inspected by travel health experts. Rapid detect test (RDT), molecular or microscopically detect method was used to diagnose the malaria. Information relating to travel, demographics and others were recorded. During the implementation of the NMEP from 2010 to 2017 in China, the indigenous cases declined continuously. However, the imported cases diagnosed at PoE increased annually. From 2010 to 2017, a total of 1564 cases were reported at PoE, the average age was  $39.1 \pm 10$  (ranged from 4 to 69) years old and male accounted for 95.3% (985/1053). A total of 981 cases are from China, distributed in Angola, Guinea, Nigeria, Ghana and other countries. Among 627 cases detected by typing methods, *Plasmodium falciparum* was the predominant, accounting 82.5%, then was *Plasmodium vivax* of 15.5%, *Plasmodium malariae* and *Plasmodium ovale* were the least of 1.4% and 0.6%, respectively. The implementation of active surveillance at PoE has successfully increased the number of reported malaria cases annually, although the indigenous cases have dramatically declined since 2010. The reason may be due to the implementation of China NMEP, as well as more strict measurements carried out at PoE when MERS, Ebola, Zika and Yellow fever spread globally, partly due to more sensitive methods used in the detection.

### Biography

Liu Lijuan has completed her Ph.D at the age of 35 years from Harbin Medical University and postdoctoral studies from Beijing Institute of Microbiology and Epidemiology. She is the senior researcher of Chinese Academy of Inspection and Quarantine. She has published more than 20 papers in reputed journals.

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## New *Cordyceps militaris* strains by crossing single ascospores and PCR determination of their mating types

**Byungjoo Lee**

Chungcheongnam-do Agricultural Research & Extension Services, South Korea

*Cordyceps militaris*, an entomopathogenic fungus is being studied and artificially cultivated as a medicinal mushroom having many valuable biological and pharmaceutical activities, substituting *C. sinensis* which has been traditionally used as a Chinese herb medicine. *C. militaris* is bipolar heterothallic and has two monokaryotic strains of compatible mating types, which are determined by the single mating type locus MAT-1 consisting of two dissimilar alleles called idiomorphs MAT1-1 and MAT1-2. Isolated ascospore monokaryons were identified on the basis of crossing, fruiting body formation ability and the production of perithecia. Each isolate was determined to be monokaryotic or dikaryotic on the basis of perithecial formation by fruiting tests. Such process is very laborious and time consuming to carry out, but molecular markers of these mating types reduce the amount of effort required for the crossing process. In the PCR tests, two opposite mating types were assayed using two sets of primers specific for *C. militaris*, which were amplified a 191-bp fragment for MAT1-1 and 233-bp fragment for MAT1-2. After the multiplex PCR assays for the rapid detection, 8 MAT1-1 and 7 MAT1-2 were detected and most of 56 mating combinations resulted in F1 hybrids with well-developed perithecial fruiting. Out of 56 hybrids screened, two strains were selected producing high quality club-shaped, bright orange-red fruiting bodies with high cordycepin contents. These new strains may serve as a valuable one for artificial cultivation and industrial-scale production of *C. militaris*.

### Biography

Byungjoo Lee completed his PhD from Chungnam National University and he is an Agricultural Researcher in the Crop Research Division, Chungcheongnam-do Agricultural Research & Extension Services in South Korea for about 20 years and has his expertise in breeding new mushroom cultivars. Recently, he got involved in environmental friendly disease and pest management, particularly against mushroom flies. He investigates the occurrence and development of sciarid flies and the use of plant extracts such as Ginkgo biloba fruits to control mushroom flies in button mushroom cultivation.

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## Development and formulation of liposome encapsulated silver nanoparticles and tea tree oil for skin infections

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Due to the overuse and incorrect prescribing of currently available conventional antimicrobials, resistance has emerged in common skin pathogens; therefore, a pre-antibiotic antimicrobial agent with broad-spectrum activity and a different mode of action against resistant microbes is urgently needed. Candidate agents include plant-derived essential oils and metal nanoparticles that have unique antimicrobial potencies. Recently, tea tree oil (TTO) obtained from *Melaleuca alternifolia* (*Myrtaceae*) leaves is frequently used as a topical antiseptic and disinfectant. On the other hand, silver nanoparticles (AgNPs) have received much attention due to their extraordinary antimicrobial effects against a wide range of microorganisms, including antibiotic-resistant strains. Their small sizes and large surface areas provide a good contact with microorganisms, confer enhanced bioactivity and bioavailability of Ag<sup>+</sup>, and allow better penetration into microbial cells. This study aims to develop liposome-encapsulated silver nanoparticles and TTO topical formulation and to evaluate the antimicrobial efficacy of the developed formula against selected skin-infecting pathogens, including bacteria, fungi, and viruses. The TTO was obtained by steam hydro-distillation of the plant leaves and the AgNPs was synthesized by green synthesis method using leaves aqueous extract. The in vitro antimicrobial activities of the TTO and AgNPs have been evaluated as single agent and/or in liposome-encapsulated combination formula against selected skin-infecting microbes, viz. *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, *Candida albicans*, herpes simplex virus type 1 (HSV-1), and herpes simplex virus type 2 (HSV-2). Bioassay results showed that both TTO and AgNPs possess good antimicrobial properties against tested strains, producing marked inhibition zones (14.8–24.7 mm). On the other hand AgNPs and TTO liposome encapsulated formulation showed better antimicrobial activities with inhibition zones (21.3–26.4 mm). Additionally, tests against HSV-1 and HSV-2 showed that AgNPs and TTO liposome encapsulated form had the strongest antiviral activity, causing 52.0% and 55.1% reduction of the cytopathic effect for HSV-1 and HSV-2, respectively. Transmission electronic microscopy analysis showed severe distortion and loss of cell integrity with cell wall disruption. In conclusion, the liposome encapsulated form of TTO and AgNPs showed a synergistic antimicrobial activity against the selected skin pathogens and these findings could support its use to treat the related skin infections. These results showed the potential of using TTO and AgNPs in liposome encapsulated formulation as a promising delivery system against the possible skin infections caused by the tested strains.

### Biography

Ashraf O Abdellatif is a PhD student at the Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University and has published five papers in reputed journals.

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### Notes:



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## Increased toxicity in long term dry age matured meat

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Long term dry age matured meat, as become a novelty in vanguard's restaurants. Many chef's produce matured meat in many ways. In general, characteristics of matured meat are: Meat from cows or oxen over 4 years, subjected to a process of maturation in a cold room ( 4°C aprox. T° refrigeration) and regulated under exhaustive parameters of temperature and humidity (from 75% approx.). Its maturation can range from 20 to more than 100 days. It is a "gourmet fetish", it is explained to the customer as an added value. The matter, is that from a legal health safety point of view, this product is "safe" for eat. Bacteria are absent, no *E.Coli*, Salmonella or Listeria are present. They can't growth on meat, at these temperature or humidity. But what about the fungi? Fungi may growth and also introduce on meat mycotoxins. Toxicity from mycotoxins isn't acute, is chronic, only high concentrations may act in a acute way. In these work, we look toxicity in long term dry age matured meat from 20 to 160 days but the meat was inoculated with *Aspergillus Brasilensis*, a strain able to produce mycotoxins at 50 days. All samples, were tested raw and fried to observe differences in toxicity induced by heat. Toxicity test are done in *Daphnia Magna*, with continuous dilutions of meat water extract. Results show a clear increase of extract toxicity with time. Up in discussion safety of these kind of matured meats.

### Biography

MS.Paula Rivera (F) – Born in Chile, MS in Food Technology at the Santiago de Chile University, Profesional Degree in Lea Artibai Ikastetxea (Basque Country), stage as innership in Basque Culinary Center at 2017-18.

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# Accepted Abstracts

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## Characterization of cyanobacteria against Gram-positive and Gram-negative bacteria

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Cyanobacteria are a very old group of organisms and represents relics of the oldest photoautotrophic vegetation in the world that occur in freshwater, marine and terrestrial habitats. Cyanobacteria from local habitats seem to be source of potential active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms. Recently, compounds from cyanobacteria have been isolated which display inhibitory effects on bacterial growth, on *Mycobacterium* spp., on fungal growth, on cancer cells, against viruses and enzymes. In the current study, antibacterial activity of different organic extracts prepared from biomass (intracellular) and culture supernatant (extracellular) of laboratory-grown were studied against some Gram-positive and Gram-negative bacteria. Cyanobacteria species viz., *Anabaena variabilis*, *Anabaena fertilissima*, *Nostoc muscorum*, *Nostoc punctiforme*, *Nostoc linckia*, *Nostoc commune*, *Spirulina platensis*, *Westiellopsis prolifica* and *Hapalosiphon* sp. were screened against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi*. Antibacterial activity of extracts varied with the test organisms analyzed. Maximum antibacterial activity was shown against *Staphylococcus aureus*, followed by *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. *Klebsiella pneumoniae* was found to be least susceptible against all tested cyanobacterial extracts. The results obtained from the present study indicate development of antimicrobial substances from different cyanobacterial extracts and their promising utilization in treatment of various infectious diseases.

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## The major risk factors and outcomes of extensively drug-resistant *Acinetobacter baumannii* acquisition in a Moroccan surgical intensive care unit

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*Acinetobacter baumannii* has emerged as an important nosocomial pathogen causing worldwide hospital outbreaks. This micro-organism can cause a wide range of infections, including bacteremia, pneumonia, urinary tract infection, peritonitis, etc. The aim of this study was to determine the risk factors and outcomes related to the acquisition of extensively drug-resistant *Acinetobacter baumannii* in a Moroccan surgical intensive care unit (ICU). This study was conducted from March, 2015 to February 2016, in a 10-bed clinical and surgical tertiary ICU of Ibn Tofail University Hospital Mohammed VI in Marrakesh-Morocco. The adult patients with a first clinical episode of infection were included in the study. The level of antibiotic resistance has been studied by the agar diffusion method, the choice of antibiotic susceptibility testing and interpretation criteria were made as recommended by the Antibiogram Committee of the French Microbiology Society (AC-FMS) and standards of the European Committee on Susceptibility (EUCAST, 2015). Obtained results showed that among 225 isolated bacteria, 85 of these isolates were represented by *A. baumannii* and all *A. baumannii* strains were resistant to imipenem, which represented 72 % of the multidrug resistant bacteria. The increasing and alarming antibiotic resistance levels were observed with gentamicin 94 %, tobramycin 93 %, ciprofloxacin 95 %, amikacin 75 % and only 48 % were resistant to trimethoprim-sulfamethoxazole. However, all *A. baumannii* tested strains were sensitive to colistin. The study shows also that infections associated to this deadly bacteria were mainly represented by pneumonia 48%, catheter-related bloodstream infection 30% and bacteremia 17%. These findings suggest the requirement of constant monitoring of MDR *A. baumannii* in order to decide which patients need isolation and prevent the transmission of this pathogen bacteria in the ICUs.

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## Biodegradation of cotton stalks for protein-rich feed production by co-cultivation of *Pleurotus ostreatus* and *Azospirillum brasilense* grown on solid state fermentation

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The alarming rate of population growth has increased the demand for food production leading to a yawning gap in demand and supply. The rapid growth, high protein content of microbes and their ability to utilize waste feed stocks have made microorganisms the prime candidates for use as animal feed protein supplement. Crop residues are generated in huge quantities every year, creating disposal problems. The potential of co-cultivation of *Pleurotus ostreatus* and *Azospirillum brasilense*, and evaluation of cotton stalks waste were studied. Solid state fermentation (SSF) was carried out for 5, 10, and 15 days at 30°C. Crude protein, cellulose, hemicellulose, lignin, and in vitro dry matter digestibility were analyzed. Activities of ligninolytic enzymes were determined. The lignin, hemicellulose, and cellulose in untreated substrate were 23, 27, and 40%, respectively. Co-cultivation resulted in the degradation of 20% lignin, 15% hemicellulose, and 17% cellulose. *P. ostreatus* degrades lignocellulose with obvious selectivity for lignin. The degradation by-products of *P. ostreatus* was metabolized by *A. brasilense*, leading to more extensive synergistic use of lignocellulosics, which reflects on the enhancement of lignin degradation as well as increased laccase and peroxidase activities. The crude protein content of the fermented cotton stalks was 15%. Extensive study of single cell protein production for bridging the gap between demand and supply is a major step in this direction.

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# 47<sup>th</sup> World Congress on Microbiology

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## Antibiotic Susceptibility Pattern of Salmonella Isolated from Enteric Fever Suspected Patients

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**Background:** Enteric fever is one of the most common diseases encountered worldwide and is endemic in Nepal. This study was conducted to access antibiotic susceptibility pattern of Salmonella isolates from culture positive cases of enteric fever.

**Methods:** Altogether 505 blood samples were collected from patients clinically suspected of enteric fever attending HAMS Hospital. All blood samples were cultured by BACTEC method and sub cultured in blood agar and MacConkey agar plates. All isolates were identified by colony characteristics, biochemical tests and serotyping methods. Antibiotic susceptibility test was performed by modified Kirby Bauer disc diffusion method interpreted with CLSI guideline.

**Result:** Isolation rate of Salmonella species was 3.6%. Among 18 Salmonella isolates, 10 were *S. typhi*, 8 were *S. paratyphi A*. The prevalence rate of infection was high among the age group 11-20 years (50%) and among the male patients. However, there was no significant association of enteric fever with gender of patients ( $p=2.47$ ). All 18 isolates were sensitive to Amoxycillin, Azithromycin, Ceftriaxone and Chloramphenicol, Ciprofloxacin and Ofloxacin. Majority of isolates were sensitive to Cefixime (94.4%), Cotrimoxazole (94.4%) and Cephotaxime (90%). There were no any MDR isolates. Higher percentage of isolates was resistant to Nalidixic acid (87.5%).

**Conclusion:** The decreased susceptibility to Fluroquinolones of *S. typhi* and *S. Paratyphi A* can be correlated with resistance to Nalidixic acid. Commonly used third generation Cephalosporins and rolled back first line drugs be the choice in case of NARS isolates.

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## Insights on Pandemic *Vibrio Parahaemolyticus* Infection

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*Vibrio parahaemolyticus* is currently one of the most important seafood-borne pathogens and is a leading cause of human acute gastroenteritis. A novel O3:K6 strain emerged in 1996, causing the first known pandemic involving this species. This pandemic O3:K6 strain has differentiated into a unique “pandemic clone”, possesses the ability to cause a rapidly spreading form of food poisoning, and is therefore an ongoing public health concern. Scientists have done a lot of work to reveal its origin and evolutionary process and to prevent outbreaks and transmission over the past 22 years. However, the disease burden from this clone remains heavy in many areas. We have to face many challenges from them, for examples:

- More attention should be paid to search for truly accurate and stable pandemic markers. This is contributing to the early identification, prevention, and control of *V. parahaemolyticus* pandemics.
- Environmental pandemic strains bring new threats to seafood safety and human health.
- Traditional typing methods can't fully meet the needs of further studies of pandemic strains.
- WGS is a challenging opportunity to fully understand the microevolution of pandemic strains.
- The emergence and dissemination of multidrug-resistant pandemic strains will be a stumbling block to seafood safety and human health if we sit idly by, while this threat grows around us.
- Thus we appeal for measures from scientists to address the problems caused by infection from pandemic *V. parahaemolyticus*, aiming to make this pathogenic bacterium disappear as soon as possible.

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## Rose-scented Geranium Essential Oil: Chemical composition, Antibacterial and Antifungal effects in vitro and a Real Food System (Orangina Fruit Juices)

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**Background:** In spite of the use of all available means of food protection, spoilage of foods is still a major problem in different parts of the world. Yeasts and filamentous fungi are widely distributed in nature and are responsible for the microbiological spoilage of an extensive range of food. Alternative sources of safe, effective and acceptable natural preservatives need to be explored, such as essential oils. Natural antimicrobials can be used alone or in combination with other novel preservation technologies to facilitate the replacement of traditional approaches in food preservation.

**Material/Methods:** The antifungal activity of Algerian rose-scented geranium (*Pelargonium graveolens* L'Hérit.) essential oil (RGEO) was evaluated against several pathogenic yeasts and filamentous fungi through disc diffusion and vapour diffusion methods. The chemical profile of RGEO, characterized through Gas Chromatography-Mass Spectrometry analysis, revealed citronellol (29.13%), geraniol (12.62%) and citronellyl formate (8.02%) as major components. RGEO exhibited promising antifungal effect against *Candida albicans* and *Aspergillus niger*, with different inhibition zone diameters (IZD) (45–30 mm). Significantly, higher anti-*Candida* activity was observed in the vapor phase. *C. albicans* and *C. famata* were inhibited completely by the RGEO vapors at 60 µL per disc. Moreover, the zone of inhibition increased with increasing oil volume. Furthermore, the anti-yeast efficacy of RGEO, alone and in combination with thermal treatment was evaluated in a real food system (Orangina fruit juices). Results confirmed the effectiveness of RGEO in providing an immediate and significant protection of Orangina juice to yeast proliferation. Using the fragrance of geranium as a food preservative seems fully justified.

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## Agents of non-gonococcal urethritis in pregnant women in onitsha metropolis: *Trichomonas vaginitis* and *candida albicans*

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An investigation on the incidence of *Trichomonas vaginalis* infection amongst pregnant women in Onitsha urban area of Anambra State, Nigeria was carried out between April 2014 and June 2015. The survey was done by random screening of 232 urine and 568 high vaginal swab samples of pregnant women in different gestational periods. The samples were collected during antenatal sessions at three different hospitals in Onitsha designated Hospitals I, II, and III. The organism, *Trichomonas vaginalis* was identified in 78 (9.8%) of the total number of samples. Some of the infected women had *T. vaginalis* infection alone while some were found to have mixed infection of trichomoniasis and candidiasis. *Candida albicans* infection was observed to be of higher incidence occurring in 284 samples (35.5%) of the total number of women studied. Some women (52%) had vaginal discharge with abnormal vaginal odour and some had vulval erythema. Some reported they had yeast infections in the past. Some have had gonorrhoeal infections, while some reported that they had slight temperature rises above 37°C in the night accompanied by abdominal pain.

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## PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCED BY *Escherichia coli* FOUND ON AUTOMATED TELLER MACHINES (ATMs) WITHIN SOKOTO METROPOLIS

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It is no longer a fallacy that environmental objects are grossly contaminated by pathogenic microbes. ATMs especially which is used on daily basis by thousands of people have been reported to be potential habitat for these microbes. The worst case scenario is the presence and ease of spread of Multi-Drug Resistant (MDR) and Extended Spectrum Beta Lactamase (ESBL) producing pathogens via these machines as a result of their huge patronage. The prevalence and fast spread of these MDR and ESBL producing strains constitute an emerging public health concern. This study was conducted to determine the prevalence of MDR and ESBL producing *E.coli* on ATMs within Sokoto metropolis. For this purpose, a total of 194 samples were obtained from 100 ATM swabs, of which 31 isolates were confirmed to be *E.coli*. The isolated *E.coli* species were subjected to antimicrobial susceptibility tests using the modified Kirby Baur disc diffusion method. Commercial antimicrobial discs (Oxoid, UK) used include: Ceftazidime (CTZ, 30ug), Cefotaxime (CTX, 30ug), Gentamycin (CN, 10ug), Augmentin (AMC, 30ug), Ciprofloxacin (CIP, 5ug) and Imipenem (IPM, 10ug). The isolates were further screened for ESBL production and phenotypic confirmatory test was done using CLSI guideline 2015. Confirmation of MBL production was also performed using antibiotic discs containing two Carbapenems (Imipenem IPM, 10ug and Meropenem MEM, 10ug). ESBLs producers were found to be 93.3% while the MBLs producers were found to be 13.3%. It can be concluded that MDR and ESBL producing *Escherichia coli* (*E. coli*) are the most prevalent species isolated and that the species isolated are more sensitive to Gentamycin, Ciprofloxacin and Imipenem.

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## Biotechnological potential of fungi associated to tropical forests of *Quercus*

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The species of the genus *Quercus* are recognized as ecologically dominant in different ecosystems, and commercially valued because of its wood [1-4]. In Mexico it is estimated that there are 174 species of this genus, distributed in temperate and tropical forests [5], even with around 109 endemic species [6]. The tropical forests of *Quercus* in Mexico are subject to strong anthropogenic pressures, such as agriculture and livestock, thus presenting a severe fragmentation [7]. Tree species of this group in native ecosystems associate with a large number of ectomycorrhizal fungi, although currently they are poorly studied [8, 9]. It has been discovered that several species of different genus of ectomycorrhizal fungi have several types of compounds: minerals, vitamins, proteins, amino acids, polysaccharides and some fatty acids among them [10, 11]. Many of these are known by having antimicrobial, antifungal, antiviral, antitumor, anti-inflammatory, antidiabetic, antiangiogenic and antioxidant properties [12, 13]. We have in development a research (CONACYT CB-252431) to recognize the ectomycorrhizal biota associated with *Quercus* species, in pure relicts, based on basidiomes and ectomycorrhizas, in two tropical forests of Mexico, in order to recognize their diversity and also to detect species with economical potential. Currently we have found two dominant *Quercus* species, associated with species of genera of fungi (*Amanita*, *Boletellus*, *Boletus*, *Cantharellus*, *Lactarius*, *Lactifluus*, *Pisolithus*, *Phylloporus*, *Russula*, *Strobilomyces*, *Tomentella*, *Tuber* and *Tylopilus*), which have been reported with some bioactive properties, such as anti-inflammatory, antibacterial, antiviral, anticarcinogenic, antifungal, antioxidant, and cytotoxic properties. Therefore, the tropical forests of *Quercus* in Mexico could be considered as natural reservoirs of fungal species and bioproducts. Sustainable use of some of the fungal species harboured in such relicts would be highly profitable and beneficial for the society. We discuss a list of species which could be subject of rational use and object of many studies aimed at the development of new drugs.

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## Antimicrobial resistance of *Brucella* isolated from seropositives cattle in the department of Tizi Ouzou, Algeria

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**B**ovine Brucellosis is enzootic and widespread in all departments of Algeria, with variable prevalence depending on the region. During the two last decades, the veterinary authorities declared an average infection rate of 1 % in cattle. All the investigations and epidemiological data were based only on serological surveys, and didn't provide the knowledge on the epidemiology of *Brucella* circulating strains. The epidemiological map of prevailing species and biotypes of *Brucella* in Algerian livestock have never been established. Our study aims (i) to isolate and to identify the different biotypes of *Brucella* prevailing in the department of Tizi Ouzou (centre north Algeria) from cattle, and (ii) to study their susceptibility to antibiotics. Between October 2011 and May 2014, 32 samples, including 14 milks and 18 lymph tissues (9 retropharyngeal and 9 supramammary lymph nodes) were collected from 15 infected cattle (detected during screening and slaughtered under the control program) coming from 11 farms, situated in six town of the department. *Brucella* strain characterization were performed according to the technique described by the French standard AFNOR NF U47-105. The sensitivity of the isolated strains to six antibiotics (streptomycin, rifampicin, gentamicin, tetracycline, doxycycline and trimethoprim-sulfamethoxazole) was tested by E-test method. A total of 11 strains of *Brucella* were isolated, 3 from milks (27%) and 8 from lymph nodes (73%), 3 from retropharyngeal (27%) and 5 from supramammary (45,5%). All isolated strains was classified to *Brucella abortus* biovar 3. The E-test revealed that 4 of the isolated strains were resistant to streptomycin (36,4%). This study represents the first investigation in Algeria on the characterisation of *Brucella* strains from cattle. This results may contribute (i) to establish the epidemiological map of the distribution of different *Brucella* strains prevailing in the this region, and (ii) alert to the existence of the antimicrobial resistance of *Brucella*.

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## COMPERATIVE ANALYSIS OF MICROORGANISMS AND HEAVY METAL CONTAMINATION OF PERIWINKLE (*Tympanotonus fuscatus* and *Pachymalania aurita*) IN AKWA IBOM STATE

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Microbiological and heavy metal concentrations of periwinkle (*Tympanotonus fuscatus* and *Pachymalania aurita*) harvested from three aquatic ecosystems in Akwa Ibom State were determined using standard microbiological and chemical techniques. The antibiotic susceptibility profiles of the isolates were determined using disc diffusion technique. The total heterotrophic bacterial counts and total coliform counts of the periwinkle samples ranged from  $3.4 \times 10^7 \pm 0.07$  to  $4.1 \times 10^7 \pm 0.29$  cfu/g and  $2.8 \times 10^5 \pm 0.33$  to  $4.1 \times 10^5 \pm 0.38$  cfu/g, respectively. The total fungal and total vibrio counts ranged from  $1.4 \times 10^5 \pm 0.44$  to  $2.2 \times 10^5 \pm 0.25$  cfu/g and  $9.2 \times 10^4 \pm 2.18$  to  $1.0 \times 10^5 \pm 2.64$  cfu/g, respectively. The faecal coliform and total salmonella-shigella counts ranged from  $1.6 \times 10^5 \pm 1.93$  to  $2.4 \times 10^5 \pm 0.14$  cfu/g and  $1.9 \times 10^5 \pm 2.13$  to  $2.5 \times 10^5 \pm 0.22$  cfu/g, respectively. Eleven bacterial genera comprising *Bacillus*, *Salmonella*, *Escherichia*, *Enterobacter*, *Pseudomonas*, *Shigella*, *Staphylococcus*, *Vibrio*, *Serratia*, *Proteus* and *Streptococcus* were isolated from the samples, while the fungal isolates were *Aspergillus*, *Mucor*, *Penicillium*, *Rhizopus* and *Candida* species. The bacterial isolates were highly resistant to Amoxicillin/Clavulanates and Cefuroxime, while high sensitivities to Gentamicin were observed among *E. coli*, *Staphylococcus* spp. and *Pseudomonas* spp. The concentrations of lead and copper in the periwinkle samples ranged from  $6.076 \pm 0.004$  to  $9.158 \pm 0.05$  mg/l and  $6.621.049 \pm 0.006$  to  $103.850 \pm 0.099$  mg/l, respectively, while the cadmium and chromium concentrations in the samples ranged from  $0.641 \pm 0.980$  to  $1.054 \pm 1.441$  and  $0.050 \pm 1.681$  to  $12.615 \pm 2.051$  mg/kg, respectively. The concentrations of mercury in the samples ranged from 0 to  $1.291 \pm 0.185$  mg/kg. There was a significant correlation ( $p < 0.05$ ) in the microbial loads and heavy metals concentration of the samples. The microbial counts and bioconcentration of heavy metals in the unprocessed periwinkle obtained in this study were beyond specified standard limits by International Commission of Microbiological Specification for Food and Federal Environmental Protection Agency. Consequently, the periwinkle should be properly processed before consumption

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## Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* clinical isolates from Alexandria

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The aim of the present study was to investigate the prevalence of genes coding four different OXA-type carbapenemases, six metallo- $\beta$ -lactamases and three insertion sequences associated with carbapenem resistant *Acinetobacter baumannii* clinical isolates collected from university hospitals in Alexandria, Egypt. A total of 74 non-repetitive carbapenem-resistant *Acinetobacter baumannii* clinical isolates were collected and identified by MALDI-TOF mass spectrometry, VITEK 2 and by amplification of the bla<sub>OXA-51</sub>-like gene. The resistance pattern of the tested isolates was determined by disc diffusion technique and the minimum inhibitory concentration values were determined by agar dilution method. The target sequences were amplified by polymerase chain reaction. bla<sub>OXA-23</sub>, bla<sub>VIM</sub> and ISAb1 were detected in all tested isolates, while bla<sub>OXA-40</sub>, bla<sub>IMP</sub>, bla<sub>GIM</sub> and bla<sub>SIM</sub> were completely absent. In addition, plasmids were isolated from 9 isolates harboring bla<sub>NDM</sub> and transformed into naturally competent carbapenem sensitive *Acinetobacter baumannii* clinical isolates. The obtained recipient transformants showed high level of resistance to carbapenems indicating that bla<sub>NDM</sub> genes were carried on the isolated plasmids. In conclusion, rapid and effective tools should be available in laboratories to detect carbapenem resistant *Acinetobacter baumannii*, a strict hospital infection control policy should be implemented and antibiotic stewardship programs should be applied to limit the spread of multi-drug resistant pathogens.

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## Metal resistant halotolerant psychrophilic bacteria from Tirich Mir glacier, Hindu Kush, Pakistan, with antimicrobial activity against clinical isolates

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Cold adapted bacterial isolates were recovered from Tirich Mir glacier, Hindu Kush, Pakistan. Subsurface ice, sediment and glacier melt water were analyzed geochemically and microbiologically. High concentrations of metals like Fe, Na, Ca, Mg and K were detected in the sediment. Total bacterial counts in melt water, subsurface ice, glacier ice and sediment were calculated as  $6.09 \times 10^4$ ,  $4.39 \times 10^5$ ,  $1.81 \times 10^5$  CFU/ml and  $4.01 \times 10^8$  CFU/g, respectively. Out of 43, 74% of the isolates were tolerant to 10% NaCl, and were able to grow at up to 36%. The isolates showed maximum tolerance to  $\text{Fe}^{+3}$  and least to  $\text{Hg}^{+2}$ . Isolates showed antimicrobial activity against ATCC (American Type Culture Collection) and bacterial and fungal strains isolated from clinical specimens. On basis of 16S rDNA gene analysis, the most abundant group was *Proteobacteria* (53%) dominated by *Beta-proteobacteria* (44%). The subgroups of *Proteobacteria* were dominated by *Beta-proteobacteria* (44%), *Gamma-proteobacteria* (40%) and *Alpha-proteobacteria* (16%). We conclude that non-polar glaciers such as of HKKH region, are a rich source of cultivable microbiota and such unexplored cold and frozen habitat should be further explored for understanding the microbial life style, microbial diversity both culturable and unculturable, the role they are playing in cycling of nutrients, climate change and for their potential industrial applications

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# 47<sup>th</sup> World Congress on Microbiology

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## Phenotypic detection of extended spectrum beta lactamases (ESBL) produced by *Escherichia coli* found on automated teller machines (ATMs) within Sokoto metropolis

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It is no longer a fallacy that environmental objects are grossly contaminated by pathogenic microbes. ATMs especially which are used on daily basis by thousands of people have been reported to be potential habitat for these microbes. The worst case scenario is the presence and ease of spread of multi-drug resistant (MDR) and extended spectrum beta lactamase (ESBL) producing pathogens via these machines as a result of their huge patronage. The prevalence and fast spread of these MDR and ESBL producing strains constitute an emerging public health concern. This study was conducted to determine the prevalence of MDR and ESBL producing *E.coli* on ATMs within Sokoto metropolis. For this purpose, a total of 194 samples were obtained from 100 ATM swabs, of which 31 isolates were confirmed to be *E.coli*. The isolated *E.coli* species were subjected to antimicrobial susceptibility tests using the modified Kirby-Bauer disc diffusion method. Commercial antimicrobial discs (Oxoid, UK) used include: ceftazidime (CTZ, 30 µg), cefotaxime (CTX, 30 µg), gentamycin (CN, 10 µg), ampicillin (AMC, 30 µg), ciprofloxacin (CIP, 5 µg) and imipenem (IPM, 10 µg). The isolates were further screened for ESBL production and phenotypic confirmatory test was done using CLSI guideline 2015. Confirmation of MBL production was also performed using antibiotic discs containing two carbapenems (imipenem IPM, 10 µg and meropenem MEM, 10 µg). ESBLs producers were found to be 93.3% while the MBLs producers were found to be 13.3%. It can be concluded that MDR and ESBL producing *Escherichia coli* (*E. coli*) are the most prevalent species isolated and that the species isolated are more sensitive to gentamycin, ciprofloxacin and imipenem.

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## Assessment of Rhizoremediation potentials of *Arachis hypogaea* Root and stem on Crude oil Contaminated Soil in Abakaliki

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The hazardous effect of crude oil spillage cannot be easily forgotten by the communities, whose major sources of drinking waters are polluted. In this work, contaminated soil was remediated by cultivation of *Arachis hypogaea*. The study revealed the rhizoremediation abilities of *Arachis hypogaea* on the crude polluted oil soil, in which the growth rate against time in weeks was significant at  $SD = 2.8 \pm 0.7$ ,  $p < 0.5$ . The under listed groups of bacteria were isolated and identified by DNA sequence, among which was *Rhizobium trifolii* reported to have ability to fix nitrogen and 64% G+C composition, with four observable mutations at 641, 666, 680 and 693 positions. *Prochlorococcus marinus* seen in the root of *Arachis hypogaea*, planted on crude oil polluted soil and had about 61% G+C composition, five different mutations at various points of 614, 648, 667, 694 and 695. *Rhodopseudomonas*, had about 62% G+C composition, with seven different mutations at various points which include: 561, 572, 630, 641, 667, 680 and 693 while *Bacillus pumilus* with about 45% of the DNA base pairs are G+C. Blast results obtained revealed 93, 72-85, 78-100, 82, 82-92, 72-87, and 86% sequence identities of *Rhizobium Trifolii*, *Rhodopseudomonas*, *Prochlorococcus marinus*, *Alcaligenes aquatillis*, *Escherichia coli*, and *Bacillus pumilus*, respectively. Sequenced prokaryotic organisms obtained from DNA sequencing reactions of 16SR marker were resolved into groups contains AMV-4\_16SF\_2016-04-18\_DO1, and AMV-1\_16SR\_2016-04-18\_AO5 and clustering with known prokaryotic organisms such as *Rhizobium leguminosarum*, *Azotobacter chroococcum*, *Enterobacteraceae* (plasmid R46), HQ 112195\_Rmaldis, *Azotobacter vinelandii*, *Rhizobium pisi* and *Azotobacter armeniacus*. Organic compounds found in the root of *Arachis hypogaea* planted on crude oil polluted soil include trimethyl-3-4 hexanedione, 2-ethyl-3-methyl-1-pentene, dimethyl-1,3,5-cycloheptatriene, 2,3-Dimethylpentane, (Z)-3-Tridecene, (Z)-2-Tridecene, n-Tetradec-1-ene, Hexadecanoic acid and n-Nonane which were mainly crude oil derivatives absorbed into the roots of *A. hypogaea* planted on crude oil polluted soil, while those organic compounds detected from the stem of *Arachis hypogaea* planted on polluted soil include p-Dimethylbenzene, 2,4-dimethylpentane, dipropylmethane, neohexane, 4-methylhexanol, (Z)-2-tridecene, (Z)-4-tetradecene and Pentafluoropropionic acid. Others are n-Tetracosanol, Dimethylfulvene and hexadecanoic acid. The rhizoremediation abilities of *Arachis hypogaea* on the polluted soil may have served as a *bulky* agent with an enhanced water and minerals uptake.

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## Isolation and characterization of amicoumacins produced by *Bacillus pumilus* 35R COPS isolated from *Acartia tonsa* copepod antagonistic agents against pathogenic strains

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A marine bacterium named 35R COPS was isolated from *Acartia tonsa* copepod eggs. The isolate was identified as *Bacillus pumilus* by matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) and by amplification and sequencing of the 16S rRNA gene followed by a phylogenetic analysis. The *B. pumilus* 35R COPS is highly inhibitory against several pathogens, including *Vibrio angularum*, *Vibrio alginolyticus* and *Staphylococcus aureus* (MRSA). The antimicrobial activity of the 35R COPS strain is mainly related to the production of several compounds of the amicoumacin family such as amicoumacin A, amicoumacin B, phosphoamicoumacin A and phosphoamicoumacin B. The Amicoumacin structures were elucidated using their specific UV absorption profiles and their MS/MS fragmentation patterns. Genomic sequencing of the *B. pumilus* 35R COPS strain associated with its bioinformatics analysis show the existence of a gene cluster coding for amicoumacin. This bacterium may be a promising candidate strain as probiotic in aquaculture and as agents for the prevention of various animals and plant infections. The assessment of the cellular toxicity are ongoing.

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## Bacteriocin—antibacterial activity of partially purified bacteriocin produced by *Lactobacillus* species against multi-drug resistant Gram-negative pathogens

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**Statement of the Problem:** Multidrug resistant Gram-negative bacteria are the cause of nosocomial infections. Rapid increase of antibiotics confers bacteria to resist and survive antimicrobials. Certain species of lactic acid bacteria are reported that secrete ribosomally synthesized antimicrobial peptides called bacteriocins. These peptides recognize and kill target cells by rendering their membrane, permeable for various small molecules. The aim of this study was to investigate the potential of lactic acid bacteria to produce antimicrobial substances, especially against Gram-negative bacteria isolated from the hospital, searching for a new alternative to control the nosocomial infection.

**Methodology & Theoretical Orientation:** The bacteriocin was purified by two methods, ammonium sulphate precipitation method and organic solvent method. The physicochemical properties of partially purified bacteriocin were determined by pH, heat and ultraviolet light.

**Findings:** Two bacteriocin producing strains *Lactobacillus plantarum* and *Lactobacillus helveticus* were isolated from traditional yogurt, which showed wide-ranging inhibitory activity against Gram-negative bacteria (*Acinetobacter baumannii* and *E.coli*). Both bacteriocins were active at acidic pH. Exposure to UV light enhanced activity of the *L. helveticus* bacteriocin and had negligible effects on the *L. plantarum* bacteriocin. The *L. plantarum* bacteriocin was heat-stable while *L. helveticus* bacteriocin was heat labile.

**Conclusion & Significance:** The study concludes that partially purified bacteriocin produced by *Lactobacillus helveticus* and *Lactobacillus plantarum* is found effective against the Gram-negative pathogens. It is considered significant because these antimicrobials could be purified and tested in vivo to further be utilized as a biocontrol agent could effectively control hospital acquired disease.

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## Characterization of Soil Actinomycete isolate (AI-2) against Gram-positive and Gram-negative food borne bacteria

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Soil microorganisms are the rich source for the isolation and identification of antimicrobial compounds. Among them, Actinomycetales are an important group. The present study was performed to isolate actinomycete from soil samples having potential activity against the bacteria isolated from food samples. Five soil samples were collected from different places around Dehradun, Uttarakhand. Potential actinomycete colonies were isolated in pure culture from five soil samples using actinomycete isolation agar. Actinomycete isolate named as AI-2 was identified based on the morphological and biochemical test carried out. A total of ninety one food samples were collected in sterilized container. Fifteen Gram-positive bacteria and twenty gram-negative bacteria were isolated using standard methodology. The bacteria isolated were morphologically and biochemically identified. The Actinomycete isolate (AI-2) was found to have moderate to high activity against fifteen gram-positive and twenty gram-negative bacteria. The hexane, ethyl acetate, methanol extracts and mycelium were tested against all bacteria. The ethyl acetate extract showed more potent activity against *S. aureus* and *E. coli*. Mycelium of AI-2 isolate did not exhibit any sort of antibacterial activity against any food borne isolates. MIC values of ethyl acetate extract were found in the range of 512-2048 µg/ml. Since extract showed inhibitory activity against food borne bacteria, it is suggestive that Uttarakhand soil could be an interesting source to explore for antibacterial secondary metabolites.

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## A study of the differences between the interaction and virulence patterns of UK and Nigerian MRSA isolates

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*Staphylococcus aureus* has been identified as the main etiological agent of many infectious diseases in Africa, yet data available about the pathogen are still relatively limited when compared with information on the pathogen from developed countries. There is an indication that African *S. aureus* isolates may be more virulent than their counterparts from other parts of the world; therefore there is need for detailed studies to confirm or refute this possibility. Consequently, this study primarily aimed to ascertain if Nigerian methicillin resistant *S. aureus* (MRSA) were more virulent than UK MRSA. In addition, differences in the level at which UK and Nigerian MRSA isolates interact with mammalian cells was also investigated in this study. Levels of interaction of the UK and Nigerian MRSA isolates with mammalian cell lines (KB epithelial and A549 lung cell lines) were investigated in association, invasion and survival assays. The toxic effects of both UK and Nigerian MRSA on mammalian cell lines (KB epithelial and A549 lung cell lines) were investigated in lactate dehydrogenase colorimetric cytotoxicity assays. No significant difference was observed in the rate at which UK and Nigerian MRSA interacted with both cell lines. However, it was only after live MRSA infected the cell lines that significant differences were observed between the toxic effects of the UK and Nigerian MRSA isolates on the cell lines.

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## Combination of invasive aspergillosis and mucormycosis in hematological patients: A prospective study results

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Invasive aspergillosis (IA) and mucormycosis are severe mycotic infections. In this prospective study during the period 2007–2017, 512 oncohematological patients with IA were included and 5% of them had a combination of IA with mucormycosis. Diagnosis of IA and mucormycosis was made according to EORTC/MSG criteria (2008). The median age of patients with IA and mucormycosis was 31 years (range 5–65), male and female ratio 2:1, adults: 83%. The main underlying conditions were: acute leukemia–64%, lymphoma–21%, chronic leukemia–6%; myelofibrosis, neuroblastoma and aplastic anemia–3% each. *Aspergillus* spp. was isolated from 51% of patients. The main agents of IA were *A. fumigatus*–55%, *A. niger*–17%, *A. flavus*–17%, and *A. nidulans*–11%. Test Platelia *Aspergillus* EIA (Bio-Rad) was positive in 62% of patients. Diagnosis of mucormycosis was confirmed by histology and direct microscopy of biopsy samples in all patients. Cultures of clinical materials were positive in 69% cases: *Rhizopus* spp. (45%), *Lichtheimia corymbifera* (20%), *Rhizomucor* spp. (10%), *Rhizomucor pusillus* (10%), *Mucor* sp. (10%), *Rhizopus stolonifer* (5%). The main sites of infection were lungs (76%), sinuses (17%), central nervous system (10%) and disseminated process was noted in 45% patients. Typical clinical feature of IA and mucormycosis combinations was hemoptysis (24%,  $p=0.008$ ), CT-signs-lesions with cavities (38%), hydrothorax (29%), and a "reverse halo" symptom (17%). Antifungal therapy was used in 76% patients, surgical treatment–34% patients. Overall 12-weeks survival was 38%. An unfavorable prognosis factor was dissemination of mycotic infections ( $p=0.009$ ). The main underlying disease in hematological patients with IA and mucormycosis was acute leukemia (64%). Twelve weeks overall survival was 38%. Disseminated mycosis was an unfavorable prognosis factor.

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# 47<sup>th</sup> World Congress on Microbiology

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## Bacterial degradation of 2, 4-dichlorophenol: Catabolic genes detection and enzyme characterization

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The widespread use of chemicals and their frequent release into the environment is the major cause of pollution, worldwide. Chlorophenolic compounds, such as 2, 4-dichlorophenol (2, 4-DCP), are classified as priority pollutants due to their recalcitrance, persistence, toxicity, carcinogenicity and mutagenicity. The aims of this study were: to isolate 2, 4-DCP degrading microorganisms indigenous to contaminated sites in Durban, South Africa, and establish their degradation potential, to detect the presence of 2, 4-DCP catabolic genes in selected isolates, and to characterize the enzymes involved in the degradation process. Following enrichment in mineral salt medium (MSM) supplemented with 2, 4-DCP (40 ppm) as the sole carbon and energy source, three 2, 4-DCP degrading bacteria were isolated and identified as *Pseudomonas chlororaphis* strain UFB2, *Klebsiella pneumoniae* strain KPNIH39 and *Klebsiella pneumoniae* strain DHQP1002001 based on the 16S rRNA gene sequence analysis. These isolates were able to degrade between 49.01% and 75.11% of 2, 4-DCP within 10 days, with the degradation rate constant ranging between 0.07 and 0.14 mg/L/d. The PCR amplification of the catabolic genes involved in 2, 4-DCP degradation revealed the presence of the phenol hydroxylase (600 and 715 bp), catechol 1, 2-dioxygenase (467 and 507 bp), muconate isomerase (651 and 494 bp), cis-dienelactone hydrolase (491 and 567 bp), and trans-dienelactone hydrolase (491 and 567 bp) in *Pseudomonas chlororaphis* and *Klebsiella pneumoniae*, respectively. The absence of catechol 2,3-dioxygenase gene in these isolates suggests that the organisms most likely follow ortho-pathway for 2, 4-DCP degradation. This study will therefore assist in contaminated sites and alleviate pollution of chlorophenolic compounds in the environment.

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## Lipid Droplets Stabilize Genomic DNA to Survive a Bacterium in Extreme Environments

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The lipid droplet (LD) is a multi-functional organelle that exists in organisms ranging from bacteria to humans. LD research has focused on lipid metabolism while other LD functions remain to be explored. Here, we report two functions of LDs in the bacterium *Rhodococcus jostii* RHA1 (RHA1). 1) The bacterial LDs bound and protected genomic DNA through the major LD protein, microorganism lipid droplet small (MLDS), which increased survival rate of RHA1 during ultraviolet exposure or extremely low nitrogen environments. MLDS expression was regulated by a transcriptional regulator, MLDS regulator (MLDSR) that bound the operator and promoter of the operon of *mlds* and *mldsr*. 2) LDs sequestered MLDSR to control its availability for transcriptional regulation. Together, this study suggests that the LD is a unique endomembrane organelle that can function in a manner analogous to the eukaryotic nucleus to facilitate bacterial survival and adaptation to extreme environments.

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## A Genomic Infection Control Study For *Staphylococcus aureus* In Two Ghanaian Hospitals

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**S**taphylococcus aureus is a notorious hospital acquired pathogen and is implicated in several serious infections such as meningitis, bacteraemia and pneumonia. Whole genome sequencing analysis (WGSA) provides the best resolution for typing of bacterial isolates and has the potential for identification of transmission pathways. We used WGSA to study carriage, environmental and clinical isolates of *S. aureus* from Korle-Bu Teaching Hospital (KBTH) and Lekma hospital (LH) in Ghana where suspected outbreaks of the organisms had recently occurred. The isolates belonged to diverse sequence types (ST) with ST15 and ST152 most common. All isolates carried the blaZ gene, with low prevalence of tetK and dfrG genes also observed. All isolates were mecA negative. The pvl genes were common and observed in distinct lineages that revealed diverse Sa2int phages. The genomics data indicated contamination of various surfaces in the paediatric emergency ward of KBTH by strains of ST15 and highlights the need for a more rigorous disinfection of environmental surfaces at this hospital.

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## SEROLOGICAL, BACTERIOLOGICAL, AND MOLECULAR INVESTIGATION OF BRUCELLOSIS IN BOVINE IN FOUR EGYPTIAN GOVERNORATES

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For the aim of validation, 347 known positive and negative serum samples of large ruminants with a history of *Brucella melitensis* infection were selected. The highest relative sensitivity was achieved by the buffer acidified plate agglutination test (BAPA). The assessed kappa ( $\kappa$ ) agreement in both species indicated a substantial agreement ( $p < 0.05$ ) in case of the BAPA, Rose Bengal plate (RBPT), indirect ELISA (iELISA) and rivanol (Riv.T) tests. According to the data obtained from the receiver operating characteristic curves (ROCs), the area under the ROCs and diagnostic odd ratio, the diagnostic performance of serological tests in cattle was arranged in descending order as follows; BAPAT, Riv.T, RBPT, iELISA, EDTA-modified micro-agglutination test (EDTA-mMAT) and MAT. The equivalent picture in buffaloes was, Riv.T, RBPT, BAPAT, iELISA, EDTA-mMAT and MAT. Eleven *Brucella* field isolates were recovered, whereas four isolates were recognized as *Brucella abortus* biovar 1 from cattle and seven as *Brucella melitensis* biovar 3 from cattle and buffaloes using phenotypic bacteriological typing and molecular speciation (Bruce-ladder PCR). As a result of better diagnostic performance offered by EDTA-mMAT over MAT under investigation, the authors recommended switching from MAT version locally adopted to EDTA-mMAT, and to a limited extent, Riv.T could be used to confirm reactors identified by screening tests. As a result of the frequent isolation of *Brucella melitensis* from the liver of slaughtered seropositive ruminants, it is necessary to amend the ministerial decree No. 1329 of 1999 to contain an explicit clause of liver condemnation as it poses hazards on public health.

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