

IUMS 2022 ABSTRACTS

IS001 / #448

Plenary OPENING CEREMONY & PLENARY 01: CLIMATE CHANGE AND MICROBIAL LIFE 07-20-2022 9:00 AM - 10:00 AM

CLIMATE CHANGE AND MICROBIAL LIFE

Sarah Gurr

Exeter University, Food Security, Exeter, United Kingdom

This paper will consider the impact of climate change on the burden of crop disease. However, of the various microbial challenges to food security, the threat of fungal (and oomycete) infection of our calorie and commodity crops outstrips that posed by bacterial and viral diseases combined (Fisher *et al.*, 2012 *Nature*; Bebber *et al.*, 2013 *Nature Climate Change*; Fones *et al.*, 2020 *Nature Food*).

We face a future blighted by known adversaries, by new variants of old foes and by new diseases. Modern agricultural intensification practices have heightened the challenge - the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance genes, and use of single target site antifungals has hastened emergence of new virulent and fungicide-resistant strains (Fisher *et al.*, 2018 Science: Fisher *et al.*, 2022 *Nature Reviews Microbiology*). Climate change compounds the saga as we see altered disease demographics - pathogens are moving poleward in a warming world (Bebber *et al.*, 2013 *Nature Climate Change*; Chaloner *et al.*, 2022 *Nature Climate Change*).

This presentation will highlight some current notable and persistent fungal diseases. It will consider the evolutionary drivers which underpin emergence of new diseases and manmade "accelerators" of spread. I will set these points in the context of a series of different disease models, initially with statistical correlative models, and thence with more recent mechanistic models - parametrised by data collected from pathogen, host, climate and with a temporal axis (Fones *et al.*, 2020 *Nature Food* 1). Such models have enabled us to look across biological scales, that is from the global level to crop to host-pathogens *per se,* in our development of predictive movement models.

IS002 / #449

Workshop Session WORKSHOP SESSION 01: VIRUS DISCOVERY AND VIROME STUDIES 07-20-2022 10:30 AM - 12:00 PM

METAGENOMIC STRATEGIES TO IDENTIFY SEQUENCES IN THE 'DARK' VIROME

Darren Obbard¹, Megan Wallace², Ben Longdon³, Oumie Kuyateh⁴

¹TBA, Tba, TBA, United Kingdom, ²University of Exeter, Biosciences, TBA, United Kingdom, ³University of Exeter, College Of Life And Environmental Science, Cornwall, United Kingdom, ⁴University of Edinburgh, Institute Of Ecology And Evolution, TBA, United Kingdom

Metagenomic sequencing has dramatically expanded our knowledge of the global virome. However, the majority of metagenomic studies depend on sequence similarity to detect and classify sequences that are viral in origin. Unfortunately, the rapid divergence and ancient relationships of viruses mean that a large proportion of viral sequences may not be easily detectable in this way. This has led to the idea of the 'dark virome': those virus genomes, segments, and fragments that cannot be found by similarity searches. Here I present metagenomic approaches that can be used to identify and classify 'dark' virus sequences. I discuss their application in our studies of the *Drosophila* virome, and particularly the discovery of new Partitiviruses, the discovery of the 'quenyaviruses', and a potential new enigmatic lineage of segmented ssDNA viruses.

IS003 / #450

Workshop Session WORKSHOP SESSION 02: ANTIVIRAL IMMUNITY 07-20-2022 10:30 AM - 12:00 PM

ANTIVIRAL IMMUNITY

<u>Tineke Cantaert</u> TBA, Tba, TBA, Cambodia

The humoral immune response to dengue is complex. Primary infection with dengue induces antibodies with potent protective capacity against homotypic re-infection but also elicits cross-reactive antibodies against other serotypes. While protection against homotypic secondary infection is often long-lasting, cross-protecting antibodies are short-lived, with a half-life from several months up to 3 years. Severe dengue, in which hemorrhage, thrombocytopenia, vascular leakage and shock are the major clinical signs, occurs almost exclusively in patients undergoing heterotypic secondary infection. This is hypothesized to be due to antibody-dependent enhancement, where virus-antibody immune complexes are taken up by FcgR expressing cells which can lead to increased infection and altered innate immune responses. Afucosylated IgG1 glycoforms can bind with higher affinity to FcgRIIIa. We investigated the role of afucosylated IgG, antibody-dependent enhancement and neutralization in a Cambodian pediatric cohort pre- and post-infection (n=18) and from individuals who were inapparent infected (n= 23) or hospitalized (n=48) and classified according to WHO1997 criteria. Neither antibody titers nor neutralizing activity correlated with disease severity in DENV-infected populations. Afucosylation is associated with dengue disease susceptibility as secondary inapparent infected individuals had lower levels of DENVspecific and total afucosylated IgG1 compared to hospitalized cases. In addition, IgG1 afucosylation is associated with dengue disease severity and correlates with biological features of severe disease. Moreover, in hospitalized patients, increased ADE is observed in more severe patients. Furthermore, we found that B cells can be infected with dengue virus which might impact plasmacell development and antibody production. .

IS004 / #620

Workshop Session WORKSHOP SESSION 03: HOST-PATHOGEN INTERACTIONS 07-20-2022 10:30 AM - 12:10 PM

DECIPHERING THE MOLECULAR SECRETS OF SEVENTH PANDEMIC V. CHOLERAE

Melanie Blokesch

EPFL, School Of Life Sciences, Lausanne, Switzerland

Cholera is a devastating diarrheal disease that sickens millions of people each year. Despite incredible progress over the past hundred years in our understanding of the pathogen's virulence mechanisms, the environmental aspects of the causative agent of the disease, the bacterium *Vibrio cholerae*, have so far been insufficiently studied at the molecular level. In my talk, I will address this knowledge gap and present insights into the pathogen's environmental lifestyle including its potential for interbacterial competition on biotic surfaces and its evolvability. I will also show how the bacterium defends itself against mobile genetic elements such as plasmids and phages, which involves dedicated DNA defense systems that are unique to the seventh pandemic clade of this cholera bcteria. I will end my talk with speculations on how these features might have shaped the evolution of the most successful lineage of pandemic *V. cholerae*.

IS005 / #820

Workshop Session WORKSHOP SESSION 04: ANTIMICROBIALS AND ANTIMICROBIAL RESISTANCE 07-20-2022 10:30 AM - 12:00 PM

ECOLOGY AND GENOMICS OF ANTIBIOTIC PRODUCTION IN STREPTOMYCES

Gilles Van Wezel

Leiden University, Institue Of Biology, Leiden, Netherlands

Central in this talk is Streptomyces, a filamentous soil bacterium with a complex life cycle that reproduced via sporulation. Streptomycetes and other members of the Actinobacteria produce some two thirds of all known antibiotics and a range of other natural products and enzymes. The treasures that lie hidden in the actinomycete genomes may well be our final resource in the fight against the rapidly emerging multi-drug resistant pathogens. Many of the biosynthetic gene clusters (BGCs) for antibiotics are poorly expressed in the laboratory, while they are likely expressed in nature.

We harness host-microbe interactions to activate antibiotic production. Where BigPharma has routinely screened bacteria in isolation, in nature bacteria live in complex communities with other organisms, and these often competitive interactions elicit specific responses involving the production of natural products. We discover novel antibiotics by combining multi-omics approaches, such as genome mining, transcriptomics, proteomics and metabolomics.

We have discovered a range of chemical elicitors and growth conditions that allow the effective activation of silent BGCs for specialized metabolites. Application of our multi-omics platform identified several new antibiotics, including the Lugdunomycins, a class of compounds with new chemical scaffold, and a novel subfamily of lantibiotics.

Thus, understanding the ecological conditions under which antibiotic-producing streptomycetes live is a key factor in approaches to activate their production and discover novel bioactive molecules. This, combined with an efficient paired omics drug discovery platform, identified several novel bioactive molecules. Novel drug-discovery approaches and antibiotics we have discovered will be discussed.

IS006 / #623

Workshop Session WORKSHOP SESSION 05: IMPORTANCE OF DATA MANAGEMENT FOR SUSTAINABLE FUTURES (COMCOF) 07-20-2022 10:30 AM - 12:00 PM

CULTURE COLLECTIONS AND SUSTAINABLE DEVELOPMENT GOALS: WFCC'S CATALYST ROLE

Dilber Kurtböke

University of the Sunshine Coast, School Of Science, Technology And Engineering, Sippy Downs, Australia

Culture Collections and Sustainable Development Goals: WFCC's Catalyst Role *lpek Kurtböke*. WFCC PRESIDENT Sustainable development goals (SDGs) defined by the United Nations (UN) in 2015, are intended to be achieved by 2030. The SDGs include: (1) no poverty, (2) zero hunger, (3) good health and well-being, (4) quality education, (5) gender equality, (6) clean water and sanitation, (7) affordable and clean energy, (8) decent work and economic growth, (9) industry, innovation and infrastructure, (10) reducing inequality, (11) sustainable cities and communities, (12) responsible consumption and production, (13) climate action, (14) life below water, (15) life on land, (16) peace, justice and strong institutions, (17) partnerships for goals. One of the key contributing disciplines toward realization of these goals is microbiology. Understanding the functional roles of microorganisms thus have great importance than ever for design and implementation of environmentally friendly and microbiallymediated technologies. The World Federation for Culture Collections (WFCC) plays a major role in all matters related to biological resource centres (BRCs). Examples include (i) standardization and best practice guidelines, (ii) networking, capacity building and education, (iii) postal, guarantine and safety regulations (iv) IP, patent and commercialization, (v) access, policies and legal frameworks and (vi) sustainability of endangered collections. Moreover, WFCC places emphasis on genome level characterization of microorganisms as well as open access to such information. All these above listed aspects have importance as they form foundational platform for microbial biotechnologies to be utilized to achieve the SDGs. Moreover, the WFCC interacts with different global organizations to promote the importance and relevance of the BRCs to the SDGs with emphasis placed on the contributions and the impact BRCs can make on science, health, education, and society. This presentation will communicate WFCC's catalyst role in linking different stakeholders and providing background support to key parties for timely delivery of the SDGs.

IS007 / #624

Workshop Session WORKSHOP SESSION 05: IMPORTANCE OF DATA MANAGEMENT FOR SUSTAINABLE FUTURES (COMCOF) 07-20-2022 10:30 AM - 12:00 PM

WDCM GCM AND 10K TYPE STRAIN SEQUENCING PROJECT

Linhuan Wu

Institute of Microbiology, Chinese Academy of Sciences, Wfcc-mircen World Data Centre For Microorganisms, Beijing, China

WFCC-MIRCEN World Data Centre for Microorganisms (WDCM, http://www.wdcm.org/) has long been committed to facilitating the application of cutting-edge information technology to improve the interoperability of microbial data, promote the access and use of data and information, and coordinate international co-operation between culture collections, scientists and other user communities. To help plenty of culture collections that cannot make their data available online, WDCM launched the Global Catalogue of Microorganisms (GCM) (http://gcm.wdcm.org/) project in 2012. Up to now, GCM (http://gcm.wdcm.org/) has become one of the largest data portals for public service microbial collections and several international culture collection networks, providing data retrieval, analysis, and visualization system for microbial resources. Furthermore, GCM gradually developed into a knowledge base linking taxonomy, phenotype, omics data as well as relative scientific papers and patents with its catalogue information, which currently has aggregated 527,215 strains and other holdings (plasmids and antibodys) deposited in 146 collections from 51 countries and regions. WDCM announced the launching of Global Microbial Type Strain Genome and Microbiome Sequencing Project in the 7th WDCM Symposium, marking the GCM project has begun to enter a new stage (GCM 2.0). Focused on exploring the genomic information of microorganisms, this project has planned to sequence all uncovered prokaryotic type strains together with select eukaryotic type strains, construct a database for genomics data sharing, and also provide online data mining environment. Working groups responsible for selecting bacterial and fungal strains, drafting SOP, managing intellectual property right and legal issues and constructing database have already embarked on the pioneer stage of GCM 2.0. The project will establish a cooperation network for type strain sequencing and functional mining, and complete genome sequencing of over 10000 species of microbial type strains in five years.

IS008 / #625

Workshop Session WORKSHOP SESSION 05: IMPORTANCE OF DATA MANAGEMENT FOR SUSTAINABLE FUTURES (COMCOF) 07-20-2022 10:30 AM - 12:00 PM

DATABASING SYSTEMS FOR THE MANAGEMENT OF BIOLOGICAL DATA

Vincent Robert^{1,2}

¹Westerdijk Institute, Software, Algorithms And Databasing, Utrecht, Netherlands, ²BioAware, Software Developments, Hannut, Belgium

The Westerdijk Institute, formerly known as the Centraalbureau voor Schimmelcultures (CBS-KNAW) is holding the largest living filamentous fungi and yeast collections in the world. To maintain the collection, preserve, use and analyse strain data, efficiently, and finally to publish them on its website, the BioloMICS software was developed and is under constant improvement. BioloMICS allows curators, technicians, and sales managers to manage all the complex aspects associated with collection's operations ranging from data entries, importations, exportations, stock management, label printing, report templates, workflows, data analyses and publications. Our system is currently used worldwide by a large diversity of scientific and microbial collections including the Microbial Resources Research Infrastructure (MIRRI, www.mirri.org) that offers access to more than 400 000 microbial resources of 50+ European culture collections. We have further developed the system to manage nomenclatural and taxonomic data and in 2004, Mycobank (www.mycobank.org) was created, which now serves as official repository for the publication of new fungal names including yeasts. Our system can also be used to manage reference databases such as the one on yeast taxonomy (www.theyeasts.org) or the Atlas of Clinical Fungi (www.clinicalfungi.org).

IS009 / #626

Workshop Session WORKSHOP SESSION 05: IMPORTANCE OF DATA MANAGEMENT FOR SUSTAINABLE FUTURES (COMCOF) 07-20-2022 10:30 AM - 12:00 PM

CULTURE COLLECTIONS AND THE NAGOYA PROTOCOL

Amber Scholz

Leibniz Institute DSMZ, Science Policy, Braunschweig, Germany

Over the past 8 years the Nagoya Protocol has significantly impacted the handling and management of microbial samples. The legal obligations of culture collections, depositors, and users can pose challenges for collection managers and scientists alike. However, there are simple procedures and compliance management strategies that can alleviate concerns and provider clarity along the microbial management chain. The Leibniz Institute DSMZ, as a Registered Collection, under the EU implementation of the Nagoya Protocol will provide some hands-on advice and practical experience gained in recent years. Furthermore, the on-going discussion around the expansion of benefit-sharing obligations to digital sequence information (DSI) will also be presented. Here, input from the scientific community, including IUMS members, is especially critical to bridge the gap between science and policymaking.

IS010 / #729

Bridging Session BRIDGING SESSION 01: MICROBIOMES AND VIROMES IN THE ANTHROPOCENE 07-20-2022 1:00 PM - 2:30 PM

MEM: MYCORRHIZAL FUNGAL NICHES AND VULNERABILITY TO CLIMATE CHANGE

Stephanie Kivlin

University of Tennessee, Knoxville, Ecology And Evolutionary Biology, Knoxville, United States of America

Climate change is affecting every organism on the planet. Yet, some organisms, in particular mycorrhizal fungi have the potential to both be impact by climate change and feedback to accelerate or dampening future warming via their key role in the carbon cycle. Determining fungal environmental tolerances and responses to climate change for unobservable and unculturable taxa belowground, such as arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi, remains one of the largest challenges in microbial ecology. Species distribution models (SDMs) can provide hypotheses for drivers of mycorrhizal fungal niches and therefore vulnerability to climate change. Using SDMs, AM fungi had up to 10x larger spatial ranges and 12x larger niches than EM fungi. Both AM and EM fungal niches were largely structured via climatic axes such as mean annual temperature or potential evapotranspiration with some influence of soil properties, disturbance regime and plant cover. Because EM fungi have smaller niches and smaller geographic extents while also occurring in colder and drier habitats, they may be more susceptible to climate change compared to AM fungi.

IS011 / #631

Workshop Session WORKSHOP SESSION 06: MECHANISMS OF REPLICATION AND ASSEMBLY 07-20-2022 3:00 PM - 4:30 PM

MECHANISMS OF REPLICATION AND ASSEMBLY

Aartjan Te Velthuis

Princeton University, Molecular Biology, Princeton, United States of America

Influenza A viruses are important human pathogens that cause moderate to severe disease. The molecular processes that determine the outcome of influenza virus infection in humans are multifactorial and involve a complex interplay between host, viral and bacterial factors. It is generally accepted that a strong innate immune dysregulation known as the 'cytokine storm' contributes to the pathology of infections with the 1918 H1N1 pandemic or the highly pathogenic avian influenza viruses of the H5N1 subtype. The RNA sensor retinoic acid-inducible gene I (RIG-I) plays an important role in sensing viral RNA and initiating a signalling cascade that leads to interferon expression. During infection, the influenza A virus RNA polymerase produces both full-length and aberrant RNA molecules, such as defective viral genomes (DVG) and mini viral RNAs (mvRNA). Subsequent innate immune activation involves the binding of the viral RNAs to RIG-I. However, it is not clear what factors determine which influenza A virus RNAs are RIG-I agonists and what molecular steps lead to the detection of the infection by RIG-I. We will discuss how innate immune activation by mvRNAs is determined, in part, by transient RNA structures, called template loops (t-loop), that stall the viral RNA polymerase. Impairment of replication by t-loops depends on the formation of an RNA duplex near the template entry and exit channels of the RNA polymerase, and this effect is enhanced by mutation of the template exit path from the RNA polymerase active site. Overall, these findings provide a mechanism that links aberrant viral replication to the activation of the innate immune response.

IS012 / #632

Workshop Session WORKSHOP SESSION 07: VIRUS EVOLUTION AND EMERGENCE 07-20-2022 3:00 PM - 4:30 PM

VIRUS EVOLUTION AND EMERGENCE

<u>Sebastian Lequime</u>¹, Ariane Düx², Philippe Lemey³, Sébastien Calvignac-Spencer² ¹University of Groningen, Groningen Institute For Evolutionary Life Sciences, Groningen, Netherlands, ²Robert Koch Institute, Epidemiology Of Highly Pathogenic Microorganisms, Berlin, Germany, ³KU Leuven, Rega Institute, Leuven, Belgium

Advances in sequencing technologies have allowed the scientific exploration of museum specimens, opening vast arrays of applications grounded in the evolutionary analyses of these genomes. The origin of infectious diseases, including viral diseases, also profit from such efforts. In this presentation, I will present how museomics, coupled with a robust phylodynamic inference framework, triggered a reanalysis of the origin of measles [Düx, Lequime, et al. 2020 Science]. We used a virus genome from a fatal measles case from 1912 in Berlin (Germany), and a molecular clock model that accounts for various sources of rate heterogeneity and long-term purifying selection implemented in a Bayesian phylogenetic framework (BEAST). Our analyses push back the emergence of the measles lineage to Antiquity, around the 6th century BC. Interestingly, this overlaps with the upsurge in population size of human communities both in the Western and Far-Eastern world, which may have reached the critical level needed to maintain the virus during this period. More recently, we used a similar approach to explore the dynamics of the 1918 influenza virus pandemic [Patrono, Vranken, Budt, et al. 2022 Nature Communications]. These results highlight the importance of medical collections in genomic research and plead for dedicating resources to preserving these valuable specimens and associated written sources.

IS013 / #633

Workshop Session WORKSHOP SESSION 08: MICROBIAL COMMUNITIES AND MICROBIOMES 07-20-2022 3:00 PM - 4:30 PM

MICROBIAL COMMUNITIES AND MICROBIOMES 01

Marnix Medema

Wageningen University, Bioinformatics Group, Wageningen, Netherlands

Plants, fungi and bacteria produce a wealth of specialized metabolites, which are of great importance from both ecological and clinical perspectives. Due to the accelerated accumulation of omics data, computational methods have become more and more important to identify these molecules and to assess their biological activities. Here, I will highlight recent work performed in my research group on developing and applying these approaches to discover these molecules and the genes and pathways encoding their production, as well as to study their roles in microbe-microbe and host-microbe interactions in human, plant and animal microbiomes.

IS014 / #635

Workshop Session WORKSHOP SESSION 09: PLANT-MICROBE INTERACTIONS 07-20-2022 3:00 PM - 4:30 PM

DOWNY MILDEW DISEASED PLANTS ARE ENRICHED FOR A DISEASE-ASSOCIATED PHYLLOPSHERE MICROBIOME THAT BENEFITS THE PLANT.

<u>Roeland Berendsen</u>, Pim Goossens, Jelle Spooren, Corné Pieterse, Guido Van Den Ackerveken Utrecht University, Plant-microbe Interactions, Institute Of Environmental Biology, Utrecht, Netherlands

Plant microbiomes have the capacity to enhance disease resistance, and can be dynamically manipulated by the plant in response to attack. Here, we studied the phyllosphere microbiome associated with infection by *Hyaloperonospora arabidopsidis (Hpa)*, the downy mildew of the model-plant Arabidopsis thaliana. In the laboratory, this obligate biotroph is typically cultured by successive weekly passaging over susceptible host plants for long periods of time. We show that the plants used for this passaging have dramatically altered phyllosphere microbiomes following the inoculation of Hpa spores and their associated microbiomes. Cultures of distinct *Hpa* isolates maintained in laboratories in Germany and the Netherlands were dominated by nearly isogenic bacterial genomes. These *Hpa*-associated bacteria increase in abundance when co-inoculated on *Hpa* resistant plants and disease-associated bacteria increase in abundance when co-inoculated microbes have been selectively enriched in the phyllosphere of distinct *Hpa* cultures as a result of downy mildew infection. Moreover, specific members of the *Hpa*-associated microbiome reduced *Hpa* spore production, whereas gnotobiotic *Hpa* consistently outperforms *Hpa* with its co-inoculated microbiome. We hypothesize that *Hpa* laboratory culture are enriched with plant-promoted microbes that reduce disease.

IS015 / #636

Workshop Session WORKSHOP SESSION 09: PLANT-MICROBE INTERACTIONS 07-20-2022 3:00 PM - 4:30 PM

PLANT-MICROBE INTERACTIONS 02

<u>Silke Robatzek</u> LMU Munich, Biocenter, Munich, Germany

Emerging and re-emerging disease epidemics represent ongoing challenges to cultivation of crop plants, a global threat to food security and social stability. One of the recent disease epidemics is caused by *Xylella fastidiosa*, a generalist bacterium that infects a broad range of hosts, now spreading in Europe and threatening olive production. Our interest is to understand how *Xylella* achieves colonization of multiple, genetically diverse species across families. Using Arabidopsis as a model host, we focus on immune responses induced by plant-encoded pattern recognition receptors (PRRs) and explore the role of bacterial-derived extracellular vesicles in shaping the outcome of plant infection. Our data show that extracellular vesicles of bacteria like *Xylella* and *Pseudomonas syringae* pv *tomato* can have diverse functions, including plant protection as well as promoting bacterial growth. This work is supported by the European Research Council (ERC), and the German Research Foundation (DFG).

IS016 / #637

Workshop Session WORKSHOP SESSION 10: TOXICOGENIC FUNGI: BIOLOGY AND TAXONOMY ICFM-TAXONOMY OF ASPERGILLUS AND PENICILLIUM 07-20-2022 3:00 PM - 4:30 PM

MYCOTOXIGENTIC FUNGI AND CLIMATE CHANGE

<u>Angel Medina Vaya</u>

Cranfield University, Environment And Agrifood, Cranfield, United Kingdom

In this talk we aim to provide a review on the most relevant data we have obtained in the last decade with regards to the temporal impacts of interacting climate change related abiotic factors of temperature (30, 37°C) water availability (0.985 and 0.93 water activity (a_w) and exposure to CO₂ (400, 1000 ppm) on A. flavus when colonizing maize. Among the data we included the examination of the temporal changes in aflatoxin biosynthesis genes, global transcriptomic response of this species using RNA sequencing and effects on the metabolite profiles using LC-MS/MS. Effects of CO₂ x temperature x water stress impacted on a number of genes co-expressed in relation to temporal colonisation patterns. Co-expressed genes with a high association with modules were also correlated with increased CO₂ levels. Heat maps of temporal differential expression of the aflatoxin gene cluster genes relative to the control conditions (30°C, 400 ppm CO₂ and 0.985 a_w) showed earlier activation at elevated temperature x CO₂ and water stress compared with control conditions. In addition, there was a stimulation of temporal aflatoxin B₁ (AFB₁) by A. flavus, especially under water stress + elevated CO₂ or temperature. There was also a change in the relative production of AFB1 and aflatoxin B2 (AFB2) and cyclopiazonic acid (CPA). Under elevated temperature and CO₂ at both a_w levels, there was a stimulation of CPA relative to the AF's. Production of up to 20 secondary metabolites showed that there was a switch in clusters of compounds during the early stages of colonization. These included from 3-nitropropionic acid to kojic acid and CPA. These results help in understanding the relationship between climate-related abiotic factors and functional effects on A. flavus in stored maize.

IS017 / #640

Workshop Session WORKSHOP SESSION 10: TOXICOGENIC FUNGI: BIOLOGY AND TAXONOMY ICFM-TAXONOMY OF ASPERGILLUS AND PENICILLIUM 07-20-2022 3:00 PM - 4:30 PM

STUDIES ON THE RELEVANCE OF SECONDARY METABOLITES FOR FUNGAL HABITAT ADAPTATION

Cristian Roder, Rolf Geisen, <u>Markus Schmidt-Heydt</u> Max Rubner-Institut, Safety And Quality Of Fruit And Vegetables, Karlsruhe, Germany

Due to their high adaptability and resilience, filamentous fungi are widespread throughout the world. In recent years there has been a sharp increase in fungal infections in humans and animals and contaminations of crops, as well as the development of resistance to common fungicides and antifungals. In particular, fungi that produce toxic secondary metabolites such as mycotoxins are of concern from a food safety perspective.

Especially in regions of the world with humid climates, epidemic outbreaks of mycotoxicosis are regularly described. In addition, climate change favors the spread and migration of pathogenic and mycotoxic fungal species. Evidence suggests that the production of secondary metabolites by fungi may thereby be viewed as a form of compensation for unfavorable conditions to which the fungus is exposed, and thus may support adaptation to, for example, new temperature regimes and competing microbial populations. Understanding the physiological drivers behind the formation of a particular secondary metabolite by the fungus will allow the development of strategies to reduce it.

But, if the production of a metabolite offers a survival advantage for the fungus, then there is a selection pressure to maintain the formation of this metabolite. This is an important aspect in the development of sustainable prevention strategies against fungal infestation and mycotoxin contamination.

IS018 / #641

Bridging Session BRIDGING SESSION 02: INNATE IMMUNITY AS A FIRST LINE AGAINST VIRUSES, BACTERIA AND FUNGI 07-20-2022 5:00 PM - 6:30 PM

BEYOND RETROVIRUSES: RESTRICTION OF FLAVIVIRUS REPLICATION BY TRIM5ALPHA

Sonja Best

National Institutes of Health, Rocky Mountain Laboratories, Hamilton, United States of America

Beyond retroviruses: restriction of flavivirus replication by TRIM5α TRIpartite Motif (TRIM) proteins belong to a large protein family, many of which are inducible by type I interferon and serve to suppress virus infection through direct interactions with viral proteins. Primate TRIM5α is a consequential inhibitor that suppresses lentivirus replication (e.g HIV-1) in a highly host species- and virus species-specific fashion to limit cross-species transmission of these viruses. Importantly, the antiviral effects of TRIM5α have been thought to function exclusively in the context of lentivirus infection. Our research interests center on the flaviviruses that include significant pathogens that have emerged into human populations from primates (e.g. dengue virus, Zika virus, yellow fever virus) prompting us to determine whether TRIM5α could also function to inhibit flavivirus replication. Surprisingly, this work has revealed a new function for TRIM5α as a potent restriction factor for replication of specific flaviviruses. The mechanisms of restriction, flavivirus escape, and the implications of TRIM5α as an early barrier to flavivirus replication will be discussed.

IS019 / #643

Bridging Session BRIDGING SESSION 02: INNATE IMMUNITY AS A FIRST LINE AGAINST VIRUSES, BACTERIA AND FUNGI 07-20-2022 5:00 PM - 6:30 PM

MEM

<u>Mihai Netea</u> Radboud UMC, Internal Medicine, Nijmegen, Netherlands

Trained immunity: a memory for innate host defense Mihai G. Netea, Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Longterm reprogramming of innate immunity, that induces adaptive traits and has been termed trained immunitycharacterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfection in a T/B-cell-independent manner. In contrast, trained immunity has been shown to be able to induce protection against reinfection in a lymphocyte-independent manner. Non-specific protective effects dependent on trained immunity have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce trained immunity, through induction of histone modifications (methylation, acetylation) and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to a long-term epigenetic reprogramming of its function. The concept of trained immunity represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines and immunotherapeutic approaches.

IS020 / #645

Workshop Session WORKSHOP SESSION 13: GENE EXPRESSION, GENE REGULATION AND DEVELOPMENT 07-20-2022 7:00 PM - 8:30 PM

GENE EXPRESSION, GENE REGULATION AND DEVELOPMENT

Egill Richard¹, Céline Loot², Didier Mazel²

¹Institut Pasteur, Genomes And Genetics, Paris, France, ²Institut Pasteur, Genomes And Genetics Department, Paris, France

Genetic Drivers of Chromosomal Integrons Stability Integrons are mainly known as the genetic agents responsible for the capture and spread of antibiotic resistance determinants among Gramnegative pathogens. They are also are found in the genomes of hundreds of environmental bacterial species, where cassettes convey much broader adaptive functions. These chromosomal integrons are the sources of both the antibiotics resistance cassettes and the integron platforms that convey these cassettes among bacterial pathogens. We are now tackling one central question linked to the integron functioning: - why is the number of cassettes carried in their arrays so different (hundreds in sedentary integrons vs less than ten in mobile ones)? I will present the recent results we obtained which allow to give convincing answers to these two questions.

IS021 / #646

Workshop Session WORKSHOP SESSION 13: GENE EXPRESSION, GENE REGULATION AND DEVELOPMENT 07-20-2022 7:00 PM - 8:30 PM

THE PROMISES AND CHALLENGES OF PROGRAMMABLE RNA ANTIBIOTICS

Jörg Vogel

University of Würzburg, Institute For Moleculare Infection Biology, Würzburg, Germany

Our body is colonized by a vast array of bacteria the sum of which forms our microbiota. The gut alone harbors >1,000 bacterial species. An understanding of their individual or synergistic contributions to human health and disease demands means to interfere with their functions on the species level. Most of the currently available antibiotics are broad-spectrum, thus too unspecific for a selective depletion of a single species of interest from the microbiota. Programmable RNA antibiotics in the form of short antisense oligomers (ASOs) promise to achieve precision manipulation of bacterial communities. These ASOs are coupled to small peptides that carry them inside the bacteria to silence mRNAs of essential genes, for example, to target antibiotic-resistant pathogens as an alternative to standard antibiotics. There is already proof-of-principle with diverse bacteria, but many open questions remain with respect to true species specificity, potential off-targeting, choice of peptides for delivery, bacterial resistance mechanisms and the host response. While there is unlikely a one-fits-all solution for all microbiome species, I will discuss how recent progress in bacterial RNA biology may help to accelerate the development of programmable RNA antibiotics for microbiome editing and other applications.

IS022 / #763

Workshop Session WORKSHOP SESSION 14: MICROBIOME AND HEALTH 07-20-2022 7:00 PM - 8:30 PM

POTENTIAL DRIVERS OF PLASTICITY AND PERSISTENCE OF THE ANIMAL MICROBIOME

Itzik Mizrahi

Ben-Gurion University of the Negev, Life Sciences, Beer Sheva, Israel

In recent years, the mammalian gut has emerged as a fundamentally important microbial environment. Intriguingly, in special cases, complete obligatory dependence exists between the host and its associated microorganisms, whereby the microbial communities perform fundamental processes, such as digestion of the feed for the host. Among the most representative and ecologically relevant examples are ruminants – foregut fermenters that rely critically on their associated gut microbes to digest their plant feed. Due to this paradigmatic obligatory dependence of the host on its microbiome, such systems serve as excellent models to understand fundamental aspects of the ecological and evolutionary relationships between hosts and their microbes. In my lecture, I will discuss some of our recent findings of the rumen microbiome ecosystem stability, development, and interaction with the host.

IS023 / #648

Workshop Session WORKSHOP SESSION 15: OOMYCETES AS BIOCONTROL AGENTS AND PARASITES 07-20-2022 7:00 PM - 8:30 PM

PHYTOPHTHORA ZOOSPORES DISPLAY KLINOKINETIC BEHAVIOUR IN RESPONSE TO A CHEMOATTRACTANT.

Michiel Kasteel

Wageningen University, Laboratory Of Phytopathology & Laboratory Of Cell Biology, Wageningen, Netherlands

Phytophthora zoospores display klinokinetic behaviour in response to a chemoattractant Michiel Kasteel^{1,2}, Joris Sprakel³, Tharun Rajamuthu^{1,2}, Tijs Ketelaar¹ & Francine Govers² ¹Laboratory of Cell Biology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen the Netherlands: ²Laboratory of Phytopathology, Wageningen University & Research, Droevendaalsesteeg 1. 6708 PB, Wageningen, the Netherlands; ³Laboratory of Biochemistry, Wageningen University & Research, Stippeneng 4, 6708 WE, Wageningen, the Netherlands Phytophthora infestans, the causal agent of potato late blight, makes use of dispersal agents called zoospores to rapidly spread and infect. Being motile, these zoospores have been thought to actively track down their hosts using chemical cues such as sugars, amino acids and isoflavonoids. In this study, we used high speed cameras to track zoospores over time and have quantified key trajectory parameters to describe their response to glutamic acid (Glu). We find zoospores to adapt their native run-and-tumble state in response to Glu by greatly increasing the frequency at which they turn. When simulated, we find tuneable tumble frequencies to be sufficient to explain aggregation, implying zoospores to have access to a klinokinetic accumulation strategy to aggregate. We used the same experimental set-up to monitor zoospores of a mutant compromised in heterotrimeric G-protein signalling, and show that their aberrant swimming behaviour is not due to a defect in Glu-chemotaxis, but to aberrantly high and consistent tumbling frequencies.

IS024 / #651

Workshop Session WORKSHOP SESSION 16: SEX DIFFERENCES IN VIRUS HOST INTERACTION & DETERMINANTS OF HOST RANGE 07-21-2022 10:30 AM - 12:00 PM

SEX DIFFERENCES IN VIRUS HOST INTERACTION

Sabra Klein

Johns Hopkins University, Molecular Microbiology And Immunology, Baltimore, United States Minor Outlying Islands

The ongoing COVID-19 pandemic has increased awareness about sex-specific differences in immunity and outcomes following respiratory virus infections. Strong evidence of a male bias in COVID-19 disease severity will be presented based on clinical data and preclinical animals models, which illustrate sex differential immune responses against SARS-CoV-2. Prior to the pandemic, data from other viral infections, including influenza viruses, showed profound sex differences in virus-specific immunity, including locally in the respiratory tract. We have used influenza A viruses to interrogate sex-specific immunity to infection and vaccination. Although males are more susceptible to most viral infections, females possess immunological features that contribute to greater vulnerability to immune-mediated pathology but also better protection following vaccination. Both sex chromosome complement and related X-linked genes (e.g., *TLR7*) as well as sex steroids, including estrogens and androgens, play important roles in mediating the development of sex differences in immunity to respiratory viral infections and vaccination.

IS025 / #652

Workshop Session WORKSHOP SESSION 16: SEX DIFFERENCES IN VIRUS HOST INTERACTION & DETERMINANTS OF HOST RANGE 07-21-2022 10:30 AM - 12:00 PM

DETERMINANTS OF HOST RANGE

Ben Longdon University of Exeter, Centre For Ecology And Conservation, Penryn, United Kingdom

Virus host shifts and the determinants of host range Emerging infectious diseases are often the result of a host shift, where the pathogen originates from a different host species. Using a panel of up to 50 species of Drosophila we have found the host phylogeny is important for understanding the ability of a virus to infect and cause virulence in a novel host. We have examined how environmental factors such as temperature affect susceptibility, and how the tissue tropism of the virus varies across host species. We have gone on to examine correlations in susceptibility to different related viruses finding evidence for virus-by-host species interactions. Finally we have examined how viruses evolve in related host species.

IS026 / #683

Workshop Session WORKSHOP SESSION 16: SEX DIFFERENCES IN VIRUS HOST INTERACTION & DETERMINANTS OF HOST RANGE 07-21-2022 10:30 AM - 12:00 PM

VIRUS DYNAMICS IN RESERVOIR HOSTS

Michelle Wille

University of Sydney, School Of Life And Environmental Science, Sydney, Australia

Human pandemics, including the ongoing pandemic of SARS-CoV-2 have brought a global focus on wildlife as a reservoirs for a variety of zoonotic diseases. Using wild birds as a as a key example, I will interrogate the virus dynamics in not only the context of "one-host, one-virus" systems, but also extend this towards understanding virus dynamics in "multi-host, multi-virus" systems. The ecology of avian influenza viruses in Mallards, the main reservoir, provide an excellent example of a "one-host, one-virus" system that has been intensively assessed. Indeed, this system has played a key role in disentangling the virus dynamics in Europe and North America. However, avian influenza is a multi-host virus; using wild birds in Australia as a model, I will reveal factors that may dictate host range, and how this may be integrated into our understanding of avian influenza virus ecology. While we have made great strides in understanding the dynamics of socioeconomically important avian viruses like avian influenza, we actually have little appreciation for the diversity of viruses found in wild birds, nor the factors that may modulate the virus communities in wild birds. Indeed, meta-transcriptomic studies have revealed both heterogeneity and connectivity of bird viromes in closely related avian species, and further illustrated that many of the factors important for viruses such as avian influenza may modulate entire virus communities. Overall, birds share our cities, and food production birds are globally with billions of birds raised each vear for human consumption. As such, understanding the diversity, ecology and evolution of avian viruses in their wild bird reservoir is imperative.

IS027 / #653

Workshop Session WORKSHOP SESSION 17: MICROBIAL EVOLUTION AND DIVERSITY, AND BACTERIOPHAGES 07-21-2022 10:30 AM - 12:15 PM

EVOLUTION OF RESISTANCE TO LAST-RESORT ANTIBIOTICS

Willem Van Schaik

University of Birmingham, Institute Of Microbiology And Infection, Birmingham, United Kingdom

Bacteria that have acquired resistance to last-resort antibiotics are a major threat to modern healthcare. Here, I will discuss recent work on the evolutionary trajectories that lead to resistance to the antibiotic colistin in the Gram-negative bacteria Escherichia coli and Klebsiella pneumoniae and to the antibiotic vancomycin in the Gram-positive bacterium Enterococcus faecium. Colistin resistance can emerge through the acquisiton of mobilisabile colistin resistance (mcr) genes, but also, and perhaps more commonly, through the accumulation of chromosomal mutations. We show that in Enterobacteriaceae strains isolated from clinical sites, mutations in genes encoding two-component systems are commonly associated with colistin resistance. In all colistin-resistant strains, the lipid A moleties of LPS were modified, through hydroxylation, palmitoylation, and/or the addition of 4-amino-4deoxy-L-arabinose (by ArnT) or phosphoethanolamine. These mutations, and their associated modifications of LPS, can have variable impacts on fitness and virulence characteristics. Vancomycin resistance in *Enterococcus faecium* is mediated through the acquisition of a gene cluster that replaces the terminal amino acids of peptidoglycan precursors from D-Ala-D-Ala to D-Ala-D-lactate or, less commonly, to D-Ala-D-Serine. At least 9 vancomycin resistance gene clusters have been identified, but the vanA-type is globally the most common cause of vancomycin resistance in E. faecium. In a recent genomic study, we studied the genomic epidemiology of the vancomycin-resistant E. faecium strains in a local hospital. We found strains that had the vanA-type vancomycin resistance genes but were phenotypically susceptible. However, these strains quickly reverted, due to plasmid rearrangements and integration in the chromosome, to a resistant phenotype upon growth in the presence of low levels of vancomvcin. Our works shows the remarkable variety of mechanisms by which opportunistic pathogens can acquire resistance to important antibiotics and highlights the need for genome-based diagnostics to rapidly identify resistance mechanisms.

IS028 / #657

Bridging Session BRIDGING SESSION 3: RETURN AFTER A PERIOD OF ABSENCE: DORMANCY, PERSISTENCE AND RELAPSE 07-21-2022 1:00 PM - 2:30 PM

BAM

Yousef Abu Kwaik

University of Louisville College of Medicine, Microbiology, Louisville, United States of America

Legionella pneumophila is an environmental bacterium present in most natural and man-made water sources, where it is present as planktonic, in biofilms or within various protozoan hosts. While protozoa graze on most bacteria as a source of food, L. pneumophila has co-evolved with protozoan species as its natural hosts. Bacterial residence within protozoa is essential for the long term survival of L. pneumophila and its protection from anti-bacterial agents, such as chlorine. Although L. pneumophila replicate within the trophozoite form of amoeba, the cyst from of amoeba is non-permissive for bacterial proliferation but is highly protective for the bacterium for a long period. Upon nutrient depletion in the water system or within dormant amoebic cysts, L. pneumophila becomes dormant but it remains viable but non-culturable (VBNC). The dormant VBNC L. pneumophila can become metabolically active and is resuscitated upon entry into an amoeba host in the trophozoite form. It is not surprising that L. pneumophila has evolved to interfere with encystation of the amoeba host to maintain it in the permissive trophozoite form. This is achieved by the secretion of a Legionella amylase A (LamA) by intravacuolar L. pneumophila into the amoeba cytosol. The injected LamA degrades most of the amoeba stored glycogen, which is the main resource for amoeba to synthesize the double-layer cellulose cell wall of the cyst form. Upon transmission to humans as the accidental host, L. pneumophila proliferate within alveolar macrophages causing pneumonia. Secretion of the amoeba host-adapted LamA by intracellular L. pneumophila into the macrophage cytosol results in an accidental and paradoxical proinflammatory macrophage response with a modest decrease in bacterial proliferation. Therefore, L. pneumophila is resuscitated from the VBNC dormant stage upon entry into amoeba natural host and the bacterium has evolved to interfere with encystation of the amoeba host.

IS029 / #661

Workshop Session WORKSHOP SESSION 18: EUKARYOTIC ANTI-MICROBIAL RESISTANCE 07-21-2022 3:00 PM - 4:30 PM

THE SUCCESSFUL GLOBAL SPREAD OF AZOLE RESISTANCE IN ASPERGILLUS FUMIGATUS; ACCIDENTAL OR INEVITABLE?

Eveline Snelders

Wageningen University & Research, Genetics, Wageningen, Netherlands

The case of resistance development in the common saprobic fungus *Aspergillus fumigatus* to agricultural fungicides and its cross-resistance to medical azoles is causing alarming mortality in vulnerable patient-groups. A crucial piece of evidence that supports the environment-to-patient route is the observation that azole-resistant strains collected from the environment share identical resistance mechanisms with those collected from patients. Only two main resistant haplotypes are dominant in the Dutch *Aspergillus fumigatus* population leading to the question whether this is an accidental or rare event, that has been able to increase in prevalence, or whether azole cross-restance development is inevitable, due to the widespread environmental of azoles.

IS030 / #662

Workshop Session WORKSHOP SESSION 18: EUKARYOTIC ANTI-MICROBIAL RESISTANCE 07-21-2022 3:00 PM - 4:30 PM

ANTIVIRAL DRUG RESISTANCE AND IMMUNE EVASION

<u>Allison Greaney</u>¹, Tyler Starr¹, Rachel Eguia¹, Andrea Loes¹, Helen Chu², Alex Sigal³, Jesse Bloom¹ ¹Fred Hutchinson Cancer Center, Basic Sciences, Seattle, United States of America, ²University of Washington, Department Of Microbiology & Division Of Allergy And Infectious Diseases, Seattle, United States of America, ³Africa Health Research Institute, Na, Durban, South Africa

The SARS-CoV-2 spike receptor binding domain (RBD) is a major target of neutralizing antibodies, but the emergence of viral variants with mutations in key antibody epitopes has raised concerns that antigenic evolution could erode adaptive immunity elicited by prior infection or vaccination. The antigenic consequences of viral mutations are not always clear due to the lag time between the identification and in vitro characterization of a new viral variant of interest. Thus, we developed a prospective approach to characterize all possible mutations to the RBD, even before they are detected in sequenced viral isolates. We used a yeast-display deep mutational scanning system to measure how all possible mutations to the RBD affect antibody binding. We applied this approach to comprehensively map mutations that escape binding by therapeutic monoclonal antibodies and polyclonal antibodies from individuals who had been vaccinated against SARS-CoV-2 or infected with different viral variants (early 2020, Beta variant, or Delta variant viruses). We also determined how the neutralizing antibody response in these individuals were affected by key mutations to the RBD. We found that different infection and vaccination histories lead to polyclonal antibody specificities that are differentially affected by viral mutations. These results indicate that individuals with different immune histories may have a differing susceptibility to erosion of antibody immunity by SARS-CoV-2 evolution.

IS031 / #664

Workshop Session WORKSHOP SESSION 20: EXTREMOPHILES AND ARCHAEA 07-21-2022 3:00 PM - 4:30 PM

EXTREMOPHILES AND ARCHAEA 01

Sonja Albers

University of Freiburg, Molecular Biology Of Archaea, Freiburg, Germany

Archaea use a rotary structure for movement, the archaellum. This structure is homologous to type IV pili and requires ATP for assembly and rotation. Interestingly, many archaea have obtained the chemotaxis system for sensing stimuli and respond to these by changing the rotation direction of the archaellum. However, as the archaellum motor is different from the bacterial flagellum motor, archaea evolved several adaptor proteins so that phosphorylated CheY can initiate a switch in the rotation direction. We have recently solved the structures of archaeal CheY and a unique adaptor protein CheF and can show how these confer the signal to the archaellum motor.

IS032 / #665

Workshop Session WORKSHOP SESSION 20: EXTREMOPHILES AND ARCHAEA 07-21-2022 3:00 PM - 4:30 PM

EXTREMOPHILES AND ARCHAEA

Guy Miezner¹, Israela Turgeman-Grott¹, Anita Marchfelder², <u>Uri Gophna¹</u>, Jutta Brendel² ¹Tel Aviv University, 1. the Shmunis School Of Biomedicine And Cancer Research, Faculty Of Life Sciences, Tel Aviv, Israel, ²University of Ulm, Biology Ii, Ulm, Germany

CRISPR-Cas systems provide heritable acquired immunity against viruses to archaea and bacteria. Cas3 is a CRISPR-associated protein that is common to all Type I systems, possesses both nuclease and helicase activities, and is responsible for degradation of invading DNA. Involvement of Cas3 in DNA repair had been suggested in the past, but later abandoned when the role of CRISPR-Cas as an adaptive immune system was realized. Here we show that in the halophilic archaeon *Haloferax volcanii* a *cas3* deletion mutant exhibits increased resistance to DNA damage compared with the wild-type strain, but its ability to recover quickly from such damage is reduced. A similar damage-resistance phenotype was observed in the naturally CRISPR-less *Haloferax gibbonsii* strain. Analysis of *cas3* mutants revealed that the helicase domain of the protein is the one responsible for DNA damage sensitivity. Epistasis analysis indicated that *cas3*, operated with *mre11* and *rad50* in the microhomology-mediated end-joining pathway of DNA repair, while co-immunoprecipitation shows interaction with RadA, the major DNA recombinase in archaea. These results demonstrate that Cas proteins not only affect DNA repair but have become an integral part of the cellular response to DNA damage.

IS033 / #666

Workshop Session WORKSHOP SESSION 21: MYCOVIRUSES 07-21-2022 3:00 PM - 4:30 PM

MYCOVIRUSES IN ASPERGILLUS FUMIGATUS

loly Kotta-Loizou

Imperial College London, Department Of Life Sciences, London, United Kingdom

Fungi, similar to all species, are susceptible to viral infection. Mycoviruses were initially discovered in the 1960s and the field continues to be active today with ground-breaking discoveries. Of particular interest are mycoviruses that infect human, insect and plant pathogens, modulating the phenotypes of their fungal hosts. Such mycovirus mediated phenotypes may include alterations in morphology, pigmentation, growth and spore production, hypovirulence, hypervirulence, control of endophytic traits, regulation of metabolite production, and drug resistance. In the context of mycovirus-fungus interactions, environmental factors, both abiotic and biotic, play crucial roles in whether and how mycovirus mediated phenotypes are manifested. The family *Polymycoviridae* was recently established and accommodates non-conventionally encapsidated viruses with segmented, double-stranded (ds) RNA genomes that are infectious as purified dsRNA. Each of the four main segments is monocistronic, encoding respectively an RNA-dependent RNA polymerase, a putative scaffold protein, a methyl transferase and an intrinsically disordered, RNA-binding, proline-alanine-serine rich protein that coats the viral dsRNA. Aspergillus fumigatus tetramycovirus-1 (AfuTmV-1) was initially discovered in the human pathogen Aspergillus fumigatus and related viruses have been reported in other Aspergilli. AfuTmV1 is a target of the A. fumigatus antiviral RNA silencing machinery and influences the interactions between A. fumigatus and Pseudomonas aeruginosa, the most common fungus and bacterium respectively in immunocompromised individuals. Beauveria bassiana polymycovirus-1 (BbPmV-1) infects the insect pathogen and popular biocontrol agent Beauveria bassiana and has been widely detected in European B. bassianaisolates, alone or in combination with other mycoviruses. Mycovirus infection differentially affects the efficacy of B. bassiana against insects, including the greater wax moth Galleria mellonella, the army mealworm Tenebrio molitor and the mosquito Anopheles coluzzii, vector of the Plasmodium parasite causing malaria. Such phenomena may be medically, ecologically and economically significant, and merit further investigation.

IS034 / #669

Bridging Session BRIDGING SESSION 4: THE DEVELOPMENT OF RESISTANCE: HOST-PATHOGEN ARMS RACE 07-21-2022 5:00 PM - 6:30 PM

VIR

Graciela Andrei

Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Microbiology, Immunology & Transplantation, Leuven, Belgium

Drug-resistance in herpesviruses is virtually not observed in immunocompetent individuals but it is a wellrecognized problem among different populations of immunocompromised patients. Therefore, in 2009 a Reference and Service Center, RegaVir [Research Group for Antiviral Resistance, (www.regavir.org)], for the diagnosis and typing of drug-resistant herpesviruses was established in Belgium, which was recognized in 2015 as National Reference Center, Phenotyping (drug-susceptibility profile) and/or genotyping {PCR amplification of viral genes involved in drug-resistance [UL97 protein kinase and DNA polymerase (DNA pol) for cytomegalovirus (CMV); thymidine kinase and DNA pol for herpes simplex virus (HSV) and varicella-zoster virus (VZV), and U69 protein kinase and DNA pol for human herpesvirus 6 (HHV-6], followed by DNA sequencing} are used to diagnose drug-resistance among human herpesviruses according to the virus and the type of sample. Today, in the context of the RegaVir platform, we have analyzed >2,500 clinical samples recovered from patients who were refractory to antiviral therapy. Our data show: a) the usefulness of rapid genotyping and/or phenotyping for the adjustment of antiviral therapy, b) a considerable number of isolates bearing mutations linked to drugresistance among the samples that proved positive for virus isolation and/or PCR amplification, c) the identification of unknown genetic polymorphisms and of novel mutations linked to drug-resistance, d) a higher risk for developing drug-resistance infections in the central nervous system, e) emergence of multiple drug-resistance due to the presence of a specific DNA pol mutation or conferred by infection with multiple viral strains, f) compartmentalization of herpesvirus populations, g) relative rapid evolution and heterogenicity of herpesviruses. h) advantage of next generation sequencing over capillary sequencing for detection of emergence of minor populations of drug-resistant viruses, i) an important number of simultaneous and concomitant infection with different herpesviruses among immunocompromised patients

IS035 / #670

Bridging Session BRIDGING SESSION 4: THE DEVELOPMENT OF RESISTANCE: HOST-PATHOGEN ARMS RACE 07-21-2022 5:00 PM - 6:30 PM

BAM

Kimberly Kline^{1,2}

¹Nanyang Technological University, School Of Biological Sciences/scelse, Singapore, Singapore, ²University of Geneva Faculty of Medicine: Universite de Geneve Faculte de Medecine, Microbiology And Molecular Medicine, Genéve, Switzerland

Among Enterococci, intrinsic and acquired resistance to antibiotics such as β-lactams and vancomycin critically limit treatment options for infection with these opportunistic pathogens. We have recently shown that Enterococus faecalis exists as both an extracellular pathogen and also replicates within a variety of mammalian cells, including macrophages, further complicates treatment of infections caused by this opportunistic pathogen. Antimicrobials that enhance the host immune response are emerging as alternative approaches, with the added advantage of overcoming bacterial resistance. Here, we investigate the antibiotic and immunological activity of an FDA-approved anticancer agent in vitro and in vivo against vancomycin resistant Enterococcus faecalis (VRE). In vitro, this drug is a potent antibiotic against Gram-positive bacteria through induction of reactive oxygen species and DNA damage. At subinhibitory concentrations, this drug synergises with vancomycin and lowers the vancomycin concentration required to kill VRE by over 140-fold. This synergy is specific to vancomycin-resistant, but not susceptible, strains because vancomycin renders the resistant strains more permeable to this drug and thus drugmediated DNA damage. In a murine wound infection model, treatment with this drug effectively reduced VRE bacterial numbers by 120-fold and with further reductions when combined with vancomycin. Wounds treated with this drug had significantly more macrophages and pro-inflammatory cytokines compared to untreated wounds. In addition, this drug augmented intracellular bacterial killing by both murine and human macrophages by upregulating the expression of lysosomal hydrolases. These results show that this drug is a potent antibiotic against Gram-positive bacteria, sensitizes VRE to vancomvcin, enhances macrophage recruitment and intracellular bactericidal activity, and represent a promising dual bacteriumand host-targeted therapeutic for overcoming vancomycin resistance.
IS036 / #671

Bridging Session BRIDGING SESSION 4: THE DEVELOPMENT OF RESISTANCE: HOST-PATHOGEN ARMS RACE 07-21-2022 5:00 PM - 6:30 PM

MEM

Pablo Chong

ESPOL University, Life Science Faculty, Cibe, Molecular Biology, Guayaquil, Ecuador

Bananas are very important global export commodities and staple food. One major problem in banana production is the black leaf streak disease (BLSD, also known as black Sigatoka) caused by *Pseudocercospora fijiensis*. Banana's susceptibility to BLSD pushes disease management to excessive chemical use, largely relying on contact and systemic fungicides. Particularly, systemic fungicides are ubiquitous in plant disease control, targeting specific cell molecules and generating resistant mechanism in the species. We will discuss *P. fijiensis* loss of sensitivity towards most common systemic fungicide and the molecular mechanisms behind the resistant. We will also elaborate how the loss of sensitivity in systemic fungicide increases the use of contact fungicide with the consequent negative impact on the environment, human health and crop sustainability. The discussion will be based on the last findings on the pathogen's field populations from various major banana production and wild zones in Colombia, Costa Rica, Dominican Republic, Ecuador, the Philippines, Guadalupe, Martinique and Cameroon. We hope these data significantly contributes to the understanding of the evolution and global distribution of the resistance mechanisms in *P. fijiensis* field populations and facilitates the search for alternative ways to control the disease. The overall reduced fungicide sensitivity calls for the deployment of a wider range of solutions for a sustainable control of this major banana disease.

IS037 / #675

Bridging Session BRIDGING SESSION 05: EMERGING DISEASES IN 'ONE HEALTH' CONTEXT 07-22-2022 9:00 AM - 10:30 AM

VIR

Linfa Wang

Duke-NUS Medical School, Emerging Infectious Diseases, Singapore, Singapore

Title: Bat-borne EIDs in the One Health context Abstract: Since 1994, we have experienced multiple zoonotic diseases outbreaks caused by bat-borne viruses or probable bat viruses: Hendra in Australia (first detected in 1994), Nipah in Malaysia/Singapore (1998/9), SARS outbreak (2002/3), MERS outbreak (2012), large scale Ebola virus outbreak (2014) and the Covid-19 pandemic (2019/20). It is now well recognized that bats are a special group of mammals exceptionally fit as natural reservoir of many different viruses. If we don't change the way we live, farm and eat, it is almost certain that such outbreak will happen again in the near future. The ongoing large scale human infections by SARS-CoV-2 and its variants also pose a real risk of spillback, resulting in the infection of animals by SARS-CoV-2 from humans, which may lead to the establishment of "unnatural" wildlife reservoir for this group of viruses. A holistic One Health approach is urgently needed for better management of animal-human interface in the context of future pandemic preparedness and responses.

IS038 / #676

Bridging Session BRIDGING SESSION 05: EMERGING DISEASES IN 'ONE HEALTH' CONTEXT 07-22-2022 9:00 AM - 10:30 AM

BAM

Ulrich Dobrindt

University of Münster, Institute Of Hygiene, Münster, Germany

Human health is closely linked to animal health and the environment. Avian pathogenic E. coli (APEC) can not only cause severe systemic infections in poultry, but are also considered potential zoonotic agents in food causing extraintestinal infections in humans. The presence of multidrug-resistant (MDR) pathogens in poultry and related foods is increasingly becoming a global public health concern. MDR-APEC pose a fundamental challenge to the effective treatment of bacterial diseases in humans and animals. Our goal is to advance our knowledge of the causes of antimicrobial resistance (AMR) transmission between humans and poultry. Whole-genome sequence analyses allow comprehensive analysis of the relationship between APEC and their resistance properties. On the one hand, we compare E. coli isolates from poultry of different husbandry systems with human clinical E. coli isolates from extraintestinal infections, but also their (AMR) plasmids. Our results confirm the observation that (i) the type of animal husbandry has a significant impact on the colonization of broilers with MDR-E. coli and (ii) healthy poultry can be a source for the spread of transmissible resistance determinants in enterobacteria that can enter the food chain and thus the human population. Our data suggest that some plasmid types and resistance cassettes in broiler chickens can potentially be shared between E. coli and other enterobacteria. We need to study this issue systematically and on a larger scale to gain a better understanding of the drivers and limits of AMR determinant transfer between food-producing animals and humans.

IS039 / #678

Workshop Session WORKSHOP SESSION 22: FRONTIERS IN BACTERIAL STRUCTURAL BIOLOGY 07-22-2022 11:00 AM - 12:30 PM

FRONTIERS IN BACTERIAL STRUCTURAL BIOLOGY 01

Meindert Lamers

Leiden University Medical Center, Cell And Chemical Biology, Leiden, Netherlands

Natural products provide a rich source of potential antimicrobials for use in treating infectious diseases for which drug resistance has emerged. Foremost among these is tuberculosis. Assessment of the antimycobacterial activity of nargenicin, a natural product that targets the replicative DNA polymerase of *Staphylococcus aureus*, revealed that it is a bactericidal genotoxin that induces a DNA damage response in *Mycobacterium tuberculosis* (*Mtb*) and inhibits growth by blocking the replicative DNA polymerase, DnaE1. Cryo-electron microscopy revealed that binding of nargenicin to *Mtb* DnaE1 requires the DNA substrate such that nargenicin is wedged between the terminal base pair and the polymerase and occupies the position of both the incoming nucleotide and templating base. Comparative analysis across three bacterial species suggests that the activity of nargenicin is partly attributable to the DNA binding affinity of the replicative polymerase. This work has laid the foundation for target-led drug discovery efforts focused on *Mtb* DnaE1.

IS040 / #656

Workshop Session WORKSHOP SESSION 22: FRONTIERS IN BACTERIAL STRUCTURAL BIOLOGY 07-22-2022 11:00 AM - 12:30 PM

VISUALIZING SPORE GERMINATION PROTEIN COMPLEXES IN ACTION IN BACTERIAL ENDOSPORES; LESSONS AND CHALLENGES AHEAD FOR THE ANALYSIS OF FUNGAL CONIDIOSPORES

Stanley Brul¹, Juan Wen², Yan Wang², Ronald Breedijk³, Norbert Vischer³, Peter Setlow⁴ ¹University of Amsterdam, Swammerdam Institute For Life Sciences, Amsterdam, Netherlands, ²University of Amsterdam, Mbmfs Swammerdam Institute For Life Sciences, Amsterdam, Netherlands, ³University of Amsterdam, Lcam Swammerdam Institute For Life Sciences, Amsterdam, Netherlands, ⁴UConn Health, Mol. Biol. And Biophysics, Farmington, United States of America

Bacterial endospores are sturdy structures that resist environmental challenges such as thermal insult. enzymatic degradation and harsh chemicals. They can be considered survival capsules of the organisms that generate them i.e. strictly anaerobic Clostridia common to the gut as well as aerobic Bacilli. The challenge for the spores is to respond to favourable environmental changes rapidly and efficiently whilst mitigating as much as possible untimely germination events thus preventing too early outgrowth and poor efficiency of the sporulation event in terms of cellular survival under adverse environmental conditions. Bacterial endospores and their molecular composition may also be seen as a model for the build-up and molecular challenges facing other survival structures in the microbial world, including fungal conidiospores. In order for the spores to sense the environment and initiate the onset of spore germination, germinant receptor proteins need to capture small molecular weight germinants such as amino acids, purines and/or bile salts. These events can be captured by assessing the presence of germination protein complexes and their possible conformational changes. Here we describe the visualization of germinant receptor proteins in bacterial spores, their interaction with scaffold proteins in the formation of a germination protein complex (germinosomes) and the dynamic changes that occur during germination in both the germinosomes and a calcium dipicolinic acid (CaDPA) specific channel protein SpoVAEa. The latter is crucial to spore formation, as CaDPA replaces water in dormant spores, a key step to spores' resistance to thermal insult. The data provide a framework for the identification and dynamic analysis of all spore germination proteins and their complexes as they occur in the inner membrane and cortex of the bacterial endospore. They may also serve, as indicated, as a model for similar experimental challenges and approaches when analysing germination processes in fungal conidiospores.

IS041 / #679

Workshop Session WORKSHOP SESSION 23: FOOD MICROBIOLOGY 07-22-2022 11:00 AM - 12:30 PM

WHAT'S ON MY FOOD? A ONE HEALTH GENOMIC EPIDEMIOLOGY APPROACH TO FOOD SAFETY

Alison Mather

Quadram Institute Bioscience, ., Norwich, United Kingdom

Whole genome sequencing (WGS) currently provides the greatest molecular resolution available to study how microbes evolve and how they differ from each other. In order to control the spread of pathogens, it is essential to understand where they arise and how they spread between populations; with the decreasing cost of WGS, genomic epidemiology to untangle the sources, reservoirs and transmission pathways of pathogens is possible. In my talk, I will use three examples to describe the use of genomic epidemiology in food safety applications. The first is an evaluation of the potential for imported chicken meat to cause disease, comparing *Salmonella* in chickens in the exporting country, and on chicken meat and in humans in the importing country. The second and third examples investigate the diversity on foods of *Vibrio* and *Campylobacter*, respectively, allowing inference of the potential risks to human health and the value of expanding WGS surveillance efforts.

IS042 / #682

Workshop Session WORKSHOP SESSION 24: SKIN NEGLECTED TROPICAL DISEASES 07-22-2022 11:00 AM - 12:30 PM

LEISHMANIASIS/SCABIES

Michael Marks

London School of Hygiene and Tropical Medicine, Clinical Research Department, London, United Kingdom

Scabies is caused by infection with the ectoparasite *Sarcoptes scabiei* var. *hominis*. Scabies occurs worldwide but an especially high burden of disease is found in low and middle income countries and in some settings the community prevalence of scabies may be as high as 20-30%. In response to the high burden of disease, individual country commitments to control the disease and the emerging evidence that ivermectin-based mass drug administration (MDA) represents an effective control strategy scabies was added to the World Health Organization (WHO) list of Neglected Tropical Diseases (NTDs) in 2017. In this talk I will cover key research and programmatic progess towards the control of scabies over the last decade and outline priorities for the coming decade.

IS043 / #684

Workshop Session WORKSHOP SESSION 25: MYCOBIOMES 07-22-2022 11:00 AM - 12:30 PM

GUT MYCOBIOME

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Because the gut mycobiome comprises a relatively small part of the human gut microbiome, the role of gut fungi in health and disease received relatively little attention so far. During recent years however, it became clear that, similar to the bacterial microbiota, mycobiota dysbiosis can be linked to several gastrointestinal diseases. Whereas these early studies showed associations only, there now is a growing (preclinical)-literature providing mechanistic evidence on the role of the gut mycobiome in e.g. inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). This presentation will mostly focus on the possible role of the gut mycobiome in these two diseases.

O001 / #590

Workshop Session WORKSHOP SESSION 01: VIRUS DISCOVERY AND VIROME STUDIES 07-20-2022 10:30 AM - 12:00 PM

THE RAPIDLY EXPANDING RNA VIROSPHERE—REPLICATORS HERE, REPLICATORS THERE, REPLICATORS EVERYWHERE?

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Background and Aims: In 2019, on the basis of a global RNA-directed RNA polymerase (RdRp) phylogeny, the International Committee on Taxonomy of Viruses (ICTV) established realm *Riboviria* with six distinct phyla (orthornaviran *Duplornaviricota, Kitrinoviricota, Lenarviricota, Negarnaviricota,* and *Pisuviricota*, and pararnaviran *Arterviricota*) to encompasses the vast majority of all then-known RNA viruses. An additional realm, *Ribozyviria*, was established in 2021 for an increasing number of hepatitis D-like RNA viruses (deltaviruses).

Methods: Novel large-scale RdRp phylogenies were established during large-scale metagenomic and metatranscriptomic studies.

Results: Here I will outline how the results of these studies will require major expansion and reorganization of both realms, possibly including the establishment of high-ranked riboviriad megataxa and novel ribozyviriad megataxa for "epsilonviruses" and "zetaviruses".

Conclusions: These developments significantly expand the RNA virosphere and therefore the future scope of the ICV meeting series.

Workshop Session WORKSHOP SESSION 01: VIRUS DISCOVERY AND VIROME STUDIES 07-20-2022 10:30 AM - 12:00 PM

STUDYING THE VIROME OF CAMELS IN CENTRAL ASIA

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Background and Aims: The emergence of new pandemic strains of the MERS-CoV coronavirus in camels necessitates regular monitoring of viral populations in this animal. The goal was to identify viruses circulating among camels in Kazakhstan that pose a potential threat to human and animal health.

Methods: Molecular-genetic and serological methods were used: the isolation of nucleic acids from the camel samples, the construction of libraries for mass parallel sequencing, bioinformatic data analysis, and serological tests.

Results: As a result of virome sequencing, contigs of viruses representing the following families were found: Flaviviridae, Circoviridae, Picobirnaviridae, Astroviridae, Parvoviridae and Hepeviridae. Of these families, all but Astroviruses and Pestiviruses have previously been found in camels. Of particular interest is the detection of short sequences, hepatitis-like viral contigs, which is potentially dangerous for humans. Serological test of camels confirmed the presence of antibodies to the hepatitis E virus, which indicates its possible circulation in camels in Kazakhstan.

Conclusions: The study of viral metagenomes of camels in the close to the Middle East region is an important epidemiological task. This will make it possible to identify not only strains of coronaviruses, but also other unknown viral pathogens that are important both for domestic animals and for human health.

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VIROME DIVERSITY AND MICROBIOME PROFILING OF BAT-ASSOCIATED CARIOS VESPERTILIONIS TICKS

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Background and Aims: Ticks harbour a broad spectrum of microorganisms, including viruses, bacteria and protozoans. Ticks of *Carios (Argas) vespertilionis* (Argasidae) are common ectoparasites of bats species in the Palearctic region and play a key vectorial role in the maintenance and transmission of zoonotic pathogens relevant to public health. The soprano pipistrelle bat (*Pipistrellus pygmaeus* - Vespertilionidae) is widely distributed in Europe, where it and can be found close to anthropogenic environments.

Methods: In the present study, we use meta-transcriptomics to investigate the RNA virome diversity and common microbiome pathogens present in recently blood fed *C. vespertilionis* ticks, collected from a soprano pipistrelle bat roosting site in Sweden.

Results: Our analysis revealed 20 newly discovered viruses classified within 12 virus families. We also identified known bat-associated and tick-borne viruses in the *Nairoviridae* and *Picornaviridae* families. Similarly, we found highly abundant bacteria and protozoans carried by *C. vespertilionis,* such as *Delftia* sp., *Coxiella* sp., *Rickettsia* sp., and *Babesia* sp.

Conclusions: These findings demonstrate the remarkable diversity of RNA viruses and microbiota components present in C. *vespertilionis* and highlight the importance of bat-associated ectoparasite surveillance.

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SURVEILLANCE OF INSECT-SPECIFIC VIROME IN FIELD-CAUGHT MOSQUITOES FROM NORTHEASTERN THAILAND

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Background and Aims: Mosquitoes are the major vector of arboviruses particularly, dengue virus (DENV), zika virus (ZIKV), and chikungunya virus (CHIKV). Regardless of the failures of current arboviral vector control and prevention strategies for their transmission, alternative approaches are necessitated to further develop and improve. Recent studies have been indicated the important roles of insect-specific viruses (ISVs) in the alteration of the vector competency, serving as alternative schemes for innovative arboviral transmission control. Northeastern (NE) Thailand has a tropical climate that provides opportune settings for mosquitoes' emergence and dissemination, supporting existing arboviruses epidemics. However, evidence concerning the representative ISVs in potential mosquito vectors of this region is lacking.

Methods: Therefore, this study aimed to survey the burden of ISVs harbored by natural mosquitoes using a Next-generation sequencing (NGS)-based metagenomic approach.

Results: Our analyses reveal that matched viral reads were highly diverse and varied in abundance among mosquito species, which were 11 taxonomic families and an unclassified group. The ISVs reads have prevailed in all mosquito species. Among these, the Guadeloupe mosquito virus (GMV) was stably found in female adults of Aedes aegypti and Culex spp. Notably, the presence of GMV contigs has firstly been suggested as a new spatial distribution of such ISV in NE Thailand as exploration with the public databases of viral metagenomic of Aedes and Culex mosquitoes.

Conclusions: Overall, this study imparts a comprehensive literacy of diverse ISV burdens circulated in the mosquito vectors from NE Thailand, which might be a potential source for innovative strategies of arboviral transmission control.

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IDENTIFICATION OF A NOVEL HLA-DR13-RESTRICTED T CELL EPITOPE OF THE HEPATITIS B VIRUS CORE PROTEIN SPANNING TWO OVERLAPPING BINDING REGISTERS

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Background and Aims: Hepatitis B virus (HBV) remains a severe health problem with 296 million people chronically infected worldwide. To address the ongoing need for curative treatment options, recent research efforts focus on immunotherapeutic approaches for patients with chronic hepatitis B. To tailor T cell-based therapies, the identification of immunogenic epitopes is vital and provides insights into the processing of viral proteins.

Methods: Through mass spectrometry analysis of the immunopeptidome of HBV core protein-loaded target cells, we identified a novel T cell epitope presented on HLA-DR13.

Results: The epitope was recognized by engineered T cells expressing MHC II-restricted HBV corespecific T cell receptors (TCRs), confirming its immunogenicity. By using truncated versions of the original sequence in combination with the identified TCRs, we found that the full-length sequence spanned in fact two distinct minimal T cell epitopes merely shifted by three amino acids. This hinted towards two overlapping binding registers of the full-length peptide sequence within the MHC II groove, which were confirmed by distinct alanine scanning motifs indicating different peptide binding cores. Through bioinformatical / homology modelling, molecular dynamics simulations and free energy calculations, the stability of both registers in the HLA-DR13 groove was analyzed. Binding studies of mutated peptides with purified HLA-DR13 as well as co-cultures with engineered T cells confirmed these findings.

Conclusions: In summary, our study identified a novel HBV T cell epitope spanning two shifting binding registers and validated a bioinformatics-based approach for the accurate modelling of such epitopes, which can be applied to the identification of other T cell epitopes.

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EFFECT OF TOLL-LIKE RECEPTOR 7 AND 9 INHIBITION ON THE SEVERITY OF EPSTEIN-BARR VIRUS DNA-EXACERBATED INTESTINAL INFLAMMATION IN A MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE

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Background and Aims: Mounting evidence suggests that the Epstein-Barr virus (EBV), found to be present in intestines of inflammatory bowel disease (IBD) patients, complicates the clinical course of the disease. Studies by our group have shown that EBV DNA increases the production of the inflammatory cytokine IL-17A through Toll-like receptor (TLR) 7 and 9 activation and that it exacerbates inflammation and disease severity in a mouse model of IBD. Hence, the aim of this study was to determine whether TLR7 and 9 inhibition ameliorates the severity of EBV DNA-exacerbated intestinal inflammation in the IBD mouse model.

Methods: Dextran sulfate sodium (DSS)-drinking C57BL/6J mice, the acute colitis model of IBD, were intra-rectally administered the viral DNA and given an intra-peritoneal injection of TLR7 and TLR9 inhibitors. The severity of colitis and inflammation was then evaluated.

Results: Both groups that were administered DSS-drinking water and received intra-rectal EBV DNA along with intra-peritoneal injections of TLR7 or 9 inhibitors had an ameliorated severity of intestinal inflammation when compared to DSS-treated groups that received the viral DNA but not the inhibitors. These groups that were treated with the TLR7 or 9 inhibitors had longer colon lengths, lower histological damage scores, and a lower disease activity index (DAI) score. Immunofluorescence assays also showed lowered numbers of inflammatory cells.

Conclusions: TLR 7 and 9 inhibitors were shown to ameliorate the severity of inflammation in the EBV DNA-exacerbated mouse model of IBD. This study uncovers a potential therapeutic/prophylactic target for IBD management in EBV-infected individuals.

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SPRAY-FREEZE-DRIED MIXED MS2-L2 VLPS: A THERMOSTABLE CANDIDATE HPV VACCINE THAT IS APPLICABLE IN LOW AND MIDDLE-INCOME COUNTRIES

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Background and Aims: Current human papillomavirus (HPV) vaccines protect against HPV types associated with up to 90% of cervical cancers. Thus, at least 10% of cervical cancers are not protected by the vaccines. This percentage is significantly important, especially in HIV/AIDS patients who can be infected with multiple HPV types.

Methods: To broaden the spectrum of protection against HPV infections, we developed an L2 candidate HPV vaccine based on bacteriophage MS2 virus-like particles (VLPs). The candidate vaccine consists of a mixture of two MS2-L2 VLPs displaying: i) a concatemer of L2 peptide from HPV31 (epitope 20-31) and from HPV16 (epitope 17-31); ii) a consensus L2 peptide representing epitope 69-86. Mice were immunized twice or thrice with a mixture of MS2-L2 VLPs.

Results: Mixed MS2-L2 VLPs protected mice against eight different HPV pseudovirus types at the genital region and against five types at the oral region. Overall, mixed VLPs protected against twelve HPV types associated with ~95.8% of cervical cancers. In addition to this, the VLPs protected against two HPV pseudovirus types associated with ~90% of genital warts and recurrent respiratory papillomatosis. Furthermore, mixed MS2-L2 VLPs protected mice against one of two HPV types (HPV5) associated with ~90% of HPV-associated skin cancers in patients with epidermodysplasia verruciformis. A spray-freeze-dried formulation of mixed MS2-L2 VLPs is thermostable at room temperature and protective.

Conclusions: Spray-freeze-dried mixed MS2-L2 VLPs is a candidate HPV vaccine that is applicable in low and middle-income countries (do not have a robust cold-chain infrastructure).

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THE EXTRACELLULAR LOOPS OF SALMONELLA TYPHIMURIUM OUTER MEMBRANE PROTEIN A(OMPA) MAINTAIN THE STABILITY OF SALMONELLA CONTAINING VACUOLE IN MURINE MACROPHAGES AND PROTECT FROM AUTOPHAGY-DEPENDENT-LYSOSOMAL DEGRADATION

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Background and Aims: Salmonella Typhimurium(STM) resides within a modified membrane-bound compartment called Salmonella containing vacuole(SCV) inside the macrophages. The biogenesis and stability of SCV are crucial for the intracellular proliferation of Salmonella. Our research aims to provide a novel mechanism behind the role of Salmonella OmpA in maintaining the stability of SCV.

Methods: Immunofluorescence, Intracellular Proliferation, Texas Red-Ovalbumin-Pulse-Chase Experiment, Acid-Phosphatase assay, Site-directed-mutagenesis, Immunoblotting, Flow cytometry



Results:







The OmpA deletion compelled STM to exit the SCV during the early stage of infection. STM $\Delta ompA$ failed to retain LAMP-1 and evaded SCV. The cytosolic STM $\Delta ompA$ population activated the autophagy machinery after colocalizing with syntaxin17 and LC3B. Subsequently, the autophagosomes harboring STM $\Delta ompA$ were targeted to the lysosomes for degradation. Inhibition of the autophagy pathway using bafilomycinA1 restored the intracellular proliferation of STM $\Delta ompA$. Furthermore, the four extracellular loops of OmpA played a crucial role in maintaining the LAMP-1 pool around the SCV. Upon alteration of the extracellular loop sequences of *Salmonella* OmpA by site-directed-mutagenesis, STM failed to preserve the interaction between LAMP-1 and the SCV and eventually escaped into the cytosol. STM $\Delta ompA$ and the extracellular loop mutants showed increased recruitment of p62 and LC3B in comparison to the wildtype-STM upon vacuole evasion into the cytosol. Surprisingly, the cytosolic population of *Salmonella* having mutations in the extracellular loops of OmpA did not activate the lysosomal degradation pathway like STM $\Delta ompA$, which helped them to survive within the murine macrophages.

Conclusions: Our study revealed an OmpA dependent novel strategy utilized by *Salmonella* to combat host autophagy by maintaining the stability of SCV.

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MANY ALTOGETHER, OR ONE ALONE, SECRETED METALLOPROTEASE(S) WOULD MAKE THE IMPACT IN BURKHOLDERIA CENOCEPACIA INFECTIONS

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Background and Aims: *Burkholderia cenocepacia* is a Gram-negative opportunistic bacterium known to cause severe lung infections in people with cystic fibrosis. Two main extracellular proteases, being zinc metalloproteases ZmpA and ZmpB, potentially shape the degree of pathogenicity^(1,2). Additionally, a BCAM1744 orthologue of serine metalloprotease PrtA (solely responsible for extracellular proteolytic activity of *B. glumae*⁽³⁾) is highly expressed in *B. cenocepacia*. However, the exact involvement of these three extracellular proteases during infection remains to be elucidated.

Methods: To understand this, we heterologously produced these proteases in *E. coli* and studied their impact on several substrates through mass spectrometry. Besides analyzing specific molecules mimicking *in vivo* target(s), the impact on several synthetic peptides was also investigated to perceive their substrate specificity. Parallel, *B. cenocepacia*K56-2 knock-out mutants were constructed which were subsequently analyzed in terms of their fitness and extracellular proteolytic activities.

Results: Intriguingly, our data demonstrated high degree of similarity in their substrate specificity, as well as their ability to compensate each other's absence. This thus shows that ZmpA, ZmpB and BCAM1744 might execute the same tasks in a synchronized fashion at a different stage of infection.

Conclusions: Results reinforce the genomic versatility of *B. cenocepacia*to express different weaponry/extracellularly means for niche-adaptation. Affirmative understanding of such pathogenic behavior will eventually unravel the intracellular survival machinery resolving novel antimicrobial targets. Acknowledgment: This work was supported by a grant from the FWO-Vlaanderen (3G005719). 1. Gingues, S. (2005) *J Bacteriol* 187(13):4421–9. 2. Kooi, C. (2006) *Infect Immunit* 74(7):4083–93. 3. Lelis, T. (2019) *Mol Plant-Microbe Interact* 31(7):841–52.

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CRISPR/CAS9 SCREENING FOR HOST FACTORS INVOLVED IN SARS-COV-2 INFECTION

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Background and Aims: To control COVID-19, elucidation of SARS-CoV-2 replication mechanisms is required. In this study, we tried to identify the host factors for SARS-CoV-2 infection in human cells through the human whole-genome screening using the CRISPR/Cas9 system.

Methods: A549-hACE2 cells expressing Cas9 were transfected with a gRNA library using lentiviral vector. The cells were inoculated with purified SARS-CoV-2/UT-NCGM02/Human/2020/Tokyo at MOI of 0.3. Three days after inoculation, DNA was extracted from the cells and applied to next-generation sequencing analysis.

Results: We identified TRIM28 and TRIM33 as new candidate genes. We suppressed the expressions of the genes in cultured cells by using CRISPRi to confirm their involvement in the viral infection. TRIM28 and TRIM33 knockdown reduced viral titer by 2.0 log and more than 5.0 log, respectively. When viral RNA levels in infected cells and supernatant were quantified, TRIM33 knockdown cells showed a decrease in viral RNA levels in both infected cells and supernatant, while TRIM28 showed an increase in RNA levels in both. Consistent with this, viral nucleocapsid protein expression in infected cells was decreased in TRIM33 knockdown and increased in TRIM28 knockdown. SARS-CoV-2 performs particle assembly and budding at the ER Golgi intermediate (ERGIC), however, in the TRIM28 knockdown cells, retention of viral Spike protein in the ERGIC was not observed.

Conclusions: These findings suggest that in the SARS-CoV-2 replication cycle, TRIM28 is involved in the assembly of infectious particles and TRIM33 is engaged in the cell entry and/or transcriptional replication.

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HBV/HDV INTERNALIZATION REQUIRES KINESIN KIF4 EXPRESSION WHICH REGULATES SURFACE NTCP LOCALIZATION AND CAN BE TARGETED BY RXR AGONISTS IN VITRO.

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Background and Aims: Hepatitis B virus (HBV) affects about 250 million individuals globally and is a major cause of chronic liver inflammation. We aimed to identify the host factors that support HBV/HDV infection and to target them as a new therapeutic modality.

Methods: We performed functional siRNA screening using an HBV reporter virus and HepG2-hNTCP cells to uncover host factors that impact the HBV life cycle.

Results: We found that The Kinesin KIF4 facilitates HBV, and iHDV, entry into human hepatocytes. Cellular fractionation and immunofluorescence analysis (IF) showed that transient KIF4 depletion reduced surface and raised intracellular NTCP levels leading to the suppression of both HBV and HDV infection. Overexpression of wild-type KIF4 but not ATPase-null KIF4 mutant regained the surface localization of NTCP and significantly restored cell permissiveness to HBV. IF revealed KIF4 and NTCP colocalization across microtubule filaments, and a co-immunoprecipitation study showed that KIF4 interacts with NTCP. KIF4 expression is regulated by FOXM1. Interestingly, we discovered that RXR agonists (Bexarotene, and Alitretinoin) down-regulated KIF4 expression via FOXM1-mediated suppression, resulting in a substantial decrease in HBV-Pre-S1 protein attachment to HepG2-hNTCP cell surface and suppression of subsequent HBV infection in both HepG2-hNTCP and primary human hepatocyte (PXB) (Bexarotene, $IC_{50} 1.89 \pm 0.98 \mu$ M) cultures.

Conclusions: Our findings show that human KIF4 is a critical regulator of NTCP surface transport and localization, which is required for NTCP to function as a receptor for HBV/HDV entry. Furthermore, small molecules that suppress KIF4 expression would be potential antiviral candidates targeting HBV and HDV entry

Workshop Session WORKSHOP SESSION 04: ANTIMICROBIALS AND ANTIMICROBIAL RESISTANCE 07-20-2022 10:30 AM - 12:00 PM

EVALUATION OF A REAL-TIME PCR ARRAY TO DETECT MULTIPLE ANTIMICROBIAL RESISTANCE GENES IN VARIETY OF FOOD WITH SUBSEQUENT CONFIRMATION AND BACTERIAL IDENTIFICATION USING NANOPORE-SEQUENCING

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Background and Aims: Microorganisms with antimicrobial resistance genes (ARGs) present in food is a public health concern worldwide. We evaluated a commercial real-time PCR array (Qiagen) that detects 87 clinically relevant ARGs for use in food for rapid and routine monitoring of ARGs.

Methods: Microbes from 63 food samples (meat=18, produce=(vegetables=21 and fruits=9) and dairy=15) collected in local retail in Canada were enriched (24 h at 37°C) using modified Schaedler media, followed by genomic DNA extraction, AMR genes detection, and identification of bacterial species by Nanopore MinION based metagenome sequencing.

Results: Among 63 food samples, 33 ARGs had at least one occurrence in all food groups with potential resistance against aminoglycoside, fluoroquinolone, tetracycline, macrolide and Class C beta-lactamase. Eight ARGs including erythromycin resistance genes were not detected. Some ARGs had unique distribution and were detected in one group of samples or one source of food, such as 4 genes including *vanB* (Vancomycin resistance) were detected in meat only, similarly 12 and 4 ARGs were detected in produce and dairy, respectively. Highest average number of ARGs were detected in meat (14 ARGs/sample), followed by produce (10 ARGs/sample) and dairy products (7 ARGs/sample). Nanopore sequencing confirmed the results of PCR and identified 23 bacterial genera in 20 representative samples.

Conclusions: In conclusion, we presented useful baseline data on ARGs presence in foods in Canada, and determined that this PCR array is a useful tool for routine ARG surveillance for food. MinION based metagenomics sequencing helps to identify bacterial species and establish link between AGRs and potential bacterial hosts.

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QUANTIFYING CONCENTRATIONS OF AND RESISTANCE SELECTION BY ANTIMICROBIAL RESIDUES UNDER IN VIVO CONDITIONS IN BROILERS

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Background and Aims: Antimicrobial usage in livestock is one of the main drivers of antimicrobial resistance, especially due to the exposure of the gut microbiome to antimicrobials. Depending on their stability, some antimicrobials can persist in the farm environment after animal treatment. We examined if persistence of the antimicrobial is a factor in the selection for antimicrobial resistant bacteria in the gut microbiota of broilers, birds with coprophagic behaviour.

Methods: Four groups of broilers were divided in three subgroups (n=12). Groups were treated with amoxicillin (non-persistent), doxycycline or enrofloxacin (persistent), and an untreated control group. Faecal droppings and caecal material were collected at different time points after the treatment. Baseline measurement occurred before the treatment. Shotgun metagenomics was performed on the faecal material to determine the resistome. Phenotypic resistance analysis of *E. coli* isolates was conducted by plating on MacConkey agar with appropriate antimicrobials. Antimicrobials were extracted from the faecal samples and analysed by LC-MS/MS.

Results: We observed higher concentrations of persistent antimicrobials (doxycycline and enrofloxacin) over time after treatment than the non-persistent antimicrobial amoxicillin. Furthermore, doxycycline treatment resulted in a larger increase in the number of resistance genes over time than amoxicillin treatment. The group treated with enrofloxacin showed a slow but continuous increase of resistance genes after treatment. Phenotypically, the same difference was observed between the persistent and non-persistent antimicrobial treatment groups.

Conclusions: Persistent antimicrobials remain longer in the farm environment with a longer selection pressure. Therefore persistence of antimicrobials should be used in the assessment of priority classification of antimicrobials.

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INVESTIGATING THE ROLE OF TRYPTOPHANASE IN E. COLI BIOFILMS

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Background and Aims: Tryptophanase (TnaA), an enzyme present in many species of bacteria, is best known for the conversion of L-tryptophan to indole, pyruvate, and ammonia. However, TnaA can also metabolise other amino acids. We recently observed that a clumping phenotype seen during the exponential growth phase of a uropathogenic E. coli strain (ATCC® 25922[™]) was absent in a tnaA knock-out.

Methods: A tnaA knock-out, its derivative (ATCC® 25922[™]), and UTI clinical isolates were assayed for biofilm development in 96-well plates. Novel inhibitors of TnaA identified in our laboratory were then screened for their effects on biofilms.

Results: The clumping phenotype, which is characteristic of early-stage biofilms, was reduced by novel TnaA inhibitors. The compounds are of interest because they could lead to the use of TnaA inhibitors as a combination therapy with existing antibiotics to mitigate recurrent UTIs and biofilms.

Conclusions: We originally assumed that the absence of clumping in the mutant was due to the loss of indole production since previous studies indicated that indole influences biofilm development. Upon closer inspection, we found that the absence of L-tryptophan in growth medium had no effect on the phenotype, but the absence of L-arginine prevented clumping. These observations shed new light on the role of TnaA and suggest a route towards a more comprehensive understanding, and therefore treatment, of biofilm formation in uropathogenic E. coli.

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COMPARATIVE ANALYSIS OF THE IMPACT OF DIFFERENT ANTIMICROBIALS ON CAMPYLOBACTER, SALMONELLA, AND E. COLI

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Background and Aims: The occurrence and persistence of foodborne pathogens on food products could be partially attributed to increases in resistance/tolerance to antimicrobials that are commonly used during food processing. Here, we assessed the impact of different antimicrobials, including organic acids, chlorine and peracetic acid (PAA), on different strains of *Campylobacter, Salmonella* and Shiga-toxin producing *E. coli*.

Methods: The minimum inhibitory (MIC) and bactericidal (MBC) concentrations were determined using a combination of the broth microdilution, kinetic growth, and colony counting assays. The antimicrobials were also tested against artificially-contaminated chicken meat and lettuce.

Results: The organic acids (formate, succinate, fumarate) had the lowest MICs (250-500mM) and MBCs (\geq 250mM) against *Campylobacter*. However, formate was superior to the other organic acids when considering solubility and *Campylobacter* control. The *Salmonella* and *E. coli* were notably less susceptible to the organic acids. Growth inhibition of all bacteria tested required high concentrations of chlorine (\geq 400ppm) while the inhibitory PAA concentrations varied considerably among bacterial species and strains (50- 200ppm). Furthermore, formate (1M) was insufficient in eliminating the bacteria on chicken breasts and lettuce. However, significant reductions in bacterial loads were noted at \geq 200ppm PAA (1log CFU/g) and at \geq 200ppm chlorine (0.5log CFU/g) for chicken samples. Lettuce decontamination showed a varying impact for PAA (50-200ppm) which depended on the bacterial species tested, with maximum reduction of 4.5 log CFU/g. Chlorine (\geq 100ppm) also significantly reduced bacterial counts (2log CUF/g) on lettuce.

Conclusions: It appears that certain bacterial strains can tolerate/resist antimicrobial concentrations that are commonly used during food production.

Workshop Session WORKSHOP SESSION 05: IMPORTANCE OF DATA MANAGEMENT FOR SUSTAINABLE FUTURES (COMCOF) 07-20-2022 10:30 AM - 12:00 PM

LINKING DARK TAXA TO FUNGAL CULTURES: DOES ENVIRONMENTAL METABARCODING OVERESTIMATE FUNGAL DIVERSITY?

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Background and Aims: High throughput environmental metabarcoding can unearth hidden microscopic biodiversity. After including environmental sequence data, the estimated global fungal diversity has increased from the commonly cited 1.5 million to 2.2-3.8 million, then to 6.3 million. However, a concern about the sequence-only analyses is that they tend to inflate the number of taxa. My lab has been uncovering a number of new fungal lineages from the pine barrens ecosystem, which is acidic and nutrient-poor. We aim to uncover new fungal taxa and understand fungal diversity using culture-based and culture-independent methods.

Methods: We collected plant roots from the New Jersey Pine Barrens. For culture-based method, we observed morphology, culture growth rate, sequenced the ITS, 18S, 28S, RPB2, ACT genes, performed phylogenetic analysis, and conducted the plant-fungal interaction experiment. For the culture-independent method, we did Illumina metabarcoding analysis of the ITS region.

Results: From the pine barrens fungal study, we observed that sequence-only analyses may overestimate the taxonomic level. For example, the new family Pygmaeomycetaceae (Umbelopsidales, Umbelopsidomycetes, Mucoromycotina, Mucoromycota) we uncovered from the pine barrens ecosystem corresponds to "clade GS23", which was recognized as a "at least class-level" new lineage based on a sequence-only soil fungal survey.

Conclusions: The disparities between sequence-only and culture-based taxonomic analyses can be explained by the problems associated with environmental sequence analyses, such as short sequence length, low quality, and chimera. Further effort on linking cultures or specimens with sequence-only "dark taxa" will provide more data to recalibrate fungal diversity calculation and to work towards a more accurate global fungal diversity estimation.

Workshop Session WORKSHOP SESSION 06: MECHANISMS OF REPLICATION AND ASSEMBLY 07-20-2022 3:00 PM - 4:30 PM

ROLE OF PLATELET FACTOR 4 IN FLAVIVIRUS REPLICATION

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Background and Aims: Recently we described that platelet factor 4 (PF4) is pro-viral for Dengue virus (DV) and Japanese Encephalitis virus (JEV). PF4 bound to receptor CXCR3 on monocytes and downregulated the interferon (IFN) production by targeting p38-MAPK-STAT2-IRF9 axis. We investigated the role of PF4 on viral replication using wild type (WT), PF4-knockout (PF4-ko) and PF4-overexpressed (PF4-oe) mice.

Methods: We infected monocyte and microglia cells with DV and JEV respectively in presence of PF4 and assessed virus replication *in vitro*. We assessed the virus replication *in vivo* in JEV infected PF4-oe and PF4-ko mice.

Results: A significantly elevated replication of both DV and JEV was observed in cells *in vitro*. A decreased replication of JEV was observed in PF4-ko mice brain. A similar observation of decreased replication of mouse-adapted DV2 strain was noted in monocytes isolated from PF4-ko mice. Decreased colocalization of JEV Capsid and LAMP1 was observed *in vitro* in cell lines in presence of PF4, similarly, in microglia isolated from PF4-oe mice, indicating a less interaction of virus containing endosomes with lysosomal vesicles. Besides, a decreased LTR stained acidic vacuoles existed in presence of PF4, indicating its role in cytosolic acidification. Thus, suggesting an involvement of endosomal-lysosomal pathway in the replication of these flaviviruses in presence of PF4. Elevation of PF4 is associated with DV infection.

Conclusions: PF4 is helping the DV and JEV propagation by affecting acidification and delaying lysosomal degradation of virus containing endosomes.

Workshop Session WORKSHOP SESSION 06: MECHANISMS OF REPLICATION AND ASSEMBLY 07-20-2022 3:00 PM - 4:30 PM

CHARACTERIZATION OF HOST FACTORS ASSOCIATED WITH THE INTERNAL RIBOSOMAL ENTRY SITES OF FOOT-AND-MOUTH DISEASE AND CLASSICAL SWINE FEVER VIRUSES

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Background and Aims: Foot-and-mouth disease virus (FMDV) and classical swine fever virus (CSFV) possess positive-sense single-stranded RNA genomes and an internal ribosomal entry site (IRES) element within their 5¢-untranslated regions.

Methods: To investigate the common host factors associated with these IRESs, we established cell lines expressing a bicistronic luciferase reporter plasmid containing an FMDV-IRES or CSFV-IRES element between the Renilla and firefly luciferase genes.

Results: First, we treated FMDV-IRES cells with the French maritime pine extract, Pycnogenol (PYC), and examined its suppressive effect on FMDV-IRES activity, as PYC has been reported to have antiviral properties. Next, we performed microarray analysis to identify the host factors that modified their expression upon treatment with PYC, and confirmed their function using specific siRNAs. We found that polycystic kidney disease 1-like 3 (PKD1L3) and ubiquitin-specific peptidase 31 (USP31) were associated with FMDV-IRES activity. Moreover, silencing of these factors significantly suppressed CSFV-IRES activity.

Conclusions: Thus, PKD1L3 and USP31 are host factors associated with the functions of FMDV- and CSFV-IRES elements.

Workshop Session WORKSHOP SESSION 06: MECHANISMS OF REPLICATION AND ASSEMBLY 07-20-2022 3:00 PM - 4:30 PM

ACTIVATION OF UNFOLDED PROTEIN RESPONSE DURING HIV-1 INFECTION AND REGULATION OF VIRION INFECTIVITY BY IRE1

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Background and Aims: HIV-1 and other viruses, depend on cellular Endoplasmic Reticulum (ER) for translation, protein folding and maturation. This causes ER stress leading to the Unfolded Protein Response (UPR). Viruses have evolved strategies to manipulate the UPR for their own benefit. However, the UPR modulation by HIV-1 and its functional significance has not been explored in depth. Our study aims to assess the effect of HIV-1 on UPR and its role in HIV-1 replication and infectivity.

Methods: HIV-1 infected CD4+T-cells were profiled for various UPR markers. Knockdown of UPR markers was conducted using sh/siRNAs and the viral gene expression and infectivity was analyzed using luciferase reporter assay, p24 ELISA and β -gal staining assay. Effect of selected viral protein on UPR was assessed by utilizing mutants and selective UPR inhibitor. Pharmaceutical ER stress inducers were tested for their anti-HIV activity.

Results: HIV-1 induces ER stress and activates UPR in CD4+T-cells with differential expression of phospho-IRE1α, phospho-PERK, cleaved ATF6α and their downstream targets. Knockdown of UPR markers leads to decrease in viral replication. IRE1α knockdown results in reduced virion infectivity through gp120. Moreover, HIV-1 Nef was found to regulate IRE1α. Also, pharmacological inducers which overstimulate ER stress, shows significant anti-HIV activity.

Conclusions: HIV-1 differentially regulates various UPR markers, suggesting a virus-specific UPR modulation. The results demonstrate that the UPR activation works in favor of HIV-1 replication. Nef mediated regulation of IRE1 α and gp120, provides a novel mechanism involved in virion infectivity. Finally, overstimulation of ER stress using chemical inducers, which can negatively regulate UPR, shows anti-HIV activity.

Workshop Session WORKSHOP SESSION 07: VIRUS EVOLUTION AND EMERGENCE 07-20-2022 3:00 PM - 4:30 PM

LARGE-SCALE EPIDEMIOLOGICAL SURVEY OF YAM VIRUSES IN GUADELOUPE SHED LIGHT ON THEIR INTRODUCTION PATHWAYS AND INFECTION DYNAMICS IN THE FIELD.

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Background and Aims: Yam (*Dioscorea* spp) is an important staple crop for hundreds of millions of people in the tropics and subtropics. Its production is increasingly threatened by pests and pathogens, including viruses whose prevalence, epidemiology and routes of invasion remain largely unknown, precluding the implementation of efficient control strategies.

Methods: Targeting Guadeloupe, a Caribbean island and yam diversification hotspot, we undertook the first large-scale epidemiological study of 13 yam viruses and addressed key issues such as their molecular diversity and entry pathways in Guadeloupe, and the recontamination dynamics of sanitised yam plants under field conditions.

Results: Our results showed that most yam plants are co-infected by up to 5 distinct viruses and that the structuration of the molecular diversity of the viruses targeted by this study differs between viruses. These results also provide insights into the role of weeds as reservoirs of yam viruses and the potential of weed management to better control these viruses. We showed that import of yam tubers for human consumption promotes the introduction of viruses in Guadeloupe, and potentially in other tropical islands with fragile agro-ecosystems. Finally, we showed that plots of fully sanitized yam plants were almost entirely infected after only two consecutive years, and that infection rates differed widely among viruses.

Conclusions: Overall, our results open the way to the implementation of a comprehensive strategy for controlling yam viruses in Guadeloupe and in other similar tropical island agroecosystems.

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Workshop Session WORKSHOP SESSION 07: VIRUS EVOLUTION AND EMERGENCE 07-20-2022 3:00 PM - 4:30 PM

DIAGNOSTIC AND GENOTYPING OF JC POLYOMAVIRUS IN PATIENTS WITH PROGRESSIVE MULTIFOCAL LEUCOENCEPHALOPATHY BETWEEN 2006 TO 2021 IN ARGENTINA

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Background and Aims: Polyomavirus JC (JCPyV) is ubiquitous and is the causative agent for Progessive Multifocal Leucoencephalopathy (PML), a rare disease that occurs in immunocompromised hosts (mostly in HIV patients). There are described eight genotypes which are strongly related to geographical areas: 1 and 4 (Europe), 2 and 7 (Asia), 3 and 6 (Africa) and 8 P.N.Guinea and Pacific Islands. The first population to settle in the Americas, brought with them type 2A from northeast Asia. The aim of this study was to describe the characteristics of JCPyV positive patients and the genotypes of the strains detected between 2006 and 2021.

Methods: A Nested-PCR in cerebrospinal fluid was carried out for diagnosis, then sequence analysis was performed using JCPyV-specific primers designed against VP1 (*Torres*2016). Samples from 1267 patients with presumptive LMP were processed.

Results: Eleven percent (n=137) tested positive for JCPyV. Ninety-nine percent were HIV+ and the age was 19-67 years old (media 39). The time from onset of symptoms was higher than 4 weeks in 71% of the cases, 68% were under HAART, CD4+ count was 114 cel/mm³ and HIV viral load 5,9 log₁₀ copies/ml in average, respectively. VP1 gene sequences were obtained from 32 samples that were selected to cover all the period. Phylogenetic analyses indicated that they belonged to genotypes 2 A (n=23), 1 (n=7), 3 (n=1) and 4 (n=1).

Conclusions: Positive cases were related to VIH advanced disease and long time of convalescence. The genotypes that we found seem to be more related to the geographical area than to the pathogenesis of the virus.

Workshop Session WORKSHOP SESSION 07: VIRUS EVOLUTION AND EMERGENCE 07-20-2022 3:00 PM - 4:30 PM

EVOLUTIONARY DYNAMICS OF THE HEPATITIS C VIRUS SUBTYPE 1A IN CROATIA

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Background and Aims: Majority of chronic hepatitis C virus (HCV) infections in Europe are caused by genotype 1 which has disseminated globally during the 20th century. High prevalence of subtype 1a is observed in Croatian patients, especially among intravenous drug users (IDU). The aim of this study was to investigate epidemic history of the HCV subtype 1a in Croatian population.

Methods: The study included 109 patients at the University Hospital for Infectious Diseases Zagreb infected with HCV subtype 1a from 2016 to 2019. Time-stamped NS3, NS5A and NS5B sequences of each patient were concatenated and phylodynamic analysis was performed using Bayesian Markov Chain Monte Carlo approach implemented in BEAST v.2.6. Models of population growth and molecular clock were selected based on Bayes factor analysis.

Results: Bayes factor analysis favored the relaxed lognormal molecular clock and the Bayesian skyline demographic model. The mean estimated substitution rate of the subtype 1a was 1.48×10^{-3} (HPD95%, $0.52 - 2.40 \times 10^{-3}$), while the mean estimate for the time to most recent common ancestor was 59 years (HPD95%, 25 - 108) (Table 1). The Bayesian skyline analysis revealed an exponential increase in HCV subtype 1a infections from 1990s onwards (Figure 1).

Table 1. Estimated time of the most recent common ancestor (tMRCA) and substitution rate (substitutions/site/year) with 95% lower and upper values from the highest posterior density (HPD95%) under various molecular clock and population growth models for HCV subtype 1a by Bayesian coalescent methods.

		population growth model		
molecular clock model		constant	exponential	skyline
	tMRCA (years, HPD95%)	56 (38, 76)	58 (39, 80)	78 (44, 126)
	substitution rate (10 ⁻³ , HPD95%)	1.42 (0.96, 1.93)	1.31 (0.84, 1.76)	1.02 (0.50, 1.52)
strict	marginal likelihood estimate (log)	-33778.19	-33691.43	-33669.42
	tMRCA (years, HPD95%)	39 (24-60)	37 (21, 56)	59 (25, 108)
	substitution rate (10 ⁻³ , HPD95%)	2.38 (1.35, 3.45)	1.99 (1.12, 3.00)	1.48 (0.52, 2.40)
lognormal	marginal likelihood estimate (log)	-33666.33	-33581.52	-33570.04
	tMRCA (years, HPD95%)	***	23 (13, 35)	53 (15, 127)
	substitution rate (10 ⁻³ , HPD95%)	***	3.61 (1.84, 5.69)	2.46 (0.46, 4.53)
exponential	marginal likelihood estimate (log)	***	-33629.85	-33617.95

*** = model did not converge



Figure 1. Bayesian skyline plots showing the epidemic history of the HCV subtype 1a in Croatia. Mean (solid blue line), upper and lower 95% HPD (solid blue area) estimates of the efective population size (Y-axis; log₁₀ scale) through time (X-axis; calendar years) from the time of the most recent common ancestor (tMRCA) are presented.

Conclusions: The increase of HCV subtype 1a in the 1990s and its continued growth throughout the first decade of the 2000s is in accordance with a huge increase in the number of IDU registered in this period in Croatia. Establishment of harm reduction programs could have contributed to stabilization of HCV subtype 1a incidence in the last decade.

Workshop Session WORKSHOP SESSION 07: VIRUS EVOLUTION AND EMERGENCE 07-20-2022 3:00 PM - 4:30 PM

LESSONS FROM DYNAMIC CHANGES IN GENOMIC AND PHENOTYPIC CHARACTERISTICS OF DENGUE, INFLUENZA, AND SARS-COV-2 VIRUSES ACROSS EPIDEMICS IN TAIWAN

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Background and Aims: A full understanding of the genomic and phenotypic changes of RNA viruses across epidemics has become critically important. **Two important questions are:** (1) under what epidemiological conditions are virus variants preferentially selected to increase epidemic severity at later time periods in epidemics or pandemics; and (2) what are the impacts of different public health prevention and control strategies on viral changes?

Methods: DENV-2-specific cDNA flanking the E gene were deep sequenced to compare quasispecies. Antibody responses to H1N1(H1N1/pdm09)-HA1-E374K mutants and replication efficiency of the two duck influenza viruses with amino acid variations in both chicken DF1 and MDCK cells were evaluated. Recently, we used the viral sequence analysis tools that we had developed in-house to integrate epidemiological characteristics with the SARS-CoV-2 sequences from the Alpha variant, which caused a 2021 outbreak in northern Taiwan.

Results: Different virus variants did appear through a series of human-to-human transmission chains with various spatio-temporal population dynamics. The over-wintering DENV-2 variants with lower genetic diversity, transmission rate, and intra-host variant numbers might play an important role in over-wintering, maintaining viral variants, and causing more severe epidemics. The H1N1/pdm09)-HA1-E374K mutant ultimately emerged as the dominant strain in high population-dense areas, and then persisted regardless of all intervention measures. The dominant SARS-CoV-2 Alpha variant did emerge from high population-density districts in Taipei and New Taipei Cities, but showed a declining trend after the implementation of a rigorous Level 3 Alert Control Policy.

Conclusions: The integration of phylodynamic and deep sequencing analysis can be very useful for public health surveillance.

Workshop Session WORKSHOP SESSION 08: MICROBIAL COMMUNITIES AND MICROBIOMES 07-20-2022 3:00 PM - 4:30 PM

MICROBIAL COMMUNITY ANALYSIS OF HYDROGEN PRODUCER THERMOPHILIC ISOLATES FROM LOCAL HOT SPRINGS WITH NANOPORE SEQUENCING

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Background and Aims: Exploring hot spring thermophilic communities and their potential use through metagenomics is crucial as these habitats host a variety of beneficial microorganisms living under extreme conditions including high temperatures, lack of oxygen and limited nutrients. Thermophilic microorganisms are reported to be capable of converting various carbon sources into biofuels. The main purpose of this study was to investigate the microbial profiles and green hydrogen production capacity of the thermophilic isolates of local hot springs.

Methods: Isolates were collected from 5 different hot springs (Temperature:38.0-77.3°C and pH:6.42-7.88). A metagenomic analysis was performed with 16S rRNA amplification products using a MinION Sequencer from Oxford Nanopore Technologies. Prokaryotic microbial profiling was acquired following the sequencing of samples from upstream, midstream and downstream of the hot spring. Two different 16S rRNA primers (universal and targeted) were used to differentiate bacterial and archaeal presence in thermal waters.



Figure: a) Schematical map of local area of hot spring locations b) Temperature measurements of upstream, midstream and downstream sections of Hot Spring 1 (HS1) c) Experimental workflow following the laboratory transfer of thermal waters.

Results: Newly designed targeted primers demonstrated the presence of archaea and bacteria while universal primers only detected bacterial communities. A significant loss of microbial variety was observed as temperature decreased towards the edge of downstream hot spring. In contrast, the microbial-rich environment of upstream hot spring resulted in an increased hydrogen production of up to 90% conversion under anaerobic conditions maintained by 100% carbon monoxide gas at 60°C.
Conclusions: The outcomes of this study successfully demonstrated the adaptation of microorganisms to altered environmental conditions together with their biohydrogen production capacity.

Workshop Session WORKSHOP SESSION 08: MICROBIAL COMMUNITIES AND MICROBIOMES 07-20-2022 3:00 PM - 4:30 PM

GUT MICROBIOME OF AN ENDANGERED FISH: TOR PUTITORA (HAM.) AND ITS IMPLICATIONS ON HOST HEALTH

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Background and Aims: The gastrointestinal tract of vertebrates harbours pathogenic bacteria in addition to symbiotic bacteria. These symbiotic bacteria suppress the growth of opportunistic pathogens by competing for nutrients available in the host and by production of various antimicrobial peptides. Alteration to the normal gut microflora leads to diseased condition. *Tor putitora* is a large freshwater fish of the Indian subcontinent and is commonly found in the foothills of Himalaya with decent-sized populations in the Gobindsagar reservoir of the Sutlej river, Himachal Pradesh, India. Commonly known as golden mahseer, this species was enlisted endangered in the International Union for Conservation of Nature and Natural Resources assessment year (2010). This necessitated the need for deciphering the microbial diversity associated with its gut and their functional aspects.

Methods: Shotgun metagenomics approach was employed to explore the composition and function of the microbial communities present in the gut of *Tor putitora*.

Results: The taxonomic diversity was mainly composed of *Proteobacteria* with predominance of genera *Escherichia*, *Klebsiella* and *Aeromonas*. The reconstruction of *Aeromonas* sp. and the genes for its secretion system also helped in enumeration of the intricate mechanisms of host-pathogen relationships.

Conclusions: In lines with the *in-silico* analysis for understanding the microbial diversity, the genes for virulence factors and antibiotic resistance were also found enriched in the community suggesting the potential role of commercial fish and seafood as reservoirs for multi-drug resistant bacteria.

Workshop Session WORKSHOP SESSION 08: MICROBIAL COMMUNITIES AND MICROBIOMES 07-20-2022 3:00 PM - 4:30 PM

SPECIES ABUNDANCE CORRELATIONS CAN DISTINGUISH SOME INTERACTION TYPES IN MICROBIAL NETWORKS

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Background and Aims: Metagenomics-based studies on communities of microorganisms are mostly limited to only a few samples in time, presenting mere 'snapshots' of the microbial ecosystem. Inferring interspecific interactions from such cross-sectional data is a daunting task and likely prone to bias, as the 'true' networks governing microbiome dynamics are often unknown. Common methods to infer microbial interactions are based on co-occurrence measures, such as correlations between bacterial phylogenetic groups. We tested whether such methods are useful tools in inferring interaction networks.

Methods: For this purpose, we simulated bacterial communities by means of the generalized Lotka-Volterra model, with variation in model parameters representing variability among hosts.

Results: Our results show that correlations can be indicative for presence of bacterial interactions, but only when measurement noise is low relative to the variation in interaction strengths between hosts. Indication of interaction was affected by type of interaction network, process noise and sampling under non-equilibrium conditions. Correlation sign mostly coincided with the strongest pairwise interaction. However, we found that competitive interactions can sometimes result in positive as well as negative correlations.

Conclusions: To summarize, correlations can distinguish some microbial interaction types, but careful interpretation and validation is required when inferring networks from cross-sectional abundance data.

Workshop Session WORKSHOP SESSION 09: PLANT-MICROBE INTERACTIONS 07-20-2022 3:00 PM - 4:30 PM

THE ECOLOGY OF ANTIBIOTIC PRODUCTION IN BACILLUS SUBTILIS BIOFILMS

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Background and Aims: Beneficial bacteria play an essential role in conferring immunity to their hosts to a wide range of bacterial, viral, and fungal diseases. *Bacillus subtilis* is a Gram-positive bacterium that protects the plant from various pathogens due to its capacity to produce an extensive repertoire of antibiotics. At the same time, the plant microbiome is a highly competitive niche, with multiple microbial species competing for space and resources. Therefore, regulating antibiotic production in the rhizosphere is of great importance for eliminating pathogens and establishing beneficial host-associated communities.

Methods: We used *B. subtilis* as a model organism to investigate how colonization by biofilm formation influences antibiotic production in *B. subtilis*. Using Imaging Flow Cytometry (IFC), we showed that colonization of *Arabidopsis thaliana* roots specifically induced the transcription of the biosynthetic clusters for the non-ribosomal peptides surfactin, bacilysin, plipastatin, and the polyketide bacillaene in *B. subtilis* [Maan et al., *Frontiers in Cellular and Infection Microbiology*, 2022].

Results: We next systemically evaluated the four non-ribosomal peptides/polyketide (NRPs/PKS) antibiotics produced by *the Bacillus subtilis* clade; we revealed that these antibiotics acted synergistically to eliminate phylogenetically distinct competitors effectively. The production of these antibiotics came with a fitness cost manifested in growth inhibition, rendering their synthesis uneconomical when growing in proximity to a phylogenetically close species, carrying resistance against the same antibiotics [Maan et al., *Nature Communications*, 2022]. Purified peptidoglycan from sensitive competitors was sufficient to activate antibiotic production.

Conclusions: We now study the synergistic interaction between the signaling molecules produced by the plant and its microbiome members to fine-tune antibiotic production.

Workshop Session WORKSHOP SESSION 09: PLANT-MICROBE INTERACTIONS 07-20-2022 3:00 PM - 4:30 PM

ISOPRENE AS ELECTRON ACCEPTOR IN ACETOBACTERIUM

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Background and Aims: Isoprene is produced by plants and is the most abundant biogenic volatile organic compound on Earth. It is an important participant in atmospheric chemistry, contributing to ozone and secondary organic aerosol formation and increasing the lifetime of methane. Soils and marine environments harbouring aerobic isoprene degrading organisms serve as isoprene sinks. The fate of isoprene under anoxic conditions is unknown. This study aimed of to observe isoprene biodegradation in the absence of oxygen.

Methods: Isoprene was incubated under anoxic conditions in the presence of activated sludge with nitrate, sulphate or ferric iron as electron acceptors and in the absence of an electron acceptor. Gas chromatography was used to monitor transformation of isoprene. Metagenome assembled genomes were retrieved from highly enriched cultures. Proteomics was used to identify a putative isoprene reductase gene.

Results: An H₂-consuming homoacetogenic enrichment utilized 1.6 µmoles isoprene h⁻¹ as an electron acceptor. The culture was dominated by *Acetobacterium wieringae* and reduced isoprene to methylbutene. In the presence of isoprene, 40% less acetate was formed suggesting isoprene reduction is coupled to energy conservation. Comparative proteogenomics identified a five-gene operon upregulated during isoprene reduction. The operon encodes an oxidoreductase, proposed as the putative isoprene reductase with a binding site for NADH, FAD and two pairs of [4Fe-4S]-clusters.

Conclusions: This study demonstrates anaerobic isoprene biodegradation for the first time and provides the first evidence for an isoprene reductase. These results have both environmental relevance in the context of furthering our understanding of electron sinks in anaerobic environments, and in our understanding of contributing mechanisms to global isoprene turnover.

Workshop Session WORKSHOP SESSION 09: PLANT-MICROBE INTERACTIONS 07-20-2022 3:00 PM - 4:30 PM

THE ROLE OF EXTRACELLULAR VESICLES OF PECTOBACTERIUM IN PLANT-PATHOGEN INTERACTIONS

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Background and Aims: The genus *Pectobacterium* gathers important pectin degrading plant pathogens. The membrane vesicles (MVs) of *Pectobacterium* and their roles are poorly characterized. This study is aimed to verify whether the MVs of *Pectobacterium* can serve as vehicles for virulence factors and might be involved in interactions with plants.

Methods: The *Pectobacterium* and *Arabidopsis* were grown in liquid MS medium with 1% sucrose. The presence of MVs was confirmed by transmission electron microscopy. The MALDI-TOF/TOF-MS analysis was conducted to identify the cargo of MVs. The activity of pectinolytic enzymes secreted via MVs was confirmed by the pectate plate test, a 1,6-diphenyl-1,3,5-hexatriene method, and pathogenicity assay on plant leaves.

Results: We have shown that both *Pectobacterium* and *Arabidopsis* secrete MVs. The morphology and yield of bacterial MVs differed depending on the medium composition. The highest MVs production was observed when the bacteria were grown in the medium containing pectin or plants extracts. Furthermore, the amount of produced MVs increased significantly during the interaction between *Pectobacterium* and *Arabidopsis* when grown as a co-culture. In contrast, the number of vesicles produced by the bacterium or plant itself was negligible. The proteomic analysis of *Arabidopsis* MVs revealed that their cargo varied whether the plant was grown in the presence of bacteria or without. Moreover, the bacterial MVs carry active pectate lyases that can macerate plant tissues in the pathogenicity test.

Conclusions: MVs participate in the interaction between plants and bacteria and serve as virulence vesicles as their cargo consists of proteins that play a role in virulence. *Funding:* OPUS18-2019/35/B/NZ9/01973

Workshop Session WORKSHOP SESSION 10: TOXICOGENIC FUNGI: BIOLOGY AND TAXONOMY ICFM-TAXONOMY OF ASPERGILLUS AND PENICILLIUM 07-20-2022 3:00 PM - 4:30 PM

SPECIES DELIMITATION IN ASPERGILLUS SECTION FLAVIPEDES AND SERIES VERSICOLORES

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Background and Aims: Aspergillus is an important genus of filamentous fungi with more than 400 accepted species and a large number of newly described species every year. Recent taxonomic studies dealing with the section *Flavipedes* left some confusion and several new species were recently described so we decided to clarify the taxonomy of this section with a proper revision. The number of species in series *Versicolores* rose to 17 in recent years, however, the identification to species level is problematic even if molecular methods are used.

Methods: We have revised the section *Flavipedes* and series *Versicolores* using modern species delimitation methods (several single-locus and one multi-locus method) and traditional morphological analysis. In the case of section *Flavipedes*, we used a dataset of 90 strains and partial DNA sequences of three genes. For series *Versicolores*, we employed an extensive dataset of more than 500 calmodulin sequences and a reduced dataset of 215 strains and partial DNA sequences of five genes.

Results: In section *Flavipedes*, we described four new species and synonymized one of the accepted species, so the section currently harbors 18 species. The analysis of our large dataset of series *Versicolores* members resulted in a dramatic reduction of species number from 17 to four. Despite the reduction in species count, phenotype-based identification remains challenging.

Conclusions: We have clarified the taxonomy of section *Flavipedes* and increased its known phylogenetic and morphological variability. The results of series *Versicolores* revision with an unprecedented large dataset revealed the importance of sampling in taxonomic studies.

Workshop Session WORKSHOP SESSION 10: TOXICOGENIC FUNGI: BIOLOGY AND TAXONOMY ICFM-TAXONOMY OF ASPERGILLUS AND PENICILLIUM 07-20-2022 3:00 PM - 4:30 PM

SPECIES IDENTIFICATION AND IN VITRO ANTIFUNGAL SUSCEPTIBILITY TESTING OF ASPERGILLUS SECTION NIGRI STRAINS ISOLATED FROM OTOMYCOSIS PATIENTS

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Background and Aims: *Aspergillus niger* is the most commonly reported etiology of otomycosis based on morphological characteristics. This fungus is a member of *Aspergillus* section *Nigri*, a set of morphologically indistinguishable species that can harbor various antifungal susceptibility patterns. The aim of this study was to accurately identify and determine the susceptibility pattern of a set of black *aspergilli* isolated from otomycosis patients.

Methods: Forty-three black *Aspergillus* isolates from otomycosis patients were identified by using the PCR-sequencing of the b-tubulin gene. Furthermore, the susceptibility of isolates to three antifungal drugs, including fluconazole (FLU), clotrimazole (CLT) and nystatin (NS), were tested according to CLSI M38-A2. The data were analyzed using the SPSS software (version 15).

Results: The majority of isolates were identified as *A. tubingensis* (32/43, 74.42%) followed by *A. niger* (11/43, 25.58%). The lowest minimum inhibitory concentration (MIC) values were observed for NS with geometric means (GM) of 4.65 mg/mL and 4.83 mg/mL against *A. tubingensis* and *A. niger* isolates, respectively. CLT showed wide MIC ranges and a statistically significant inter-species difference was observed between *A. tubingensis* and *A. niger* isolates (P < 0.05). FLU was inactive against both species with GMs > 64 mg/mL.

Conclusions: Species other than *A. niger* can be more frequent as observed in our study. In addition, considering the low and variable activity of tested antifungal drugs, empirical treatment can result in treatment failure. Accurate identification and antifungal susceptibility testing of isolates is, however, recommended.

Workshop Session WORKSHOP SESSION 11: PATHOGENESIS OF VIRAL DISEASES 07-20-2022 7:00 PM - 8:30 PM

ORGAN-SPECIFIC THREE-DIMENSIONAL VESSEL-ON-CHIP MODELS TO STUDY ORTHOHANTAVIRUS PATHOGENESIS

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Background and Aims: Orthohantaviruses are emerging zoonotic viruses with rodents as their main reservoir. Upon respiratory transmission, human infection can result in hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome (HCPS) depending on the causative virus. Endothelial cells are predominantly infected with endothelial cell activation and increased vascular permeability likely to play a central role in pathogenesis. Although microvasculature of multiple organs can become infected, distinct clinical outcomes between HFRS and HCPS exist. Endothelial cells cultured in conventional static two-dimensional conditions are physiologically not representative due to their lack of shear-stress and their inherent pro-inflammatory state. Novel culturing platforms that allow endothelial cell culture under these physiological conditions are needed to unravel organ-specific vascular host responses.

Methods: Here, we present novel organ-specific three-dimensional primary endothelial vessel-on-chip microfluidic models for studying orthohantavirus infection. We established a high-throughput method for culturing these vessels originating from lungs, kidney, liver, heart, skin and umbilical vein.

Results: Upon TNF α -stimulation, endothelial cell activation leads to increases of inflammation marker ICAM-1 expression, monocyte adhesion, platelet binding and vascular permeability. Furthermore, we demonstrated that Puumala orthohantavirus infection causes endothelial cell activation in primary human umbilical vein endothelial cells resulting in increased monocyte adhesion without directly causing vascular permeability increases.

Conclusions: These results characterize a novel three-dimensional vessel-on-chip platform to study orthohantavirus pathogenesis. This platform can be expanded by addition of co-cultures with other (immune) cell types to optimize representation of organ-specific microenvironments. Ultimately, this platform will pave the way for studying pathogenesis or assessment of possible therapeutics for a wide range of endotheliotropic viruses.

Workshop Session WORKSHOP SESSION 11: PATHOGENESIS OF VIRAL DISEASES 07-20-2022 7:00 PM - 8:30 PM

USUTU VIRUS: COMPARING VIRULENCE OF CIRCULATING STRAINS IN A MOUSE MODEL

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Background and Aims: Knowledge of emerging infectious diseases helps us better prepare for outbreaks in changing future scenarios. Due to the emergence of vector mosquitoes the threat of arboviral diseases is increasing. Usutu is a flavivirus spreading through Europe, causing death in bird populations, but also sometimes severe infection in humans. There are currently few tools available to study this disease and little understanding of the difference in pathogenicity of different strains. We aim to better study and compare strains circulating within the Netherlands using a mouse model.

Methods: IFNAR-/- mice were injected subcutaneously with decreasing titres of Africa-3 lineage Usutu virus to determine a minimal lethal dose, or with 100pfu of three different Usutu virus lineages in order to compare virulence. Weight and survival was monitored for 16 days. Blood samples were taken, and when the animals reached a humane end point organs were harvested for pathology, immunohistochemistry and to determine viral titres by qRT-PCR.

Results: Much lower titres of virus were required in order to achieve a sublethal dose than expected. We also observed higher virulence in two of the Usutu lineages which showed more rapid disease progression and weight loss, resulting in earlier lethality. This was also evident in the earlier increase in viral titres determined from the blood samples taken during the course of infection.

Conclusions: We determined a minimal lethal dose for the Africa-3 lineage and compared the differences in virulence caused by three Usutu lineages, which will help to develop further tools and understanding to be better prepared for future outbreaks.

Workshop Session WORKSHOP SESSION 11: PATHOGENESIS OF VIRAL DISEASES 07-20-2022 7:00 PM - 8:30 PM

PERSISTENT EXPRESSION OF HPV ONCOGENES AMONG HIV-INFECTED WOMEN ON HAART IN IBADAN NIGERIA

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Background and Aims: The natural history of HIV infection has been greatly changed by the introduction of highly active antiretroviral therapy (HAART) with a significantly improved immunity and increased life expectancy but its impact on HPV-associated cancer is uncertain. This study aims to investigates persistent HPV infection among HIV infected women on HAART by measuring the expression of HPV oncogenes over a period of 18 months after HAART initiation.

Methods: A total of 350 HIV infected women who were newly initiated on HAART in South-western Nigeria and consented to participate were enrolled in the study. Cervical swab was collected from these women and tested for HPV DNA using primers targeting the L1 gene. Total RNA was extracted from the swab and used for quantification of expression of HPV E6/7 genes relative to the actin gene on the samples that tested positive for HPV DNA using Sybr real-time. The data collected were analysed using descriptive statistics chi square and ANOVA.

Results: A prevalence of 35.6% (124) of HPV infection was observed among the 350 HIV infected women in this study. Persistent higher expression of HPV oncogenes relative to the actin gene was observed in 63.7% of the 124 women with detectable HPV DNA at the three check-point over the 18 months period.

Conclusions: Persistent active HPV infection was observed in majority of HIV infected women. Placement of HAART was observed not to have effect on the expression of the virus oncogene and persistent infection among these women.

Workshop Session WORKSHOP SESSION 12: NOVEL APPROACHES TO VIRUS CONTROL 07-20-2022 7:00 PM - 8:30 PM

MACROCYCLES TARGETING INFLUENZA VIRUS HA SHOW ANTIVIRAL EFFECTS BY HAMPERING ADSORPTION AND FUSION, WHICH RESULT IN VIVO ANTIVIRAL EFFECTS IN MURINE AND MACAQUE MODEL

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Background and Aims: The influenza A virus is a great public health issue. Most anti-influenza drugs currently used, such as oseltamivir and zanamivir, inhibit the enzymatic activity of neuraminidase (NA). NA inhibitor-resistant viruses, however, have already been isolated among seasonal H1N1 pandemic (H1N1) 2009, and even highly pathogenic avian H5N1 viruses. These resistant viruses can only be controlled by a new antiviral with a mechanism of action that is totally different from NA inhibition.

Methods: To devise smaller molecules capable of binding to the influenza viral HA as potential antiviral agents, we used an emerging technology called Random non-standard Peptides Integrated Discovery (RaPID). Sequencing of 69 molecular clones from the selected cDNAs revealed 28 candidates for an inhibitor of HA (iHA).

Results: Of the 28 candidates, eight iHA macrocycles inhibited plaque formation of the high pathogenic H5N1 avian virus . Among these macrocycles, iHA-24 and iHA-100 remarkably reduced the plaque number and size. Virus replication was inhibited by iHA-100 treatment at -1 h, 0 h, 0.3 h, 1 h, and 1.5 h after infection, but uncoating at 3 h after infection was not inhibited. They showed powerful efficacy in preventing severe pneumonia at later stages of infection in mouse and non-human primate cynomolgus macaque models.

Conclusions: iHA-100 is a candidate antiviral agent that inhibited both virus replication and pathogenesis *in vivo*. The RaPID system can attain a broader anti-viral spectrum of macrocycles. Thus, building on this current work, we expect that more potent, broad-spectrum anti-influenza macrocycles will be developed in the near future.

Workshop Session WORKSHOP SESSION 12: NOVEL APPROACHES TO VIRUS CONTROL 07-20-2022 7:00 PM - 8:30 PM

DELIVERY OF A COVID-19 VACCINE TO THE SKIN USING A HIGH DENSITY-MICROARRAY PATCH (HD-MAP)

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Background and Aims: SARS-CoV-2 has infected hundreds of millions globally and resulted in nearly 6 million deaths to date, with ongoing waves of infection in communities with relatively high vaccination rates. We still face many challenges in the rollout of vaccines and subsequent boosting of initial protective immune responses. A high-density microarray patch (HD-MAP) has been developed at the University of Queensland and Vaxxas that can deliver vaccines to the skin, with significant dose sparing and high thermostability.

Methods: This presentation will cover the background HD-MAP technology and its application to COVID-19 vaccine development. We have used the HD-MAP to deliver a SARS-CoV-2 spike subunit vaccine directly to the skin of mice in order to examine protective efficacy.

Results: We have shown that the vaccine, dry-coated on the patch is thermostable, and delivery of spike via HD-MAP induces higher cellular and antibody immune responses than traditional needle delivery, with serum able to potently neutralize clinically relevant isolates including alpha, beta, delta and omicron lineages. Finally, a single dose of HD-MAP-delivered spike provided complete protection from a lethal virus challenge.

Conclusions: We have shown that HD-MAP delivery of a SARS-CoV-2 vaccine has the potential to significantly impact the ongoing COVID-19 pandemic.

Workshop Session WORKSHOP SESSION 12: NOVEL APPROACHES TO VIRUS CONTROL 07-20-2022 7:00 PM - 8:30 PM

IN VITRO ANTIVIRAL ACTIVITY OF SOLANUM OVALIFOLIUM A NATIVE COLOMBIAN PLANT AGAINST DENV, ZIKV AND CHIKV

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Background and Aims: Background. Diseases caused by viruses (DENV, ZIKV, and CHIKV) are a global public health problem since there are no specific treatments. The major source of molecules of pharmacological interest have been natural products, and despite Colombia's biodiversity, few studies evaluate their antiviral potential. **Objective** To evaluate the *in vitro* antiviral potential of methanolic extract and fractions obtained from *Solanum ovalifolium* native from Colombian coffee region belonging to the Solanaceae family.

Methods: The extract viability was assessed by the MTT method in serial dilutions (7.8µg/mL to 500µg/mL). Screening for antiviral activity against (CHIKV/Col, ZIKV/Col, and DENV-2/S16803) was performed *in vitro* with a combined strategy on VERO cells, quantifying the number of infectious viral particles by plating supernatants.

Results: The methanolic extract of *Solanum ovalifolium* exhibited a high antiviral activity with 100% of inhibition against the three arboviruses. Afterward, we analyzed the chemical compounds in this extract by TLC, finding steroidal-like compounds, alkaloids, and polyphenols. Subsequently, we fractionated the extract using a sintered funnel and different solvents (hexane-Fraction A, ethyl acetate-Fraction B and, methanol-Fraction C). Finally, the fraction C showed antiviral activity against DENV, ZIKV, and CHIKV with inhibition of 54.67%, 61.1% and 92.3%, respectively. Next studies will focus on isolate these molecules and evaluate again the antiviral activity.

Conclusions: Solanum ovalifolium is a potential source of compounds with anti-arbovirus activity. **Acknowledge** Founded by the Universidad Cooperativa de Colombia and FCTel of the SGR-Colombia

Workshop Session WORKSHOP SESSION 13: GENE EXPRESSION, GENE REGULATION AND DEVELOPMENT 07-20-2022 7:00 PM - 8:30 PM

INVESTIGATION OF THE GLUCOSE-MEDIATED REGULATORY MECHANISM OF TRANSCRIPTIONAL ACTIVATION OF FRUR.

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Background and Aims: In most bacteria, efficient use of carbohydrates is primarily mediated by the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) which concomitantly phosphorylates the substrates during import. Therefore, transcription of the PTS-encoding genes is precisely regulated by transcriptional regulators, depending on the availability of the substrate. Fructose is transported mainly through the fructose-specific PTS (PTS^{Fru}) and simultaneously converted into fructose 1-phosphate (F1P). The PTS-dependent utilization of fructose is inhibited by glucose in *Vibrio cholerae*, but the underlying mechanism remains unknown.

Methods: RNA extraction and quantitative real-time reverse transcription-PCR (qRT-PCR), Determination of the amount of sugars in the culture medium using High-Performance Liquid Chromatography system, Protein ligand fishing experiment, Protein binding assay using native polyacrylamide gel electrophoresis (native-PAGE), Electrophoretic mobility shift assay (EMSA), DNase I footprinting

Results: We previously show that, FruR acts as a transcriptional activator of the *fru* operon and is indispensable for the growth of *Vibrio cholerae* on fructose. Several lines of evidence suggest that binding of the FruR-F1P complex to an operator which is located between the –35 and –10 promoter elements changes the DNA structure to facilitate RNA polymerase binding to the *fru* promoter. Here, we investigate the regulatory mechanism by which glucose inhibits the FruR-mediated transcriptional activation of the *fru* operon.

Conclusions: We found that the fructose operon transcription was inhibited when cultured in glucose and fructose. This results in aberrant transcription of the genes encoding the fructose-specific PTS components. Through this results, we were able to confirm the carbon catabolite repression mechanism between PTS sugars.

Workshop Session WORKSHOP SESSION 13: GENE EXPRESSION, GENE REGULATION AND DEVELOPMENT 07-20-2022 7:00 PM - 8:30 PM

INTERACTIONS BETWEEN STREPTOCOCCUS THERMOPHILUS AND LACTOCOCCUS LACTIS STRAINS SHAPE THE FLAVOR PROFILE OF CHEESE

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Background and Aims: The extent to which microbial interactions play a role in the production of cheese is still an open question.

Methods: We used a strategy where one of the components of the original culture have been left out. We monitored the microbial community dynamics and metabolic changes during a year-long Cheddar cheese-making experiment. Additionally, we performed a controlled milk fermentation experiment using the same culture. We devised an approach that combined: the analysis of the microbial genomes, the construction and analysis of their genome-scale metabolic models, the analysis of the metatranscriptomes across the leave out conditions and the quantification of key metabolites.

Results: A significant benefit was observed on the overall *L. lactis* community population when *S. thermophilus* was present. Also, the presence of *S. thermophilus* strain was found to have an effect on the final metabolic profile of the cheeses. We obtained evidence that *S. thermophilus* may relieve the nitrogen limitation of the *L. lactis* community, which is necessary for de novo nucleotide biosynthesis. Interestingly, closely related strains of *L. lactis* subspecies *lactis* exhibited different interaction patterns with *S. thermophilus*, highlighting the importance of strain specificity. Also, we observed increased accumulation of key metabolites, such as diacetyl and acetoin, when the major *L. lactis* strain was left out. Based on experimental evidence, we hypothesize that this is due to competition between *L. lactis* strains for the available citrate.

Conclusions: We show a range of novel interactions both competitive and cooperative that take place to shape the flavor profile of the cheese.

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POSITIVE FEEDBACK, AND NOT INDUCER EXCLUSION, DRIVES MOST OF THE GLUCOSE-MEDIATED CATABOLITE REPRESSION OF THE LAC OPERON OF ESCHERICHIA COLI

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Background and Aims: Glucose-mediated *lac* repression in *Escherichia coli* is a classical problem in bacterial physiology. It is widely believed this repression is due to CRP-mediated transcriptional inhibition and PTS-mediated LacY inhibition (*inducer exclusion*). In 1996, Aiba and coworkers showed that CRP-mediated effect is weak, and hypothesised that the repression must be due to inducer exclusion (1).

Methods: To test this hypothesis, we measured the magnitude of inducer exclusion in *E. coli* cells induced to various levels. We found that LacY was never repressed more than ~5-fold, which is ~200-fold smaller than the observed ~1000-fold *lac* repression observed in presence of glucose. To find the source of this discrepancy, we measured the LacZ activities and *intracellular inducer levels* observed upon addition of glucose to *E. coli* cultures growing in the presence of lactose as well as TMG (2, 3).

Results: These transient measurements show that inducer exclusion accounts only for the initial ~5-fold repression that occurs within the first 5 min of glucose addition. The subsequent ~200-fold repression that occurs over several hours is due to amplification of the repression by positive feedback that is generated because the lactose enzymes promote the accumulation of the inducer, which in turn induces the synthesis of even more enzymes.

Conclusions: References 1. Inada, T., Kimata, K. and Aiba, H. (1996), *Genes to Cells*, 1(3), pp. 293–301. 2. Aggarwal, R. K. and Narang, A. (2022), *Biophysical Journal*. Cell Press, 121(5), pp. 820–829. 3. Aggarwal, R. K. and Narang, A. (2022), *Biophysical Journal*. Cell Press, 121(5), pp. 808–819.

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THE IMPACT OF CIPROFLOXACIN TREATMENT ON MICROBIAL AND METABOLIC REGIME SHIFTS IN A FERMENTATIVE MIXED CULTURE

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Background and Aims: Little is known about the effects of antibiotics on microbial communities. We analysed the impact of the broad-spectrum antibiotic ciprofloxacin on the microbial composition and metabolic functionality of a fermentative mixed culture.

Methods: A 0.75-L chemostat was sterilized, inoculated with standardized healthy-donor human faeces, and operated (dilution rate 0.16 h⁻¹) for glucose fermentation in mixed culture. After establishing the fermentative baseline, ciprofloxacin was administered over 3 days, prior to letting the bioreactor recover after treatment. Switches in product spectrum and rates, microbial selection, and metabolic regulation were tracked by on-line monitoring, liquid chromatography, metagenomics, and metaproteomics.

Results: The short-term impact of ciprofloxacin was profound. Fermentative bacteria (*Lactobacillus, Enterobacter, Enterococcus*) were outcompeted by the yeast *Candida albicans* (30% of DNA and protein read counts). Bacterial growth was not completely inhibited. The treatment resulted in a metabolic switch in fermentation product fluxes from mainly lactate ($0.20\pm0.04 \text{ mol}\cdot\text{h}^{-1}\cdot\text{C}-\text{mol}x^{-1}$) to ethanol and CO₂ (both 0.22 mol·h⁻¹·C-molx⁻¹). This matched with the emergence of *Candida* and the increased expression of key proteins associated with ethanol production (*e.g.*, alcohol and acetaldehyde dehydrogenases, pyruvate decarboxylase). The bacterial community and lactate production recovered to baseline level within 3 days after treatment, although instabilities persisted over the two-week recovery period.

Conclusions: Quantitative biotechnology and multi-omics helped identify the predominant regime shifts under ciprofloxacin administration in fermentative mixed culture derived from human faeces. Our findings provide a deeper understanding of microbial and metabolic perturbations, and antibiotic resistance development, upon antibiotic treatment of fermentative mixed cultures like gut microbiota.

Workshop Session WORKSHOP SESSION 14: MICROBIOME AND HEALTH 07-20-2022 7:00 PM - 8:30 PM

METAGENOME-SCALE SPECIES-RESOLVED FUNCTIONAL PROFILING OF THE GUT MICROBIOTA IN INFANTS AT RISK OF CELIAC DISEASE

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Background and Aims: Alterations in the gut microbiota have been associated with Celiac Disease (CeD); however, the contribution of individual microbial species to the overall functional activity of the microbiota (i.e., the gut metabolome) and their role in CeD pathogenesis are largely unknown. This is because existing functional profiling approaches merely enumerate functional pathways encoded by metagenomes but do not provide any information about what microbes carry what pathways or produce what metabolites. To address this gap, we leveraged data from a perspective birth cohort study of infants at high risk of CeD to functionally profile their gut microbiota at species and molecular level resolution.

Methods: We used 167 fecal metagenomes collected longitudinally from ten children who developed CeD and ten controls to construct genome-scale models of metabolism for 359 microbial species in these samples. The models for species present in each sample were then integrated to build sample-specific species-resolved models of the entire gut microbiota metabolism.

Results: By computationally simulating these models, we could track back individual microbial species producing specific secreted metabolites. We identified 18 (out of 2,120) species-metabolite pairs involving nine species and 14 metabolites, that were significantly different between cases and controls (Wilcox, p < 0.05). Some of these metabolites were previously implicated in CeD or inflammation, e.g., L-tryptophan, arabinose, xanthine, and cholic acid that are linked to species of Bacteroides, Bifidobacterium, and Blautia in our models.

Conclusions: Overall, this study provides a roadmap for mechanistically linking microbial and molecular markers of CeD in the gut.

Workshop Session WORKSHOP SESSION 14: MICROBIOME AND HEALTH 07-20-2022 7:00 PM - 8:30 PM

METAPROTEOMICS ANALYSIS REVEALS MICROBIAL AND FUNCTIONAL DIFFERENCES RELATED TO COW'S MILK TOLERANCE AND NUTRITIONAL INTERVENTIONS

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Background and Aims: Development of the gut microbiome occurs in early life. Previous studies provide evidence for an association between gut microbiota modifications and the development of food allergies. Nutritional interventions have been proposed to support food tolerance development by altering the gut microbiome. We aim to gain insight into microbial and functional differences related to outgrowth of cow's milk allergy (CMA) and nutritional interventions.

Methods: This study included 120 faecal samples of the PRESTO clinical trial (NTR3725), where 40 infants with CMA were monitored after diagnosis, as well as 6 and 12 months after a nutritional intervention with standardized amino acid formula or similar formula with a synbiotic blend (oligosaccharides (oligofructose, inulin) + Bifidobacterium breve). Twenty-four out of forty infants showed outgrowth of CMA after 12 months. We performed 16S rRNA sequencing and metaproteomics analysis of the bacterial gut microbiome.

Results: Outgrowth of CMA was characterized by significantly higher levels of proteins produced by some members of the core gut microbiota. Significantly higher levels of Bifidobacteriaceae and several microbial proteins were observed after treatment with synbiotics. The synbiotic group also had higher levels of several bifidobacterial carbohydrate-active enzymes, known to metabolize oligosaccharides.

Conclusions: Apart from studying the microbiome, metaproteomics also allows to study protein expression. By combining metaproteomics and 16S rRNA sequencing, several microbial and functional differences related to outgrowth of CMA and nutritional interventions could be revealed. Acknowledgement: Guus Roeselers and Heleen de Weerd (Danone-Nutricia Research) for input in the study design and pre-processing 16S rRNA data respectively.

Workshop Session WORKSHOP SESSION 14: MICROBIOME AND HEALTH 07-20-2022 7:00 PM - 8:30 PM

INFLUENCE OF GEOGRAPHICAL VARIATIONS ON THE STRUCTURE OF HUMAN GUT MICROBIOME

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Background and Aims: Human gut microbial communities are known to vary geographically. It is important to understand the nature of geographical influences in order to get an overall picture of how gut microbial community affects overall health. In this study, we have explored available human gut microbiome sequence datasets of individuals representing different geographical realms to map geographical influences on gut microbial community structure and identified factors that shaped the observed trends.

Methods: Selected datasets representing United States of America (PRJNA471742), Chile (PRJEB16755), South Africa (PRJEB40733), Kuwait (PRJNA554702) and Malaysia (PRJNA631204) were considered based on commonality in sequencing chemistry approach and overlap of the 16S rRNA molecule. As part of this study 13,52,59,399 number of sequences were analysed using computational biology approaches.

Results: It was observed that diet, lifestyle and geographical separation causes significant difference in gut microbial community structure. Firmicutes and Bacteroidetes were the most dominant phyla among all populations but Actinobacteria was exclusively present in high abundance in Malaysian populations (15.99%). Alpha diversity analysis (Shannon indices) showed that Malaysian population had highest diversity. Beta diversity analyses estimated using PCA and PCoA analyses showed that Chilean population formed a distinct cluster among all the studied populations.

Conclusions: The study suggests that not just geographical separation but a combined effect of geographical separation, modern lifestyle, food habits and several linked factors structure gut microbiome their functions and ultimately influence overall health.

O045 / #90

Workshop Session WORKSHOP SESSION 17: MICROBIAL EVOLUTION AND DIVERSITY, AND BACTERIOPHAGES 07-21-2022 10:30 AM - 12:15 PM

IDENTIFICATION OF PNEUMOCOCCAL FACTORS RELATED TO CLINICAL SYMPTOMS USING GENOME-WIDE ASSOCIATION STUDY

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Background and Aims: *Streptococcus pneumoniae* inhabits the normal nasopharyngeal flora of human infants, though often causes life-threatening bacterial infections, including pneumonia, bacteremia, and meningitis. However, pneumococcal factors that determine host pathophysiology remain largely unknown. In this study, pneumococcal genetic factors related to clinical symptoms were explored using a bacterial genome-wide association approach.

Methods: A total of 6037 whole genome sequences of *S. pneumoniae* from 13 countries registered in the NCBI database were obtained, in addition to 58 sequences that we previously determined. Using Pyseer, v.1.3.4, a genome-wide association study (GWAS) of genetic variants including single nucleotide polymorphisms (SNPs) in core genes was conducted. To elucidate the influences of SNPs on protein functions, protein structures were predicted with use of AlphaFold2.

Results: The GWAS results revealed 329 SNPs that were significantly related to clinical symptoms. Furthermore, 32 significant SNPs were detected in the *aroE* gene, which encodes shikimate dehydrogenase and has been reported to have effects on expressions of other virulence factors. Protein structure prediction obtained with AlphaFold2 suggested that an amino acid substitution in AroE is located in a domain that interacts with nicotinamide adenine dinucleotide phosphate.

Conclusions: The present GWAS identified SNPs significantly related to clinical symptoms, of which approximately 10% were found in the *aroE* gene. These results indicate that AroE mutations may have important effects on metabolic processes and pneumococcal pathogenesis.

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EVOLUTION TOWARDS SMALL COLONY VARIANTS OF PANDEMIC MULTIDRUG RESISTANT ST131 ESCHERICHIA COLI ISOLATES FROM A 10-YEAR BONE INFECTION

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Background and Aims: Chronic wounds can readily become infected by microorganisms due to failure of mechanical and physiological first line innate immune responses. We report the characterization of host adaptation of three *Escherichia coli* ST131 genomes that occurred during a 10-year chronic wound infection after a foot fracture.

Methods: The three *E. coli* strains were characterized by various microbiological and genomic approaches as assessment of antimicrobial resistance, growth in different media, biofilm formation and genome sequencing by PacBio RSII. Phylogenetic analyses and genome alterations such as single nucleotide polymorphisms, deletion and rearrangements that led to pseudogenes and chromosomal inversions were documented. Relevant selected metabolic and physiological pathways were analyzed for integrity.

Results: The three *E. coli* ST131 strains showed a heavily host-adapted genome with high number of pseudogenes and a large chromosomal inversion compared to ST131 reference strains. Furthermore, two of three *E. coli* ST131 isolates were small colony variants with its genetic basis in multiple genome alterations including pseudogenes and deletions in the pathway for heme biosynthesis. Pseudogene analysis indicated three ST131 strains to be mutator strains. Enhanced capability of biofilm formation of the ST131 isolates was indicated by the agar plate assay.

Conclusions: ST131 clone members are ubiquitously found in patients and the environment. ST131 strains have perhaps already been acquired from the environment upon foot fracture and persisted in wounds showing outmost genome plasticity and adaptability which might causing the chronic infection. Although co-infection with *E. faecalis* might have supported chronicity, these findings indicate that in individuals with underlying metabolic diseases wound infection by ST131 *E. coli* isolates can be a health risk.

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PHENOTYPIC CHARACTERIZATION OF STRAINS OF SALMONELLA ENTERICA SEROVAR TYPHIMURIUM GENOTYPE ST213

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Background and Aims: Salmonella enterica serovar Typhimurium is mainly associated with enteric selflimiting disease. Its virulence mechanism has been studied using reference strains belonging to the worldwide prevalent ST19 genotype. However, the emergence of strains from different genotypes associated with multidrug resistance and non-typhoidal invasive infections has increased. In Mexico, during an epidemiological surveillance program, Typhimurium strains, mostly belonging to the ST19 and ST213 genotypes, were isolated; the latter was the most abundant and was linked to five cases of systemic infection. ST213 strains possess distinctive characteristics such as a broad profile of antibiotic resistance encoded by large plasmids and lack the pSTV virulence plasmid. They have been mainly identified across North America and represent a public health risk. This study aimed to phenotypically characterize ten representative ST213 strains isolated in Mexico compared to a prototype ST19 strain.

Methods: Biofilm formation was assessed by crystal violet staining and RDAR morphotype. Their ability to invade and survive/replicate in epithelial cell lines and macrophages was assessed by aminoglycoside protection assays.

Results: ST213 strains displayed a stronger ability to form biofilms than the prototype ST19 strain but exhibited lower internalization capacity and no difference in intracellular replication.

Conclusions: Even though the reductionistic approach used for the study of the pathogenesis of Typhimurium led the field to great advance, the emergence of new strains stresses the need for integrative approaches to further our understanding of host-Typhimurium interactions, as the reference strains do not always represent the genomic diversity present in clinical or food isolates. *Acknowledgements: DGAPA-PAPIIT-(IN215119/IN218322) and CONACyT-354699-ISF.*

Workshop Session WORKSHOP SESSION 19: MICROBIAL BIOTECHNOLOGY AND APPLIED MICROBIOLOGY 07-21-2022 3:00 PM - 4:30 PM

ESCHERICHIA COLI BIOSENSORS BASED ON SURFACE ENHANCED RAMAN SCATTERING FOR MULTIPLEX DETECTION

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Background and Aims: The progress in synthetic and computational biology has significantly improved our capability to fabricate robust bacterial biosensors. These and other advancements have made possible, for instance, the engineering of E. coli as a programmable living tool for diagnostic and environmental applications. However, the dependency of bacterial biosensors on bioluminiscence, fluorescence, or colorimetric reporters limits their use for those applications requiring the simultaneous detection of multiple targets in the same sample. Surface-enhanced Raman scattering (SERS) spectroscopy is an analytical technique that employs plasmonic nanoparticles as optical enhancers for increasing the inherently weak intensity of the Raman signal. The main features of SERS include its high specificity, sensitivity, and multiplexing capabilities owing to the narrow spectral bandwidths that characterize the Raman spectra. In previous studies we successfully applied SERS for the in situ detection and imaging of secreted bacterial metabolites^{1, 2}. Herein, we report the development of bacterial biosensors based on SERS. 1. Bodelon G., et al. Nature Materials. 2016; 15(11):1203-1211 2. Bodelon G., et al. ACS Nano. 2017; 11(5):4631-4640

Methods: To this aim, we evaluated the inducible expression of heterologous Raman-active metabolites in E. coli and, in combination with multivariate statistics and machine learning tools, we investigated their potential use as SERS reporters.

Results: We show the unambiguous identification of the selected metabolites by SERS, as well as their simultaneous detection in mixtures of biosensor strains.

Conclusions: Our results demonstrate the suitability of the proposed approach, thereby paving the way for a novel class of bacterial biosensors with improved multiplex detection capabilities.

Workshop Session WORKSHOP SESSION 19: MICROBIAL BIOTECHNOLOGY AND APPLIED MICROBIOLOGY 07-21-2022 3:00 PM - 4:30 PM

A DYNAMIC ANTIBACTERIAL T6SS IN PANTOEA AGGLOMERANS PV. BETAE DELIVERS A LYSOZYME-LIKE EFFECTOR TO ANTAGONIZE COMPETITORS

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Background and Aims: The type VI secretion system (T6SS) is deployed by numerous Gram-negative bacteria to deliver toxic effectors into neighboring cells. The genome of Pantoea agglomerans pv. betae (Pab) phytopathogenic bacteria contains a gene cluster (T6SS1) predicted to encode a complete T6SS. We examined structural genes of the T6SS1 cluster and associated putative effector and immunity genes. In addition, we experimentally validated a predicted effector and immunity pair and its activity.

Methods: Computational analysis was used to characterize genes in the Pab T6SS1 cluster, while secretion and competition assays were employed to test T6SS1 functionality. These analyses revealed a specialized VgrG antibacterial effector and numerous putative effectors and immunity proteins. We tested functionality of the specialized VgrG using protein expression, toxicity and competition assays.

Results: The Pab genome contains two T6SS clusters: T6SS1 and T6SS2, which encode a complete and a partial T6SS, respectively. T6SS1 can be divided in three rapidly evolving genetic islands. Each island displays arrays of orphan immunity and toxin immunity modules acting as an antibacterial system and possibly conferring Pab the ability to resist aggression by competitors. Furthermore, the VgrG C-terminal domain encodes a peptidoglycan hydrolyzing toxin that targets a periplasmic component of prey cells.

Conclusions: Pab T6SS1 is a functional antibacterial system secreting toxic effectors to eliminate competitors and it is equipped with immunity proteins to avoid self-intoxication. Using bioinformatics, biochemical, and genetic assays, we identified T6SS-secreted effectors and determined that VgrG is a specialized antibacterial effector.

Workshop Session WORKSHOP SESSION 19: MICROBIAL BIOTECHNOLOGY AND APPLIED MICROBIOLOGY 07-21-2022 3:00 PM - 4:30 PM

A SUSTAINABLE REPLACEMENT FOR PIGMENTS? ENGINEERING BRILLIANT STRUCTURAL COLOUR IN FLAVOBACTERIUM IR1.

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Background and Aims: Structural colour (SC) is an optical mechanism by which ordered nanostructures reflect light to generate vivid, angle-dependent hues. A familiar example is a feather of the peacock. The optics of SC have been studied for centuries, in birds and insects, since the time of Newton and Hooke. Optical physics has now demonstrated of a rich variety of SCs in many living organisms. However, very little is known about the genes that encode SC in any branch of the tree of life. Some bacterial colonies also display SC, particularly strains from Class *Flavobacteriia*. Organised and aligned cells, such as those of *Flavobacterium* IR1, can form a 2D photocrystalline structure, resulting in vivid, angle-dependent colour when illuminated. We aim to use IR1 as a model, genetically amenable system to understand how living matter can organise to create SC.

Methods: Proteomics, transposon mutagenesis and genomics approaches have been used to identify the underlying genes/proteins and pathways involved in SC. A Crispr-Cas12 gene editing system was adapted to IR1 and is being used to make targeted changes to the genome to map the genes involved in SC and create new strains with improved photonic properties.

Results: We have created mutants with altered properties in SC, for example the colony b is 'red shifted' due to a transposon insertion, compared to the WT in panel a. Genomics/proteomics data and Crispr-mediated KOs will also be



Conclusions: We can create strains with improved photonic properties to understand the role of SC in nature and to make sustainable, photonic biomaterials to replace unsustainable pigments.

Workshop Session WORKSHOP SESSION 19: MICROBIAL BIOTECHNOLOGY AND APPLIED MICROBIOLOGY 07-21-2022 3:00 PM - 4:30 PM

GENETIC ENGINEERING AND IMPROVEMENT OF HYDROGEN PRODUCTION IN CYANOBACTERIA

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Background and Aims: Cyanobacteria are a diverse group of photoautotrophic prokaryotes that are found in a variety of environments, and can be grown in the laboratory. With genomic information available for more than 130 cyanobacterial strains, many can be engineered to enhance hydrogen production. Cyanobacteria are ideal cell factories for hydrogen production because they have low nutrient requirements and are capable of using light to generate biomass from water and CO₂. Cyanobacteria produce hydrogen through two key enzymes, nitrogenase and bidirectional hydrogenase, and oxidize molecular hydrogen by uptake hydrogenase.

Methods: Cyanobacterial uptake hydrogenase has two subunits; the small subunit directs electron transport to the large subunit, which has an active site that binds H₂. These subunits are encoded by *hupS* and *hupL* genes, respectively, that can be genetically modified to reduce activity of the uptake hydrogenase and increase hydrogen production.

Results: Genetic deletion of uptake hydrogenase in cyanobacteria affects strains in various ways. In *Anabaena* sp. PCC 7120 it increased hydrogen production by 4-7 fold while in *Anabaena variabilis* ATCC 29413 it reached a 5-fold improvement, compared to the wild type strain. Uptake hydrogenase deletion strains can be the starting point for further genetic modifications for the purpose of enhancement of their hydrogen-producing capacity.

Conclusions: Hydrogen production makes cyanobacteria promising for renewable fuel. Accumulation of biomass by cyanobacteria is linked to carbon dioxide sequestering. This reduces the carbon footprint by producing polymers of carbon. The ability to reduce carbon dioxide pollution as well as produce energy dense hydrogen makes cyanobacteria significant in biofuel research.

Workshop Session WORKSHOP SESSION 22: FRONTIERS IN BACTERIAL STRUCTURAL BIOLOGY 07-22-2022 11:00 AM - 12:30 PM

STRUCTURAL BASIS FOR THE ALLOSTERIC BEHAVIOUR AND SUBSTRATE INHIBITION OF LACTOCOCCUS LACTIS PROLIDASE

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Background and Aims: Prolidase (E.C 3.4.13.9) is an enzyme that specifically hydrolyzes Xaa-Pro dipeptides into free amino acids. We studied the kinetic behaviours and solved the crystal structure of *Lactococcus lactis* prolidase (*Ll*prol), showing that this homodimeric enzyme has unique characteristics: allosteric behaviour and substrate inhibition. Mutagenesis studies revealed that the allosteric behaviour of *Ll*prol was eliminated in *Ll*prol D36S and R293S mutants, and substrate inhibition was not detected in *Ll*prol H38S and R293S mutants.

Methods: We solved the crystal structures of D36S, H38S, and R293S *LI*prol mutants with X-ray crystallography.

Results: Structural comparison between the mutants suggests that *Ll*prol can adapt to different conformational states with distinct substrate affinities, and various interactions between the monomers are the keys to separating these conformational states. We speculate that in allosteric *Ll*prol (WT and H38S), the domain movements required by productive substrate binding are restrained. In addition, we also identified a secondary binding site in *Ll*prol variants that show substrate inhibition. The secondary binding site shares two amino acid residuals with the productive substrate-binding site of *Ll*prol.

Conclusions: In conclusion, we proposed that the relative positioning of monomers and the resulting variation in the structural rearrangements levels and the completeness of certain conformational changes upon substrate binding lead to the allosteric behaviour of *Ll*prol. The substrate inhibition is caused by the presence of a secondary binding site that is partially overlapped with the active site. The damaged secondary binding site explains the removal of substrate inhibition in *Ll*prol H38S and R293S.

Workshop Session WORKSHOP SESSION 23: FOOD MICROBIOLOGY 07-22-2022 11:00 AM - 12:30 PM

MICROBIAL FERMENTATION AS A MEANS OF INCREASING PROTEIN CONTENT AND REDUCING CYANIDE IN CASSA DERIVED FOOD PRODUCTS

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Background and Aims: Even though cassava is a potential energy-rich food crop, the shortage of protein content and the presence of toxic cyanogenic glycosides are considered inferior food. This study aimed to study the contribution of microbial fermentation to increasing protein content and reducing cyanide.

Methods: For the study, operating conditions were selected microorganisms (Saccharomyces cerevisiae, Lactobacillus plantarum, and Lactobacillus coryneformis), inoculum level (0.5 and 1.5ml), and fermentation time (24 and 48h). Thus, three hundred grams (300 g) of cassava-teff four were fermented with each of single starter culture at 24 and 48 h.

Results: The analysis of pH, crude protein, and cyanide content indicated that fermentation samples with Lactobacillus plantarum and Lactobacillus coryneformis for 48 h with 1.5 ml inoculum resulted in the highest pH and cyanide reduction were recorded. Whereas the least cyanide reduction was recorded in fermentation samples of Saccharomyces cerevisiae at 24 h of 1 ml of inoculum level, this value was higher when compared upon boiling (47.77 mg/kg. The highest levels of crude protein were observed in fermentation samples with 1.5 ml inoculum of Saccharomyces cerevisiae for 48 h.

Conclusions: Therefore, microbial fermentation is a promising candidate for improving nutrient content and removing toxic substances in cassava and is suggested as a choice of the processing method, as this method significantly reduced cyanide content, and increases the protein content of cassava derived products.

Workshop Session WORKSHOP SESSION 23: FOOD MICROBIOLOGY 07-22-2022 11:00 AM - 12:30 PM

BACILLUS AMYLOLIQUEFACIENS ENRICHED CAMEL MILK ATTENUATED COLITIS SYMPTOMS IN MICE MODEL

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Background and Aims: Fermented camel's milk has various health beneficial prebiotics and probiotics. This study aimed to evaluate the preventive efficacy of Bacillus amyloliquefaciens enriched camel milk (BEY) in 2-, 4- and 6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis mice models.

Methods: To this end, the immune modulatory effects of Bacillus amyloliquefaciens (BA) on TNF- α challenged HT29 colon cells were estimated using the cell proliferation and cytokines ELISA method. BEY was prepared using the incubation method and nutritional value was quantified by comparing it to commercial yogurt. Furthermore, TNBS-induced colitis was established and the level of disease index, pathological scores, and inflammatory markers of BEY-treated mice using macroscopic and microscopic examinations, qPCR and immunoblot were investigated.

Results: The results demonstrate that BA is non-toxic to HT29 colon cells and balanced the inflammatory cytokines. BEY reduced the colitis disease index, and improved the body weight and colon length of the TNBS-induced mice. Additionally, Myeloperoxidase (MPO) and pro-inflammatory cytokines (IL1 β , IL6, IL8 and TNF- α) were attenuated by BEY treatment. Moreover, the inflammatory progress mRNA and protein markers nuclear factor kappa B (NF κ B), phosphatase and tensin homolog (PTEN), proliferating cell nuclear antigen (PCNA), cyclooxygenase-2 (COX-2) and occludin were significantly down-regulated by BEY treatment. Interestingly, significant suppression of PCNA was observed in colonic tissues using the immunohistochemical examination. Treatment with BEY increased the epigenetic (microRNA217) interactions with PCNA.

Conclusions: In conclusion, the BEY clearly alleviated the colitis symptoms and in the future could be used to formulate a probiotic-based diet for the host gut health and control the inflammatory bowel syndrome in mammals

Workshop Session WORKSHOP SESSION 23: FOOD MICROBIOLOGY 07-22-2022 11:00 AM - 12:30 PM

METAGENOMICS HIGHLIGHTS STRAIN-LEVEL SELECTION OCCURRING IN ITALIAN TYPICAL FERMENTED FOODS

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Background and Aims: In modern times, many traditional fermentation technologies have been industrialized, relying on the use of selected microbial starters to standardize the process and the fermented food properties. However, this leads to the global diffusion of few microbial strains and to a huge decrease of the microbial diversity in fermented foods. The project FOODMICROHERITAGE has the goal to characterize the microbiome of typical Italian fermented foods by using shotgun metagenome sequencing and to create a collection of microbial genomes of lactic acid bacteria (LAB) species and strains from artisanal fermented foods, to preserve and depict the microbial diversity of these traditional products.

Methods: More than 200 Italian fermented foods were collected from different producers, including fresh, medium- and long-ripened cheeses and fermented meats. Metagenomics sequencing and Volatile Organic Compounds (VOCs) analysis were carried out.

Results: We identified microbial genes involved in the production of the typical sensorial properties of each fermented food, highlighting the presence of different LAB strains in each product. Microbial profiles of each food type are unique and can be used to track fermented foods origin and production according to traditional practices.

Conclusions: Microbiome of fermented foods can be used as marker to track the origin and the typical production tecnology. Acknowledgements: This work was funded by a grant from the Italian Ministry of Foreign Affairs and International Cooperation to the project FOODMICROHERITAGE–Quality and authenticity protection of artisanal fermented foods through the characterization and conservation of their microbial and genetic heritage, CUP E79J21002000001.

Workshop Session WORKSHOP SESSION 24: SKIN NEGLECTED TROPICAL DISEASES 07-22-2022 11:00 AM - 12:30 PM

THE HUMAN PATHOBIONT MALASSEZIA FURFUR SECRETED PROTEASE MFSAP1 REGULATES CELL DISPERSAL AND EXACERBATES SKIN INFLAMMATION

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Background and Aims: Malassezia forms the dominant eukaryotic microbial community on the human skin. The Malassezia genus possesses a repertoire of secretory hydrolytic enzymes involved in protein and lipid metabolism which alter the external cutaneous environment. The exact role of most Malassezia secreted enzymes, including those in interaction with the epithelial interface, are not well characterized.

Methods: In this study, we compared the expression level of secreted proteases, lipases, phospholipases, and sphingomyelinases of Malassezia globosa in healthy subjects and seborrheic dermatitis or atopic dermatitis patients. We observed upregulated gene expression of the previously characterized secretory aspartyl protease MgSAP1 in both the lesional and non-lesional skin sites of affected compared to healthy subjects. To explore the functional role of MgSAP1 in skin disease, we generated a knockout mutant of the homologous protease MfSAP1 in the genetically tractable Malassezia furfur.

Results: We observed the loss of MfSAP1 resulted in dramatic changes in cell adhesion and dispersal in both culture and a human 3D reconstituted epidermis model. In a murine model of *Malassezia* colonization, we further demonstrated that MfSAP1 contributes to inflammation as indicated by the reduced edema formation and myeloid cell infiltration with the knockout mutant versus wildtype *M. furfur*.

Conclusions: Taken together, we show that the secretory *Malassezia* aspartyl protease MfSAP1 has an important role in enabling a planktonic cellular state that can potentially aid in host colonization and additionally act as a virulence factor in barrier-compromised skin, further highlighting the importance of the contextual relevance when evaluating the functions of secreted microbial enzymes.

Workshop Session WORKSHOP SESSION 24: SKIN NEGLECTED TROPICAL DISEASES 07-22-2022 11:00 AM - 12:30 PM

MYCETOS: USING AN OPEN SOURCE DRUG DISCOVERY APPROACH TO IDENTIFYING NOVEL COMPOUNDS ABLE TO INHIBIT MADURELLA MYCETOMATIS, THE MAIN CAUSATIVE AGENT OF THE MYCETOMA

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Background and Aims: Mycetoma is a Neglected Tropical Disease characterized by large subcutaneous swellings and the formation of grains. Mycetoma is treated with itraconazole and surgery with low success rates, resulting in amputations and social sigma. To improve the therapeutic success rates a novel drug is needed. Due to the lack of interest of pharmaceutical industry, the open source drug discovery program mycetOS was established.

Methods: In total 1360 compounds were screened for in vitro activity against M. mycetomatis. Compounds able to inhibit growth at 100 μ M, 25 μ M, and had an IC50 < 8 μ M were selected for studying in vivo efficacy in our M. mycetomatis grain model in the invertebrate Galleria mellonella.

Results: In total 302 compounds were able to inhibit growth at 100 µM and 23 of those met all criteria to be screened in vivo. Of these 23, nine did prolong larval survival. These included 3 out of 7 azoles tested, olorofim, fenbendazole, MMV006357, MMV022478, MMV675968 and MMV1782387. Based on these results, 6 compound series were selected for further studying: fenarimols; aminothiazoles; phenotiazines; dihydrofolate reductase inhibitors; benzimidazoles and ketoximines. For the fenarimols in total 185 additional compounds were screened. By analyzing the in vitro activity and in vivo efficacy in relation to the chemical properties of the fenarimol it appears that the LogD value of a compound was important for in vivo efficacy.

Conclusions: Here we demonstrated that an open source drug discovery project such as MycetOS can be an effective way to identify novel lead compounds for fungal skinNTDs.

Workshop Session WORKSHOP SESSION 25: MYCOBIOMES 07-22-2022 11:00 AM - 12:30 PM

IMPROVED EXTRACTION OF LIGNOCELLULOSE DEGRADING ENZYMES FROM IRPEX LACTEUS

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Background and Aims: Lignocellulosic biomass can be used as a source for energy, fuel and valuable chemical production. Nevertheless, practical and industrial scale application is still limited due to either high conversion costs, low efficiency or environmental issues (unsustainable technologies for conversion like concentrated acid hydrolysis). From all technologies, biological approaches have been recognized as the most sustainable ones, however, the need for specific and expensive lignocellulose degrading enzymes and generally slow conversion rates (from one to several days) still set this technology aside. The need for new enzyme formulations that have lower production costs, compatibility with current commercial products (currently being produced in a limited group of large enterprises) and better use conditions is still topical. Within this research we aim to develop enzyme products from white rot fungi *Irpex lacteus* and other wood decay fungi.

Methods: Fungal growth was performed in laboratory scale reactor system under various mixing conditions. Enzyme extraction was performed with protein sedimentation or membrane separation. Subsequent enzyme characterization was performed by representative FPU, CMC assays and hydrolysis tests within project No.1.1.1.1/18/A075.

Results: The results demonstrated comparable conversion efficiency in less than 30 hours at mild environmental conditions. Introduction of ultrasound pre-treatment during enzyme extraction, have improved the recovered enzyme yields for more than 20%. Furthermore, the production is less sensitive than previously described, e.g., in terms of fungal growth and incubation conditions.

Conclusions: Subsequent validation of the produced enzyme products has demonstrated the ability to produce as much as 0.4 g of fermentable carbohydrates per g of dry biomass.
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Workshop Session WORKSHOP SESSION 25: MYCOBIOMES 07-22-2022 11:00 AM - 12:30 PM

STRONG IMPACT OF MICROCLIMATIC, CLIMATIC, AND ANTHROPOGENIC FACTORS ON CULTIVABLE SOIL MICROFUNGAL COMMUNITIES IN A CANYON AT THE NORTHERN ISRAEL.

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Background and Aims: The study aimed to examine the effect of microclimatic, climatic, and anthropogenic factors on soil culturable microfungal communities at the Nahal (wadi) Metzar (the Golan Heights, Israel) consisting of the mesic temperate north-facing slope (NFS) and the more xeric south-facing slope (SFS).

Methods: For isolation of microfungi, the soil dilution plate method was employed.

Results: A total of 94 fungal species from 47 genera was isolated. The communities' composition was subjected to the pronounced spatial (interslope) and seasonal (summer-winter) variations. While xerotolerant melanin-containing species and thermotolerant Aspergillus spp. predominated in the soil of SFS, peaking up in the summer, mesophilic Penicillium spp. were especially abundant at the NFS. The quantitative parameter – density of isolates, exhibited the strongest seasonal variations being more sensitive to the fluctuation in soil temperature and moisture. Comparison of microfungal communities in the currently recovered and previously disturbed soil at the SFS displayed the variations in communities' composition and diversity level. Comparison of the NFS microfungal communities isolated from undisturbed soil in the summer 2002 and 2019 revealed changes in the community structure, which could be related to global warming.

Conclusions: The microclimatic and edaphic differences of the wadi slopes caused significant variations in the diversity of mycobiota. The intensive pasturage followed by soil degradation led to the simplification of microfungal communities, decreasing their diversity level, and caused the prevalence of species with different ecological preferences. The changes possibly related to global warming were associated with stress-tolerant fungal traits, which might be useful under increasing soil temperature and desiccation.

EP001 / #210

E-Poster Viewing Topic: AS01 Virus discovery and virome studies

VIROME DISCOVERED IN THE SURFACE WATER OF THE CASPIAN SEA

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Background and Aims: The Caspian Sea is the largest inland waterbody on Earth, which involved in intensive human production activities since the discovery of oil and gas fields. Research of the ecological state of the sea is based on the studies of the five countries of the Caspian Sea region. Unfortunately, despite significant progress in the study of the region's flora and fauna, there are only sporadic studies of the diversity of fish, mammalian, prokaryotic and protozoan viruses. In our research, the study of virosphere in the surface waters of the Caspian Sea in Tyup-Karagan Bay was carried out by metagenomic sequencing method for the first time.

Methods: Water samples were collected in September 2021 with a volume of 10 l each. The samples were filtered to remove phyto- and zooplankton. Virus particles were concentrated from the samples by ultracentrifugation. Total nucleic acid was isolated from the precipitate for subsequent sequencing on MiSeq. Sequencing data were analyzed using Kaiju software, using a reference database of non-redundant proteins with default parameters.

Results: The taxonomic classification revealed that the most numerous and diverse group were viruses of the Heunggongvirae kingdom, affecting prokaryotes, among which 39% belonged to the family Siphoviridae, 9% Podoviridae, 5% Myoviridae and 2% Autographiviridae. The remaining viral sequences belonged to the Baculoviridae (Alphabaculovirus), Phycodnaviridae, Mimiviridae, Marseilleviridae, Microviridae families and others.

Conclusions: The obtained data provide the basis for a comprehensive study of the Caspian Sea virome and an understanding of the ecological interactions therein between populations of viruses, bacteria, protozoa and macroorganisms.

EP002 / #535

E-Poster Viewing Topic: AS01 Virus discovery and virome studies

DIVERSITY PHAGES AS A PROMISING SOURCE FOR THE CREATION OF NEW ANTIMICROBIAL DRUGS

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Background and Aims: Bacterial infections are known to cause high mortality worldwide, however, there has been little successful development of new drugs against multidrug-resistant pathogens. Of great concern are human diseases caused by commensal microflora combined into an acronym ESCAPE and Escherichia. Therefore, to combat this group of infections, a relatively new and effective direction of therapy by bacteriophages began to be used. For this, it is of great importance to search for sites of isolation of lytic viruses. For a comparative study of the diversity of phages capable of lysing enterobacteria, metagenomic studies of water samples from some reservoirs of the Aral-Syrdarya basin were carried out.

Methods: The samples were freed from microflora by filtration through membrane filters and concentrated to obtain total preparations of nucleic acids. Total nucleic acid preparations were shotgun sequenced using a MiSeq sequencer (Illumina).

Results: An analysis of the obtained databases of nucleic acid fragments from 150 to 300 nucleotides in length showed that viruses that infect ESCAPE and Escherichia are the most relevant for the creation of antimicrobial drugs. It has been established that sequences of 7 families of phages belonging to the Caudovirales order (Myoviridae, Demerecviridae, Siphoviridae, Drexlerviridae, Podoviridae, Autographiviridae, Schtoviridae), capable of lysing ESCAPE and Escherichia representatives, were found in the total nucleic acid samples of water bodies.

Conclusions: Thus, it has been shown that the search for new phages has a high level of promise for the creation of new antimicrobial drugs.

EP003 / #260

E-Poster Viewing Topic: AS01 Virus discovery and virome studies

USING ELECTRON MICROSCOPY TO UNCOVER LATENT PLANT VIRUSES

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Background and Aims: A combination of electron microscopy and genome sequencing is highly efficient in screening for latent virus infections independent of the virus morphology or genome. One example is hoya tobamovirus-2 (HoTV-2 genbank accession number: MT750216.1) that has been reported from symptomatic mixed infected samples in 2011 and also as single, asymptomatic, infection in several *Hoya* species (Richert-Pöggeler et al. 2018; Gaafar et al. 2020) in Germany.

Methods: 1- Symptom expression 2- Initial screening for systemic infection 3- RNA extraction, RT-PCR 4- Sequence analysis 5- Immuno-electron microscopy 6- Ultrathin sectioning

Results: The virus induces no symptoms in experimental host plants. 14 days post inoculation, plant sap homogenates were examined using negative staining. Rod shaped virions of 300nm length were detected in newly developing leaves, confirming systemic infection. The virions' morphology and size indicated presence of a tobamovirus. The virus titer was lower when compared to tobacco mosaic virus (TMV) infected plants. The assembled virus genome could be assigned to the genus *Tobamovirus*. Phylogenetic analysis showed it is closely related to Youcai mosaic virus (YoMV). The heterologous antiserum reacted with HoTV-2. The binding of antibodies along the virion was irregular when compared to a homologous antisera.

Conclusions: 1- YoMV antisera (DSMZ AS 0527) can be used for detection of HoTV 2 2- A homologous antiserum for HoTV 2 needs to be developed for specific identification 3- HoTV 2 infection seems to induce autophagy, an antiviral mechanism in the host plant 4- It is important to maintain phytosanitary measures to contain tobamoviruses from uncontrolled spreading

EP004 / #320

E-Poster Viewing Topic: AS01 Virus discovery and virome studies

ISOLATION AND DETECTION OF BACTERIOPHAGES THAT COULD INFECT PSEUDOMONAS AERUGINOSA FROM THE FRESH WATER ENVIRONMENT

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Background and Aims: In this study, we focused on bacteriophages that live in freshwater environments. Specifically, we have investigated whether phages that can infect *Pseudomonas aeruginosa*, could be isolated from these environments.

Methods: Small particles including bacteria were removed from the sampled environmental lake-water by passing through a membrane filter with a pore size of 0.22 µm, after which the phage or virus fractions were collected by ultracentrifugation. The phage fractions were then subjected to a plaque assay with *Pseudomonas aeruginosa*. Afterwards, the single plaque sample was suspended in the buffer and then re-purified. Large scale of culture of the phages have done using the same plate-plaque method. Phages were collected by ultracentrifugation and extracted the DAN for sequences by next-generation of DNA sequencer. The isolated phages morphology were also observed using field emission scanning electron-microscopes (FE-SEM).

Results: As the screening result, eleven strains of phage using *Pseudomonas aeruginosa* PAO1 as a host were isolated. Using an FE-SEM, phage-like particles having a polyhedral structure with diameter of 90 nm were observed. The results of fluorescence microscope observation show that the phages have double-stranded DNA in their genomes. The sequence of the DNA genome using a next-generation sequencer represents about 61.6 kb circular genome has been observed from a strain of isolated phage. The eighty-nine open reading-flames were detected.

Conclusions: We have isolated two type of phages that could infect *Pseudomonas aeruginosa* PAO1 from the same environmental water. The details of the genome structure, the host-range and characteristics of the isolated phages will be presented.

EP005 / #491

E-Poster Viewing Topic: AS01 Virus discovery and virome studies

POTENTIAL EVIDENCE FOR THE INFECTION OF A RECENT H3N2 INFLUENZA A VIRUSES IN SWALLOWS WITH OCULAR DISEASE AND ANOREXIA

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Background and Aims: Poxvirus, Flavivirus, Influenza virus (H5N1), etc. were previously known as viruses that cause ocular diseases in avian species. Four swallows with ocular disease and anorexia were found in 2021, Jeju-Island, Korea. They were treated with antibiotics, but there were no improvement and eventually died. Therefore, we were suspicious of ocular diseases caused by unknown viruses.

Methods: The samples of oral, cloaca, and tears were collected from them. First, DNA and RNA were extracted after pooling samples. Then, they were amplified by T4 Polymerase and K-Random Primer for NGS analysis which was performed by Macrogen (Seoul, Korea). The obtained raw data was analyzed using CLC Genomics Workbench 21.0.05 in the order of trimming, de novo assemble, and BLAST analysis.

Results: Several contigs from one of 4 swallow samples were found to match human Influenza A (H3N2) virus genes (PB2, PA, M2, HA, PB1) in the BLAST analysis showing 94.77-99.75% of sequence identities. All matched contigs were most similar to H3N2 discovered in 2021 (A/Bethesda/001/2021(H3N2), A/France/IHUMI_InfluenzaAH3N2_genome_60678_3/2021(H3N2), A/Minnesota/02/2021(H3N2), A/NewYork/01/2021(H3N2), A/SaoPaulo/HIAE001/2021(H3N2), A/Virginia/01/2021(H3N2)).

Conclusions: Influenza A virus (H3N2) has been known to be able to infect humans, swine, avian species, and so on, and resulting in frequent reassortment to generate diverse variants. As the virus closely related with recently reported human Influenza A (H3N2, 3C.2a1b) in 2021 was found in swallows which is a migrating bird sharing their breeding habitat with humans during summer season in Korea, there should be further study about the interspecies transmission of human Influenza A (H3N2, 3C.2a1b) between swallows and humans.

EP006 / #599

E-Poster Viewing Topic: AS01 Virus discovery and virome studies

PHAGES OF ROOT NODULE BACTERIA NATIVE TO PRIMARY GENE CENTER OF CULTIVATED PLANTS AT THE CAUCASUS

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Background and Aims: Phages can significantly reduce bacteria titer in soil and in plant rhizosphere as well. When bacteriophages integrate into bacteria chromosome as a result of lysogenic infection then they can impact on cultural and/or symbiotic properties of rhizobia. The purpose of our research was to test bacteriophages diversity in the Primary Origin of cultivated plants at the Caucasus, which was discovered by N.I. Vavilov.

Methods: Negative colony morphotypes were assessed using Adams method. Virion morphology was assessed using electron microscopy methods. Genomes were sequenced using MiSeq, Illumina, assembled (SPAdes, Flye, Racon and Medaka modules, Pilon), annotated with Prokka and analysed using BLASTn and BLASTp tools.

Results: The three bacteriophages differing in morphotypes of negative colonies were obtained. According to electron microscopy research, recovered phages belong to Siphoviridae, Podoviridae and Myoviridae families. Phage of the Siphoviridae family had a genome larger than 60 kb and it showed similarity to Rhizobium phage 16-3 (Ref Seq NC_011103). Another phage of the Podoviridae family had a genome larger than 120 kb, and the last one phage was assigned to Emdodecavirus order of the Myoviridae family since its genome was more than 400 kb. No similarity was revealed between nucleotide sequences of above both phages with any known bacteriophages.

Conclusions: Origins of diversity of plants are well known for genetic diversity of legumes and for their root nodule nitrogen fixing bacterial symbionts as well, and according to our research the diversity of rhizobiophages could be predicted in a such regions as well. This work supported by RSF 20-16-00105

EP007 / #745

E-Poster Viewing Topic: AS02 Antiviral immunity

INHIBITION OF FELINE INFECTIOUS PERITONITIS VIRUS REPLICATION BY NATURAL EXTRACTRS OF MALLOTUS PHILIPPENSIS

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Background and Aims: Feline infectious peritonitis virus(FIPV) causes a fatal and devastating disease in young cats. It usually leads to death with symptoms such as severe vomiting, diarrhea, ascites, and pleural effusion. In addition, as the virus infect persistently, it may recur in stressful situations. We have searched for antiviral candidates that could be used to treat the FIPV. Finally, we obtained a natural extract from the Mallotus philippensis tree and confirmed that it inhibits the replication of the virus.

Methods: Our research team made nanoparticles by inserting the natural extract into a liposome. Next, the potency of suppressing the replication of the FIP virus within the range in which toxicity is not observed. After inoculating cells with the nanoparticles of various concentrations and FIP virus, the degree of inhibition of replication was compared at RNA level, protein level, and viron level. Additionally, the timing of action was investigated using the time of addition test.

Results: When using natural extract-nanoparticles, the effect of suppressing the virus was higher than when using the natural extract alone. The particle inhibited the virus at all three levels effectively: RNA, protein, and virion. As a result of the time of addition test, the natural extract appears to inhibit the stage of virus endocytosis in cells.

Conclusions: Therefore, nanoparticles made using *Mallotus Philippensis* fruit extract are an antiviral agent that can be used for the treatment of FIP virus. For clinical use, further toxicity evaluation and clinical trials are required.

EP008 / #235

E-Poster Viewing Topic: AS02 Antiviral immunity

SERUM BIOMARKER LEVELS PRIOR AND AFTER DAA TREATMENT IN HCV CHRONIC-INFECTED PATIENTS

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Background and Aims: The treatment with direct acting antivirals (DAA) for hepatitis C infection (HCV) has offered an opportunity to analyze kinetics profiles of serum biomarkers and the changes in the immune system. The role of complex biological response modulators in the immunopathogenesis of HCV have not been sufficiently investigated. The aim of this study was to analyze the serum biomarker levels prior and after DAA treatment in HCV chronic-infected patients.

Methods: Serum concentrations of 13 growth factors (Angiopoietin-2, EGF, EPO, FGF-basic, G-CSF, GM-CSF, HGF, M-CSF, PDGF-AA, PDGF-BB, SCF, TGF- α , VEGF) and 12 cytokines (IL-5, IL-13, IL-2, IL-6, IL-9, IL-10, IFN- γ , TNF- α , IL-17A, IL-17F, IL-4 i IL-22) were analysed in 56 chronic HCV patients before and after sustained virological response (SVR) and compared with 15 controls by using bead-based flow cytometry. Statistical analysis was performed using R. Statistical significance was set at p< 0,05.

Results: The results shown the importance of variables in the classification of patients before and after DAA treatment compared with controls. Significantly increased concentrations of IL-4, SCF, and IL-5 were observed in patients with chronic hepatitis C before and after DAA treatment.



Figure 1.The importance of variables in the classification of patients before starting treatment and in control group.



Figure 2. The importance of variables in the classification of patients after treatment and in control group.

Conclusions: The association of SCF and IL-4 in patients with HCV infection compared to controls suggests an important role for mast cells in HCV. Also, IL-4, SCF and IL-5 could be considered as a specific biomarkers of DAA treatment. Therefore, differences in SCF concentrations between HCV patients and controls are extremely important, as SCF is one of the key factors in liver regeneration.

EP009 / #122

E-Poster Viewing Topic: AS04 Virus evolution and emergence

MOLECULAR AND GENOMIC CHARACTERISTICS OF ROTAVIRUS CIRCULATING IN THE SOUTH OF BENIN, 2016-2018.

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Background and Aims: We report the genotype prevalent and whole genome sequence data prior to ROTAVAC® vaccine introduction in Benin. The study aim was to monitor circulating RVA strains during this baseline examination period for changes that may affect vaccine performance in Benin.

Methods: A total 72 selected RVA strains collected in Benin during the 2016-2018 RVA season, were sequenced by NGS, using the Illumina MiSeq with 500 cycles and the standard 250 bp paired-end reads method. Maximum-likelihood trees were constructed using MEGA 6 with 1000 bootstrap replicates. Alleles were assigned based on phylogenetic clusters with bootstrap support of \geq 70% and nucleotide identity of \geq 95.8% within a cluster

Results: Common genotype combinations were G1P[8] (32%), G2P[4] (26%), G3P[6] (16%), G12P[8] (13%) and mixed G and P types (1%). Most of the NGS-sequenced strains exhibited Wa-like genetic constellations, G1/G9/G12-P[6]/P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 or DS-1-like G2/G3/G9/G12-P[4]/P[6]-I2-R2-C2-M2-A2-N2-T2-E2 or E6-H2. At the allelic level, in-depth analysis of the Benin strains identified 2-13 and 1-17 sub-genotypic alleles for DS-1-like and Wa-like strains, respectively. Most of the study strains clustered into previously defined alleles, but we defined 3 new sub-genotypic alleles each for VP7 (one for G3 and two for G12) and VP4 (one for P[4] and two for P[6]) which formed the basis of the VP7 and VP4 gene clusters, respectively.

Conclusions: These pre-vaccine baseline data provide information on the genetic composition of RVA in Benin and will help identify changes in genotypes and alleles that occur post vaccine introduction

EP010 / #120

E-Poster Viewing Topic: AS04 Virus evolution and emergence

MOLECULAR PHYLOGENY OF GAMMAHERPESVIRUSES IN BATS AND MOLES IN VIETNAM AND JAPAN

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Background and Aims: Bats (Order Chiroptera) serve as reservoir hosts of multiple zoonotic pathogens, including Ebola virus, Nipah virus, rabies virus and SARS coronaviruses. Also, recent molecular evidence indicates that moles (Order Eulipotyphla), captured in Eurasia and North America, harbor genetically distinct hantaviruses.

Methods: To establish cell cultures for use in virus isolation attempts, kidney tissues were collected from 28 bat species in Vietnam and one mole species in Japan.

Results: Cytopathic effect was observed in primary kidney cell cultures from two *Rhinolophus acuminatus*, one *Rhinolophus chaseni* and one *Mogera wogura*. Analysis by next-generation sequencing showed gammaherpesviruses (GHV) in *R. acuminatus*, *R. chaseni* and *M. wogura*. To ascertain the presence of the *Rhinolophus* gammaherpesvirus (RGHV, 139,222-bp) and *Mogera* gammaherpesvirus (MGHV, 136,749-bp), RNA*later*TM-preserved lung tissues of 503 bats (75 species) and 27 moles (4 species), collected in Vietnam, Cameroon, the United States and Japan, during 2002–2019, were screened by PCR using newly designed oligonucleotide primer sets. RGHV and MGHV sequences were confirmed in 14 *R. acuminatus*, three *R. chaseni* and five *R. microglobosus* in Vietnam and in two *M. wogura* in Japan. Phylogenetic analysis indicated that the newfound RGHV was similar to other bat-borne GHV from *Rhinolophus blythi* in China. Interestingly, the MGHV shared a common ancestry with the bat-borne GHV.

Conclusions: Future studies are warranted to determine the genetic diversity and geographic distribution of RGHV and MGHV, as well as other GHV, in bats and moles.

EP011 / #389

E-Poster Viewing Topic: AS05 Pathogenesis of viral diseases

UTILITY OF MEDICAL IMAGING IN MONITORING FILOVIRAL DISEASE

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Background and Aims: The pathophysiology of filoviral infection and disease is relatively poorly understood and is typically interrogated using biomarkers, clinical pathology and ex vivo assays, some of which provide only single snapshots in time. Complementing these with non-invasive in vivo medical imaging in well-established animal models may enable longitudinal disease characterization that can not only be mapped to known disease biomarkers and clinical pathology but also provide high resolution insight into poorly understood disease phenotypes, e.g. meningoencephalitis associated with filoviral persistence in filovirus disease survivors. Importantly, longitudinal imaging potentially works around ethically debated and logistically challenging serial euthanasia studies.

Methods: Toward this end, we performed several pilot studies in nonhuman primate models of filoviral (Marburg virus and Ebola virus) infections, during which we acquired whole-body imaging with multiple imaging modalities (computed tomography, positron emission tomography, and magnetic resonance imaging) at several timepoints after virus exposure.

Results: We identified imaging abnormalities in liver, spleen, lymph nodes, bone marrow, lungs, pancreas, brain, vasculature, and mesentery—corresponding to clinical, virological, and gross pathologic hallmarks of filoviral infections in these animal models. Each modality provides unique insight: some sample images are shown in the

figure.



Figure: (A) Computed tomography images of liver (left panels) and spleen (right panels) in nonhuman primates exposed to Marburg virus, showing hepatomegaly and splenomegaly at the terminal day compared to baseline reading. (B) Time course of magnetic resonance imaging (T2-weighted sequence) in a nonhuman primate exposed to Ebola virus, showing progressively increasing signal in the liver, which likely reflects late-stage congestion and/or edema. BL: baseline, D2: Day 2 (after virus exposure), D5: Day 5, DT: terminal day (euthanasia criteria met), a.u.: arbitrary units.

Conclusions: Historically, imaging characterization of human filoviral disease has not been routinely available. In the absence of human data, *in vivo* medical imaging adds an additional tool to qualitatively and quantitatively characterize disease otherwise opaque to investigation in the absence of serial euthanasia. Furthermore, understanding and benchmarking the imaging correlates of known disease markers may provide previously unavailable readouts in the evaluation of medical countermeasures.

EP012 / #718

E-Poster Viewing Topic: AS05 Pathogenesis of viral diseases

PATHOGENICITY OF VARIEGATED SQUIRREL BORNAVIRUS

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Background and Aims: Since 2015, variegated squirrel bornavirus (VSBV) was detected in four people with lethal encephalitis and in squirrels. Borna disease virus (BoDV) closely related to VSBV also causes lethal encephalitis in human, however, difference of pathogenicity between VSBV and BoDV is unclear. Although we tried to generate VSBV LN713681 strain using reverse genetics system, virus rescue was failed. To elucidate the pathogenicity of VSBV, we improved the reverse genetics system of VSBV and evaluated the pathogenicity of chimeric viruses between VSBV and BoDV.

Methods: Consensus sequence of VSBV was obtained by alignment of complete genome sequences from 23 isolates. We constructed template plasmid for expression of VSBV anti-genome RNA encoding the consensus sequence. We rescued chimeric viruses recombining N, X/P, or M/G/L genes between VSBV and BoDV.

Results: VSBV was successfully rescued from the consensus sequence of VSBV but not from that of LN713681 strain. Between the consensus sequence and LN713681 strain, we found 12 differences in nucleotide and 5 substitutions in amino acids sequence, indicating that these differences are responsible for the rescue efficiency of VSBV. To evaluate viral pathogenicity, three-week-old rats were nasally inoculated with 10⁴ of chimeric viruses. Mortality rate of chimeric BoDV having X and P genes of VSBV was 100%, whereas wild-type BoDV showed asymptomatic infection.

Conclusions: These results suggest the possibility that X and P genes of VSBV are involved in higher pathogenicity of VSBV than BoDV. We are currently investigating the association of these characteristics with pathogenicity of VSBV.

EP013 / #527

E-Poster Viewing Topic: AS06 Novel approaches to virus control

DIETARY A-KETOGLUTARATE INHIBITS SARS COV-2 REPLICATION AND RESCUES INFLAMMATION AND THROMBOSIS TO RESTORE 02 SATURATION

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Background and Aims: Our recent findings indicate inhibition of hypoxia inducible factor "HIF- α " and phosphorylated Akt "pAkt" via prolyl hydroxylase-2 "PHD2" is augmented by α -ketoglutarate " α KG". We aim to investigate if α KG can reduce the pAkt/HIF α -mediated pathogenesis like inflammation and hypoxemia in SARS CoV-2 infection.

Methods: We infected cells with SARS CoV-2 in presence of α KG and measured viral genome and spike protein. For in-vivo experiments, SARS CoV-2 infected hamsters and mice were supplemented with α KG. We measured viral replication, inflammation, thrombosis, apoptosis in the lungs. Also measured O₂ saturation in these animals.

Results: α KG decreased SARS CoV-2 replication along with decrease in pAkt/HIF α in cells, but failed to inhibit the same in PHD2-knockdown cells. Dietary α KG inhibited viral infection and rescued hamsters and mice lungs from thrombosis and inflammation. α KG reduced inflammatory immune cells accumulation, apoptotic cell death and cytokines, including IL6, IL1 β and TNF α , in lungs and circulation of infected animals, alongside downmodulation of pAkt and HIF2 α . α KG supplementation neither affected IgG levels nor altered the neutralization antibody response against SARS CoV-2. Interferon- γ + T cells were unaltered with α KG supplementation in infected animals. Importantly, α KG supplementation restored the O₂ saturation in circulation of SARS CoV-2 infected animals.

Conclusions: α-ketoglutarate inhibits SARS CoV-2 replication and rescues inflamed lungs, thus restoring normal O₂ saturation, without affecting immune responses.

EP014 / #530

E-Poster Viewing Topic: AS06 Novel approaches to virus control

INFLUENCE OF SOME XYLOPHYTE MUSHROOMS EXTRACTS ON THE REPRODUCTION OF THE INFLUENZA VIRUS

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Background and Aims: Mushrooms are used in their natural form as a food supplement and food additive. In addition, several bioactive compounds beneficial for human health have been derived from mushrooms. In this work, antiviral activity extracts from the mycelium of some xylophyte fungi: Pleurotus eryngii, Auricularia auriculajudae, Tremella fuciformis were investigated.

Methods: The main criterion for studying the specific antiviral effect of compounds was the CTI (chemical-therapeutic index), that is determined by the ratio of the average toxic concentration of the substance (TC50) to the average effective viral inhibitory concentration (EC50). The antiviral activity of the obtained extracts was studied on a model of human, animal and bird influenza viruses, in the dose range from 0.025 mcg/ml to 1.25 mcg/ml according to methodological recommendations of the "Guidelines for conducting Preclinical studies of Guidelines for conducting Preclinical studies of medicines".

Results: In the study of the ability to suppress the reproduction of various strains of the influenza virus, it was found that the extracts of the fungi Auricularia auricula-judae and Tremella fuciformis possessed antiviral properties and are superior to commercial anti-influenza preparations in terms of the chemical-therapeutic index.

Conclusions: Xylophyte fungi may become a new source of effective antiviral drugs

EP015 / #333

E-Poster Viewing Topic: AS06 Novel approaches to virus control

REDUCTION OF VIRAL INFECTIVITY IN HPV-POSITIVE WOMEN BEFORE AND AFTER VACCINATION WITH 9VHPV.

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Background and Aims: Human Papillomavirus (HPV) is a sexually transmitted virus, and it is the causal agent of cervical cancer. Viral particles released through the anogenital mucosa, are responsible for the transmission of the virus. Vaccines against HPV such as Gardasil-9 have been shown effective and safe for the prevention of new infections. However, in most countries, adult population is not vaccinated and remains as a reservoir of HPV. We propose a functional assay to assess whether vaccination with Gardasil-9 in HPV16/HPV18 positive women may reduce viral infective capacity, and in turn, be an adequate strategy to limit the infection of their sexual partners.

Methods: We have adapted an in vitro functional assay previously described to analyze the infective capacity of body fluids. This assay is based on the expression of E1^E4 mRNA in human keratinocytes incubated with cervical, anal, and oral exudates. We designed a non-randomized, open-label trial to assess the reduction of HPV viral infectivity in HPV16/18-positive women before and after vaccination with 9vHPV, a multivalent L1 Virus-like particle vaccine, evaluated in samples obtained after one, two, and three vaccine doses.

Results: E1^E4 mRNA expression together with the evaluation of HPV viral clearance, virion production and seroconversion will generate clear evidence of whether vaccination, in addition to preventing the acquisition of new infections, can be used as an efficient tool to limit viral infective capacity.

Conclusions: The evaluation of the infective capacity of HPV16/18-positive body fluids is the nuclear part of the RIFT-HPV1 and RIFT-HPV2 projects, which are currently ongoing.

EP016 / #268

E-Poster Viewing Topic: AS06 Novel approaches to virus control

PHARMACOLOGICALLY INDUCED ENDOLYSOSOMAL CHOLESTEROL IMBALANCE THROUGH CLINICALLY LICENSED DRUGS ITRACONAZOLE AND FLUOXETINE IMPAIRS EBOLA VIRUS INFECTION IN VITRO

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Background and Aims: Outbreaks of Ebola Virus Disease (EVD), a haemorrhagic fever caused by Ebola viruses (EBOV), occur again and again, with high mortality rates of up to 90 %. In this context, the last major EVD outbreak from 2013 to 2016 in West Africa in particular highlighted the potential for epidemic and pandemic spread. So far, anti-EDV therapy is limited to the Zaire strain, leaving only symptomatic treatment for the rest. Due to the acute and urgent need for a strain-independent host-directed therapy, we investigated a new approach by using the antifungal drug itraconazole and the antidepressant fluoxetine, both clinically approved for use in humans, for their antiviral activity against EDV and thus, a repurposing application.

Methods: To this end, we performed infection experiments with human and bat lung cells under BSL-4 conditions to determine the amount of newly formed virus particles with and without drug treatment using RT-PCR and TCID50 assay. We found that replication rates were significantly reduced compared to untreated controls.

Results: We assume that viral membrane fusion at endolysosomes is non-specifically and speciesindependently blocked by itraconazole through direct binding of the endosomal membrane protein Niemann-Pick C1 and by fluoxetine through induced endolysosomal cholesterol accumulation and alteration of the endolysosomal pH. Cell biological analyses indicate that a lipid imbalance at the endolysosome prevents fusion.

Conclusions: Consequently, targeting the endolysosomal interface between host and pathogen is an effective strategy to reduce the risk of EBOV infection and could therefore be used as an adjunctive intervention to established protective measures in outbreak situations.

EP017 / #484

E-Poster Viewing Topic: AS06 Novel approaches to virus control

BLACK SILICON SURFACES WITH DIFFERENT DEGREES OF WETTABILITY INFLUENCE INTERACTION OF HUMAN PARAINFLUENZA TYPE-3 RESPIRATORY VIRAL PARTICLES

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Background and Aims: Respiratory viruses, such as SARS-CoV-2, show extended survivability on many abiotic surfaces. The global coronavirus pandemic has highlighted the importance of surface transmission for respiratory viruses, alongside air transmission routes. Huge efforts have focused on disinfecting surfaces, but this strategy is extremely tedious, costly and prone to lead to resistance in the long term. The fabrication of nanotextured surfaces promises to be an innovative way of terminating viral particles and preventing viral transmission through transfer from contaminated surfaces.

Methods: The fabricated topography of nano-protrusions on silicon (black-Si) by plasma etching has been inspired by the anti-wetting and anti-biofouling properties of insect wings (and other topologies found in nature). Herein, hydrophilic black-Si (water contact angle ~10) was coated with trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (PFTS) monolayer using chemical vapour deposition (CVD) method to achieve a superhydrophobic surface (water contact angle ~180°).

Results: We show that hydrophilic black-Si with proven antibacterial efficacy demonstrate a high degree of human parainfluenza virus type 3 (hPIV-3) particle termination via RT-qPCR (98.4% reduction) and plaque assay (95.4% reduction). Whereas superhydrophobic black-Si showed a substantial reduction in the hPIV-3 particle attachment density.

Conclusions: Results of these findings will lead to more studies in the area of antiviral surface development to help combat the next respiratory virus pandemic or other viruses that transmit via contaminated surfaces.

EP018 / #51

E-Poster Viewing Topic: AS06 Novel approaches to virus control

INFECTION BY THE WMEL STRAIN OF THE BACTERIUM WOLBACHIA INFLUENCES FEMALE POST-MATING RESPONSES IN THE YELLOW FEVER MOSQUITO AEDES AEGYPTI.

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Background and Aims: Wolbachia bacteria are endosymbionts that naturally infect ~66% of insects, but Aedes aegypti mosquitoes are not among these species. However, artificial infection of Ae. aegypti by the wMel Wolbachia strain diminishes female ability to transmit arboviruses and modifies reproduction to allow infected populations to become dominant over time. Due to these characteristics, Ae. aegypti males and females infected with Wolbachia are being introduced in various cities to replace native Ae. aegypti populations to suppress disease transmission. It is generally assumed that Wolbachia-infection has a minimal effect on Ae. aegypti female fertility. Female mosquitoes undergo several physiological and behavioral changes in response to mating—called the female post-mating response (PMR)—that collectively are required for optimal fertility and that facilitate the production of progeny.

Methods: To assess how Wolbachia alters the female PMR in Ae. aegypti, we collected Wolbachiainfected adults released in Medellín, Colombia, and backcrossed this strain for 7 generations to our laboratory strain (Thai), generating a Wolbachia-infected strain in the same genetic background as the Thai strain.

Results: We find that the presence of Wolbachia influences female fecundity, fertility, longevity, and female re-mating incidence, with some effects observed in a sex-specific manner. Further, female PMR changes are not due to defects in sperm transfer by infected males, or sperm storage by infected females.

Conclusions: Thus, artificial infection of Ae. aegypti by Wolbachia bacteria influences post-mating processes in this species. Understanding how Wolbachia alters post-mating physiology and behaviors will ultimately aid control programs that release Wolbachia-infected Ae. aegypti.

EP019 / #366

E-Poster Viewing Topic: AS06 Novel approaches to virus control

TWO-COMPONENT NANOPARTICLE VACCINE FROM INSECT CELLS PROTECTS AGAINST SARS-COV-2

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Background and Aims: Vaccination is essential to reduce disease severity and limit the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Protein-based vaccines are useful to vaccinate the world population and to boost immunity against emerging variants. Their safety profiles, production costs, and vaccine storage temperatures are advantageous compared to mRNA and adenovirus vector vaccines. Here, we use the versatile and scalable baculovirus expression vector system to generate a two-component nanoparticle vaccine to induce potent neutralizing antibody responses against SARS-CoV-2 variants. These nanoparticle vaccines can be quickly adapted as boosters by simply updating the antigen component.

Methods: Baculoviruses were constructed encoding SARS-CoV-2 spike proteins: full-length S, stabilized secreted S, or the S1 domain. Since subunit S only partially protected mice from SARS-CoV-2 challenge, we produced S1 for conjugation to bacteriophage AP205 VLP nanoparticles using tag/catcher technology. Immunogenicity of S1-VLP vaccine was assessed by prime-boost immunization and vaccination-challenge of mice.

Results: The S1 yield in an insect-cell bioreactor was 11 mg/liter, and authentic protein folding, efficient glycosylation, partial trimerization, and ACE2 receptor binding was confirmed. Prime-boost immunization of mice with only 0.5 ug S1-VLPs showed potent neutralizing antibody responses against Wuhan and Alpha/B.1.1.7 SARS-CoV-2 variants. Non-adjuvanted S1-VLPs protected K18 hACE2 mice from disease after SARS-CoV-2 challenge.

Conclusions: This two-component nanoparticle vaccine can now be further developed to help alleviate the burden of COVID-19.

EP020 / #28

E-Poster Viewing Topic: AS06 Novel approaches to virus control

A SIMPLIFIED, FLEXIBLE, AND SENSITIVE SALIVA-BASED PCR TEST FOR ACCESSIBLE SARS-COV-2 TESTING

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Background and Aims: Detecting SARS-CoV-2-positive individuals is essential for limiting further virus transmission. Policy makers rely on case numbers to make decisions, and individuals use this information to evaluate risk in public spaces. Testing strategies have been plagued with limited authorized assays and high test prices, with large-scale implementation hampered by worldwide supply chain issues.

Methods: We simplified saliva-based COVID-19 diagnostics by not requiring specialized tubes or preservatives; developing clear self-collection guidance; replacing RNA extraction with a simple enzymatic step; and testing specimens in dualplex RT-qPCR. We validated this approach ("SalivaDirect") with materials from multiple vendors to permit flexibility and circumvent supply disruptions. We expand the protocol in response to laboratory-specific needs to further aid the implementation of saliva testing to local communities.

Results: SalivaDirect's simplified protocol does not compromise on sensitivity. We demonstrate stable detection of SARS-CoV-2 RNA in unsupplemented saliva for prolonged periods at elevated temperatures. Test limit of detection ranges from 1.5-12 virus RNA copies/ul of saliva (workstream-depending). Importantly, we maintained sensitivity when adding heat pre-treatment for safer sample handling, and pooled testing (up to 5 samples). With 3.5M+ SalivaDirect tests reported from 170+ labs in 40+ U.S. states, low sample rejection (0.53%) and invalid results (0.61%) demonstrate the robustness of test implementation across this unique laboratory network setting.

Conclusions: Saliva is a sensitive alternative to swabs for SARS-CoV-2 testing. SalivaDirect enables labs to utilize existing infrastructure, expediting test implementation and requiring minimal investment to scale-up for mass testing needs. Our vision is to help provide accessible and equitable testing solutions, especially in low-resource settings.

EP021 / #277

E-Poster Viewing Topic: AS08 Determinants of host range

THE HOST PHYLOGENY DETERMINES VIRAL INFECTIVITY AND REPLICATION ACROSS STAPHYLOCOCCUS HOST SPECIES

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Background and Aims: Emerging infectious diseases are often the result of a host shift, where a pathogen transmits to a novel host species. Understanding the patterns and determinants of cross-species transmissions may provide insights into the process underlying pathogen emergence. Here, we measure the susceptibility of 64 strains of *Staphylococcus* bacteria (16 non-*aureus* species and 48 strains of *S. aureus*) to the bacteriophage ISP, to investigate the role of bacteria host relatedness in susceptibility to viral infection.

Methods: We used three methods to assess the susceptibility of our *Staphylococcus* strains: spot tests, optical density assays, and qPCR. The resulting susceptibility data was analysed using generalised linear phylogenetic mixed models.

Results: We find that the host phylogeny explains a large proportion of the variation in susceptibility to ISP across the host panel, with susceptibility measured by qPCR providing the highest phylogenetic signal. These results were consistent across models of *S. aureus* only and *Staphylococcus* species, suggesting that the phylogenetic effect is conserved both within and between host species. We find a strong positive correlation between optical density and qPCR but poor correlations between spot tests and either OD or qPCR, suggesting that, for assessments of phylogenetic signal, optical density assays and qPCR are the most appropriate.

Conclusions: Together, our results demonstrate the significance of bacteria host evolutionary relatedness on susceptibility to phage infection, with implications for the development of ISP as a phage therapy treatment, and as an experimental system for the study of virus host shifts.

EP022 / #252

E-Poster Viewing Topic: AS09 Virus dynamics in reservoir hosts

SCREENING OF HELSINKI URBAN RATS FOR RODENT-BORNE VIRUSES

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Background and Aims: Rats are considered one of the vectors of diseases transmitted to humans due to their close contact. There are disease concerns with rats including hantavirus, leptospirosis, lymphocytic choriomeningitis (LCMV), and poxviruses. As a part of the Helsinki Urban Rat Project, which is a multidisciplinary research project dedicated to understand the dynamics of urban rats and their effects on urban life in Helsinki, our aim was to understand how rats share and spread viruses that might cause infection risk to humans.

Methods: Animals were collected mainly from Helsinki and Vantaa between 26.3.2018-and 25.11.2020. Using the IFA technique, 102 heart samples in PBS were screened for Puumala (PUUV), Dobtava (DOBV), and Seoul (SEOV) hantaviruses, poxviruses, and LCMV antibodies. The only determined species among collected animals was *Rattus norvegicus*.

Results: The overall seroprevalence detected among collected rats was LCMV 2,94%, SEOV 1,9%, POX 5,88%, DOBR 3,92%, PUUV 4,9%. These results based only on IFA screening.

Conclusions: These results confirmed the circulation of these viruses among urban rats in Helsinki. The exact virus species needs to be confirmed by sequencing.

EP023 / #786

E-Poster Viewing Topic: AS09 Virus dynamics in reservoir hosts

HEPATITIS B AND C INCIDENCE IN BLOOD DONORS: RETROSPECTIVE STUDY FROM BEJAIA CITY, ALGERIA

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Background and Aims: Blood transfusion is a medical therapeutic act, but it's also a risk for transmission of infectious agents to recipients, despite the progress made in transfusion safety. For this reason, it is essential to systematically detect and screen all blood donations for hepatitis B and C, HIV viruses and *Treponema pallidum*. HCV and HBV cause liver damage with varying severity that can slowly progress to cirrhosis, then to hepatocellular carcinoma.

Methods: This retrospective epidemiological study, focusing on donors and recipients, was conducted over a 10-year period (2010 to 2020) at the blood transfusion center in the city of Bejaia, Algeria. The objective was to assess the risks of transfusional transmission of HCV and HBV agents in the wilaya of Bejaia and to estimate the prevalence of infections with these infectious agents in the population of Bejaia. ELISA Techniques and western blot were used (BIO-RAD).

Results: In this study, about two persons for thousand tested positive for one of the two hepatitis viruses. Seroprevalence for HCV and HBV was 0.08% (n=107) and 0.10% (n=134), respectively, with a very high male tendency compared to female . At Bejaia city, the seroprevalence of HCV and HBV is lower than in the general population.

Conclusions: A large screening within population should be implemented and improved means of screening are needed to prevent a wide dissemination of these two diseases

EP024 / #563

E-Poster Viewing Topic: AS11 Host-pathogen interactions

A PROTEOGENOMICS FRAMEWORK TO IMPROVE PROKARYOTIC GENOME ANNOTATIONS

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Background and Aims: Address dilemma that accurate prediction of all protein coding genes is still an unsolved issue[1]. Develop a broadly applicable proteogenomics solution that allows researchers to identify missed protein-coding open reading frames (ORFs) in prokaryotes.

Methods: To capture the entire protein coding potential, we hierarchically integrate reference genome annotations that can differ substantially, results from *ab initio* gene prediction algorithms and a modified six-frame translation and create a large but minimally redundant integrated proteogenomics search database (iPtgxDB) where ~95% of the peptides unambiguously identify one protein [1]. A general feature format (GFF) file, when loaded in a genome browser, transparently visualizes all annotation differences and can be overlaid with experimental data.

Results: Our proteogenomics framework identifies novel small proteins, new start sites and expressed pseudogenes in prokaryotic genomes using tandem mass spectrometry (MS) data. Complete genomes sequences provide the optimal basis, as a fragmented Illumina short-read based genome assembly of a closely related reference strain can differ and even miss essential and disease-relevant genes, as for the clinically relevant *Pseudomonas aeruginosa* MPAO1 [2]. We successfully applied our strategy to microbiome consortia [3] and to *M. tuberculosis* clinical isolates [4]. We host a public web server (https://iptgxdb.expasy.org) [1] where researchers can create iPtgxDBs and GFF files for their organisms of interest.

Conclusions: Our approach identified novel ORFs in any prokaryote tested. [1]. Omasits U et al. GenomeRes 2017, 27: 2083-2095. [2]. Varadarajan AR et al. NPJ Biofilms&Microbiomes 2020, 6:46. [3]. Petruschke H, Schori C et al. Microbiome 2021, 9:55. [4]. Heiniger B et al. (*under review*).

EP025 / #447

E-Poster Viewing Topic: AS11 Host-pathogen interactions

DIFFERENTIAL PNEUMOCOCCAL GROWTH FEATURES IN SEVERE INVASIVE DISEASE MANIFESTATIONS

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Background and Aims: Introduction: The nasopharyngeal commensal *S. pneumoniae* can become invasive and cause metastatic infection. This requires the pneumococcus' ability to adapt, grow and reside in diverse host body environments. Therefore, we studied whether the likelihood of severe disease manifestations to occur was related to pneumococcal growth kinetics.

Methods: Methods: For 383 *S. pneumoniae* blood isolates and 25 experimental mutants we observed highly reproducible growth curves in nutrient-rich medium. Derived growth features were lag time, maximum growth rate, maximum density, and stationary phase time before lysis. First, the pathogenicity of each growth feature was probed by comparing isolates from patients with and without marked pre-existing comorbidity. Then, growth features were related to the propensity of causing severe manifestations of invasive pneumococcal disease (IPD).

Results: Results: A high maximum bacterial density was the most pronounced pathogenic growth feature, that was also an independent predictor of 30-day-mortality (p=0.03). Serotypes with an epidemiologically higher propensity for causing meningitis, displayed a relatively high maximum density (p<0.005), and a short stationary phase (p<0.005). Correspondingly, isolates from patients diagnosed with meningitis showed an especially high maximum density and short stationary phase when compared to isolates from the same serotype that had caused uncomplicated bacteremic pneumonia. In contrast, empyema-associated strains were characterized by a relatively long lag phase (p<0.005), and slower growth (p<0.005).

Conclusions: Discussion: The course and dissemination of IPD may partly be attributable to the pneumococcal growth features involved. If confirmed, we should tailor the prevention and treatment strategies for the different infection sites that can complicate IPD.

EP026 / #143

E-Poster Viewing Topic: AS11 Host-pathogen interactions

HYPERSENSITIVITY REACTION INDUCED BY IMMUNIZATION WITH EXTRACELLURA VESICLES OF STAPHYLOCOCCUS AUREUS

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Background and Aims: Staphylococcus aureus is an opportunistic pathogen that causes a variety of infections ranging from impetigo to septicemia. Extracellular vesicles (EVs) from methicillin-resistant S. aureus (MRSA) have been characterized. They are recognized to promote pathogenicity by transferring the virulence factors to host cells. We have demonstrated that the purified EVs from a clinical isolated MRSA (SaEVs) significantly stimulated proinflammatory cytokine production from mouse immune cells, suggesting that SaEVs have a high potential to be used as a vaccine. In this study, we investigated host immune response after SaEV immunization and its protective effect against MRSA infection in a mouse model.

Methods: Mice were subcutaneously immunized twice with SaEVs and infected with MRSA. Survival of mice were observed, and spleen and serum were collected. Spleen cells were stimulated with SaEVs. Cytokine production and IgE titer in serum, spleen and/or culture supernatant were determined by ELISA.

Results: SaEV immunization had no protective effect against MRSA. On the other hand, all SaEVimmunized mice died within 1 day after infection. Production of IL-6, TNF- α and IL-17A in the spleen of SaEV-immunized mice was dramatically higher than that in control mice after MRSA infection for 3 h. On Day 5 after the second immunization, total IgE in the serum increased significantly, and ex vivo stimulation of the spleen cells with SaEVs resulted in a remarkable increase of Th2-related cytokines.

Conclusions: MRSA-derived EVs act as an immunostimulant that induces inflammatory response and IgE-mediated hypersensitivity after immunization.

EP027 / #93

E-Poster Viewing Topic: AS11 Host-pathogen interactions

SELECTIVE RECOGNITION OF HYPERMUCOVISCOUS KLEBSIELLA PNEUMONIAE CLINICAL ISOLATES BY MEMBERS OF THE INNATE IMMUNE GALECTIN AND SIGLEC FAMILIES.

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Background and Aims: Since first described in the mid-80's, hypervirulent Klebsiella pneumoniae has globally spread and pose a serious public health threat, as they can cause invasive infections even in healthy, immunocompetent individuals. These variants frequently exhibit an hypermucoviscous (HMV) phenotype detected by string-test. A recurrent trait of HMV K. pneumoniae is the production of a thick polysaccharide capsule, which is assumed to provide protection from the host immune response. Hypercapsule production is associated with the hypermucoviscosity gene A (magA) and/or the gene regulator of the mucoid phenotype A (rmpA), although the HMV phenotype has also been detected in magA-/rmpA- isolates. In this work, we explored the carbohydrate structures present on the surface of HMV clinical isolates, compared to non-HMV isolates, and their recognition by different galectins and Siglecs.

Methods: A collection of HMV (magA+/rmpA+, magA-/rmpA+, and magA-/rmpA-) and non-HMV (magA-/rmpA-) isolates was examined. Binding assays to microarray-printed bacteria were used as main tool.

Results: First, the binding of model lectins with known carbohydrate-binding specificities was tested. Lectin-selective binding was almost exclusively detected for magA-/rmpA- isolates, indicating that the hypercapsule, in general, is not recognized by the tested lectins and hides potential ligands on the bacterial surface. On the other hand, strong isolate- and galectin/Siglec-dependent binding signals were detected, with preference for magA+/rmpA+ and magA-/rmpA+ isolates but with distinct or even contrasting binding intensities between particular galectins and Siglecs.

Conclusions: Overall, the results suggest that, besides providing protection from host immune responses, K. pneumoniae hypercapsules could have developed to benefit from recognition by galectins and Siglecs.

EP028 / #82

E-Poster Viewing Topic: AS11 Host-pathogen interactions

STANDARDIZATION OF A METHOD TO USE LARVAE OF THE COLEOPTERA TENEBRIO MOLITOR AS AN INFECTION MODEL TO ASSESS STAPHYLOCOCCUS SPP. VIRULENCE

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Background and Aims: Staphylococcus spp. comprise more than 60 different species of bacteria, that may be commensal or opportunistic/primary pathogens. Studying their mechanisms of pathogenicity often requires mammal models, which is hampered by ethical issues, increased costs, and a tendency of the scientific community to decrease the use of these animals. To address these limitations, the use of invertebrates as infection models has greatly increased in the past decades, supported by the fact that their immune system presents components that are similar in function to that of mammals. In this study, we used larvae of the coleopter Tenebrio molitor, to evaluate the best conditions to test the virulence of a highly pathogenic Staphylococcus aureus strain, and a harmless Staphylococcus nepalensis strain.

Methods: Larvae were fed with a mixture of seed and grains and kept away from the light, at 4°, 22°C and 37°C. Larvae were infected with micro-syringes with concentrations of bacteria ranging from 0 to 10° CFU/mL, followed by incubation at 37°C to allow bacterial growth. Survival was accompanied for 72h.

Results: During larval breeding, they pupated more quickly when kept at 22 or 37°C, although no differences in survival was observed. Because of that, we performed further studies with larvae kept at 4 °C. Increasing inoculum concentrations was directly related the number of larvae killed, which was accompanied by melanization. Also, the lethal dose of the pathogenic strain was significantly lower than that of the commensal

Conclusions: Our results indicate that T. molitor can be used to study Staphylococcus strains infections.

EP029 / #359

E-Poster Viewing Topic: AS11 Host-pathogen interactions

SENSOR HISTIDINE KINASE, ATSR: A POTENTIAL COMMANDER FOR DIFFERENTIAL PATHOGENESIS IN B. CENOCEPACIA INFECTION WITH VARIOUS ORDERS

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Background and Aims: *Burkholderia cenocepacia* is an opportunistic pathogen associated with chronic lung infections and increased mortality in cystic fibrosis (CF) patients. For this, *B. cenocepacia* is equipped with a complex regulatory network as two component-regulatory systems, to subtle weaponrymeans for intracellular niche-adaptation and host-immune evasion. Of which, the <u>a</u>dhesion and <u>type</u> 6 <u>secretion system regulator</u> **AtsR** was proven to negatively regulate biofilm formation, T6SS expression, and homoserine lactone (AHL-QS) signaling suggesting it to be a virulence regulator. Yet, the global influence of AtsR on proteome and transcriptome has not been investigated.

Methods: Here we report the details using a global label free proteomics analysis of the proteome/secretome of the AtsR-deficient mutant compared to the wild type (WT) strain on two time-points (9 and 18 hours). Several registered effectors were validated using multiple reaction monitoring (MRM). Parallel, RNA-sequencing was performed at the latter time-point. Bacterial cells were grown in a synthetic CF sputum medium mimicking the CF lung-environment.

Results: Transcriptomics and proteomics revealed anticipated targets, notably of genes involved in adhesion, biofilm formation, and T6SS-dependent effectors. Intriguingly, our data proven that AtsR not only controls the AHL-QS but also acts upstream the valdiazen QS and phenylacetic acid signaling pathways.

Conclusions: These results cumulatively consolidate the role of AtsR as a master regulator of *B. cenocepacia* virulence possibly-implicated in adhesion and biofilm formation. This demonstrates that asyet-undefined host-environmental factor(s) triggered AtsR toward differential pathogenesis of *B. cenocepacia* at different infectious stages resolving novel antimicrobial pathways/agents. <u>Acknowledgement</u>: This work was supported by a grant from the FWO-Vlaanderen (3G005719).

EP030 / #353

E-Poster Viewing Topic: AS11 Host-pathogen interactions

INVESTIGATING THE OUTCOMES OF COINFECTION WITHIN AND BETWEEN HOST SPECIES

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Background and Aims: Interactions between coinfecting pathogens have the potential to alter the course of infection, increasing phenotypic variation in susceptibility between hosts. This phenotypic variation may influence the evolution of host-pathogen interactions within host species and interfere with predictions of the outcomes of infection across host species.

Methods: To investigate these hypotheses, we have examined experimental coinfections of two *Cripaviruses* – cricket paralysis virus (CrPV), and Drosophila C virus (DCV) – across *Drosophila* Genetic Reference Panel (DGRP) lines, immune mutant lines, and a large panel of *Drosophilidae* host species.

Results: We find small yet credible effects of coinfection with these viruses across DGRP lines, but little evidence of a host genetic basis for these effects. Mutations in multiple immune genes caused virus-specific changes in the outcomes of coinfection, showing that host immune responses have the potential to moderate coinfection interactions between viruses. Across host species, we find little evidence of systematic changes in susceptibility with most host species showing no apparent effect of coinfection.

Conclusions: Together, these results suggest that phenotypic variation in coinfection interactions within host species can occur independently of natural host genetic variation, and that predictive models of the outcomes of infection across host species built on single infection data may not be invalidated by coinfection in all systems.

EP031 / #327

E-Poster Viewing Topic: AS11 Host-pathogen interactions

THE SYNTHETIC AGONISTS TO SQSTM1/P62 ENHANCE HOST DEFENSE AGAINST MYCOBACTERIAL INFECTION THROUGH ACTIVATION OF XENOPHAGY

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Background and Aims: The N-degron pathway is a proteolytic system in which the N-terminal degrons (N-degrons) of proteins, such as arginine (Nt-Arg), induce the degradation of proteins through ubiquitin-proteasome system (UPS) or autophagy process.

Methods: Here, we report that the chemical mimics of the autophagic N-degron enhance host defense and ameliorate pathologic inflammation through activation of xenophagy.

Results: We developed synthetic agonists to the ZZ domain of p62/SQSTM1 (p62 ligands) and found that the p62 ligands significantly increased the antimicrobial responses in macrophages during mycobacterial infection including *Mycobacterium tuberculosis* (Mtb), *M. bovis* Bacillus Calmette–Guérin, and multidrug-resistant Mtb. The p62 ligands upregulated the autophagosome formation and the recruitment of autophagic membranes to intracellular bacteria via SQSTM1, leading to lysosomal degradation. In mice, these drugs exhibited suppressed replication of BCG, Mtb, and even multidrug-resistant Mtb and decreased the expression of inflammatory cytokines and chemokines and the inflamed lesions from lung tissues.

Conclusions: Together, these results suggest that the N-degron pathway as a therapeutic target in hostdirected therapeutics for drug-resistant mycobacterial infection.

EP032 / #509

E-Poster Viewing Topic: AS11 Host-pathogen interactions

RECOMBINANT LEPTOSPIRA BIFLEXA AS A TOOL FOR CHARACTERIZATION OF THE LIC12587 PROTEIN FROM THE PATHOGENIC LEPTOSPIRA INTERROGANS

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Background and Aims: Leptospirosis is a worldwide zoonosis and the lack of the disease control may be associated to the limited understanding of the molecular basis of pathogenic *Leptospira* activity in the host. In this work, we performed a functional characterization of LIC12587 protein of *L. interrogans* using saprophytic *L. biflexa* as a surrogate host for heterologous expression.

Methods: Firstly, *lipL32* promoter (P32) and *lic12587* were genetically fused by PCR. The amplicon was cloned into the pMaOri shuttle vector and wild *L. biflexa* serovar Patoc was transformed. The heterologous expression of LIC12587 in *L. biflexa* was evaluated by western blotting, while cellular localization was performed by ELISA. Binding to complement system components was performed to evaluate a phenotypical gain of recombinant *L. biflexa*.

Results: The first PCR amplified the P32 from *L. interrogans* serovar Copenhageni genome. The amplicon was used as a forward megaprimer in a second PCR for amplification of *lic12587* coding sequence, resulting in the P32LIC12587 fusion, which was digested and ligated into pMaOri. Recombinant *L. biflexa* was able to transcribe *lic12587* at a higher level than native *L. interrogans* due to P32 activity, while western blotting analysis showed the expression of LIC12587 exclusively in recombinant *L. biflexa*. LIC12587 was detected in recombinant *L. biflexa*'s surface, suggesting the localization of the native LIC12587 in *L. interrogans* and heterologous expression enhanced the binding to C7, C8 and C9 components.

Conclusions: The results indicates that LIC12587 may play a role in *L. interrogans* resistance against the complement system during infection.
EP033 / #476

E-Poster Viewing Topic: AS11 Host-pathogen interactions

THE ROLE OF POLYKETIDE SYNTHASE IN POLYUNSATURATED FATTY ACIDS (PUFAS) SYNTHESIS BY MALASSEZIA ON HUMAN SKIN

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Background and Aims: *Malassezia* are lipid-dependent fungal skin commensal that do not possess any fatty acid synthase - hence they have to uptake fatty acids from the environment which suggest their niche adaptation to oily regions of the skin. Polyunsaturated fatty acids (PUFAs) are produced by Pokyketide synthase (PKS) in marine organisms; while its secondary metabolites, oxylipins, are known to play a role in fungal development and pathogenity. We hypothesize that PKS in *Malassezia* is responsible for the production of the the PUFAs and oxylipins. PKS was replaced with a selection marker and the change in the oxylipin profiles between the wildtype (WT) and the knockout (KO) was measured with LC-MS.

Methods: PKS was replaced through homology directed recombination (HDR) based on cotransformation of *M. furfur* with two A. tumefaciens strains, one bearing the binary vector with the HDR template and the other with a binary vector engineered for the CRISPR/Cas9 system without a gene marker. The co-culture was maintained on induction agar for 4 days, and, transferred onto selection agar as reported in DOI: https://doi.org/10.1534/genetics.119.302329.

3 clones were sucessfully isolated and they were verified at DNA and RNA level. The oxylipins were measured as reported in DOI: 10.3390/jof7090693



he detectable PUFAs and oxylipins in the Wildtype were significantly lower in Knockout.

Conclusions: We have shown that by removing PKS, there are lower amounts of PUFAs and the oxylipins. We seek to determine PKS's role in host-interaction by investigating skin cells' responses to WT versus KO.

EP034 / #246

E-Poster Viewing Topic: AS11 Host-pathogen interactions

A NOVEL SILKWORM INFECTION MODEL FOR ELUCIDATING THE VIRULENCE OF THE FUNGAL PATHOGEN TRICHOSPORON ASAHII

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Background and Aims: *Trichosporon asahii* is a pathogenic fungus that causes deep-seated mycosis in patients with neutropenia. Establishing an experimental animal model for quantitatively evaluating pathogenicity and developing a genetic recombination technology will help to elucidate the infection mechanism of *T. asahii* and promote the development of antifungal drugs. Here we established a silkworm infection model for quantitatively evaluating the virulence of *T. asahii*.

Methods: The *T. asahii* clinical strains and JCM2466, a type strain, were used in this study. Silkworm fifth instar larvae were fed the artificial diet, Silkmate 2S, overnight. *T. asahii* grown on Sabouraud agar plates was suspended in physiologic saline solution and filtered through a 40- μ m cell strainer. A 50- μ l suspension of *T. asahii* cells was injected into the silkworm hemolymph. Silkworms injected with *T. asahii* cells were placed in an incubator and their survival was monitored.

Results: Injecting *T. asahii* into silkworms eventually killed the silkworms. Moreover, the administration of antifungal agents, such as amphotericin B, fluconazole, and voriconazole, prolonged the survival time of silkworms infected with *T. asahii*. By comparing the pathogenicity of *T. asahii* clinical isolates in a silkworm infection model, *T. asahii* MPU129 was identified as a highly pathogenic strain. A mutant lacking the *cnb1* gene, which encodes the beta-subunit of calcineurin, was generated. The *cnb1* gene-deficient mutant showed reduced pathogenicity against silkworms compared with the parental strain.

Conclusions: Our findings suggest that a silkworm infection model with *T. asahii* is useful for elucidating the molecular mechanisms of *T. asahii* infection.

EP035 / #480

E-Poster Viewing Topic: AS11 Host-pathogen interactions

CYTOTOXICITY OF SALMONELLA ENTERICA SEROVARS ENTERITIDIS AND GALLINARUM BIOVAR GALLINARUM AGAINST CHICKEN INTESTINAL CELLS AND MACROPHAGES

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Background and Aims: *S.* Enteritidis and *S.* Gallinarum are presently the major causative agents of *Salmonella* infections in poultry. The serovars are closely related genetically, but the clinical manifestations of infections are different. *S.* Enteritidis typically causes self-limiting gastroenteritis, and *S.* Gallinarum is responsible for systemic infection. Despite the intensive studies, the reasons of such differences are poorly understood. The key steps in *Salmonella* infections are the interactions of bacteria with host intestinal cells and macrophages, and the *Salmonella* cytotoxicity against host cells plays important role in the pathogenesis of *S.* enterica infection, but there is limited data about this process in the chicken cell lines. The aim of the study was to evaluate and compare the cytotoxicity of *S.* Enteritidis and *S.* Gallinarum against chicken intestinal cells and macrophages.

Methods: Chicken intestinal CHIC-8E11 cells and chicken macrophage HD-11 cells (untreated or LPS-treated) were infected *S*. Enteritidis 327 and

S. Gallinarum 589/02. Cytotoxic effect after 24 hours of infection was measured by LDH assay. Type of cell death was assessed by FLICA test using flow cytometry.

Results: S.Enteritidis was not cytotoxic against intestinal CHIC-8E11 cells, however, it caused death of HD11 macrophages. The cytotoxicity increased twice, when macrophages were treated with LPS. In contrast, S. Gallinarum was not cytotoxic against epithelial cells and macrophages. S. Enteritidis caused death of HD11 cells mainly by activation of caspase-1. The differences in cytotoxicity between S. Enteritids and S. Gallinarum were not dependent on the number of intracellular bacteria.





Conclusions: S. Enteritidis causes pyroptotic cell death of chicken macrophages.

EP036 / #806

E-Poster Viewing Topic: AS11 Host-pathogen interactions

AN INNOVATIVE, SAFE AND EFFECTIVE DECONTAMINATION DEVICE FOR NON-TOXIC REMOVAL OF PATHOGENS FROM SURFACES AND SKIN

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Background and Aims: Glycosylation is a key modification of proteins in nature and proteincarbohydrate interactions are essential in many host-pathogen interactions. These interactions often trigger the first steps of infection. Glycomimetics have become attractive tools to hamper pathogen adhesion to host cells. In order to overcome the shortcomings of current decontamination methods, an antipathogen decontamination glycomaterial that is effective against a range of pathogens was developed for use on human skin and other surfaces. The objective of this work was to incorporate nature's inherent pathogen capturing mechanisms into a prototype pathogen decontamination material, that was aqueous based and formulated from non-toxic ingredients and comprised of a sustainable biodegradable matrix material.

Methods: The antipathogen decontamination glycomaterial incorporates a biodegradable cellulose matrix containing irreversibly attached active agents (glycoconjugates). This material mimics natural cell membranes with multiple presentations of predominant binders and effectively captures the pathogens, resembling a microbiological Velcro[™] system. The binders were carefully selected using cutting-edge microarray technology and bioinformatic research. Efficacy tests were conducted to demonstrate the capture and removal of target pathogens from selected surfaces.

Results: An average of 90-99% pathogens (bacteria, virus, fungi and toxins) were removed from contaminated surfaces. During the early stages of the COVID-19 pandemic, an independent laboratory of expertise has proven the efficiency of the adapted glycopolymer to capture and remove up to 99.99% of SARS-CoV-2 from human skin explants.

Conclusions: Our results showcase an excellent tool for pathogen management and decontamination from surfaces and human skin. The prototype was developed using safe, natural and biodegradable components.

EP037 / #200

E-Poster Viewing Topic: AS11 Host-pathogen interactions

COMPARISON OF THE VIRULENCE ATTRIBUTES OF CLINICAL AND ENVIRONMENTAL ISOLATES OF PSEUDOMONAS AERUGINOSA GROWN IN BIOFILM AND NON-BIOFILM MODE OF GROWTH

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Background and Aims: Background and Aim: The capacity to form biofilm by Pseudomonas aeruginosa is reported to contribute to the virulence of this opportunistic pathogen. In this study, we compared several virulence attributes of P. aeruginosa grown in biofilm and non-biofilm states.

Methods: Crystal violet dye staining method was used in biofilm assays. Skim milk agar was used in protease assays. Spectrophotometric haemoglobin estimation was used to measure haemolytic activity. Disk diffusion assay was used in antibiotic sensitivity testing.

Results: Comparison of production of biofilm, protease and haemolysin by clinical and environmental isolates revealed that (a) environmental isolates produced relatively leaser amount of biofilm (b) both clinical and environmental isolates produced protease to similar levels, (c) Clinical strains were more haemolytic in nature. Interestingly, clinical strains, which did not produced protease was also negative for haemolysin production. Comparison of strains grown in biofilm and non-biofilm states in parallel showed no significant change in production of these virulence factors in general, however one environmental isolate exhibited 4 fold increase in haemolytic activity when grown in biofilm mode. Clinical strains uniformly exhibited enhanced resistance to a panel of antibiotic tested, however, no correlation was observed between antibiotic resistance, production of haemolysin and protease or biofilm formation.

Conclusions: Conclusion: The findings of this study indicate that the potential to form biofilm and production of protease and haemolysin in environmental and clinical P. aeruginosa isolates are differentially regulated. Further studies are needed to delineate the mechanism(s) involved.

EP038 / #385

E-Poster Viewing Topic: AS11 Host-pathogen interactions

STRUCTURES AND GANGLIOSIDE SPECIFICITY OF BK POLYOMAVIRUS VP1 MUTANTS OBSERVED IN KIDNEY TRANSPLANT RECIPIENTS REVEAL A SIALIC-ACID INDEPENDENT ENTRY PATHWAY

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Background and Aims: BK Polyomavirus (BKPyV) is an opportunistic virus that can cause nephropathy and graft loss in kidney transplant recipients. In a kidney transplant cohort followed in Nantes University Hospital, persistent BKPyV replication was associated with mutations in the BKPyV VP1 protein. The goal of this study was to determine how these mutations influence the structure and tropism of the virus, focusing on two variants found in patients: VQQ and N-Q.

Methods: Tropism was characterised in patient biopsies and through functional assays: glycan microarray screening, cell binding and infection assays. Protein structures were determined by X-ray crystallography.

Results: In graft biopsies, both variants were associated with atypical histology, suggesting altered *in vivo* tropism, while in cell culture they showed divergent infectious profiles in 293TT and RS (SV40 TAg immortalised RPTEC) cells. Microarray and cell binding assays showed that unlike Wild-type (WT) VP1, the VQQ variant binds GD1a ganglioside, which was present in RS cell membranes, consistent with increased infectivity of VQQ in RS cells. The N-Q variant was sialic-acid independent for both binding and infection. These results were explained through structural studies: the VQQ variant had a conserved sialic acid binding site but with a shift of the loop orientation for mutated amino acids 72 and 73. For the N-Q variant, K69N leads to a steric clash which precludes sialic acid binding.

Conclusions: The change in glycan binding properties of the mutant BKPyV VP1 influences the viral cell tropism. Infectivity of the N-Q variant indicates that a sialic-acid independent entry (co)receptor exists for BKPyV.

EP039 / #124

E-Poster Viewing Topic: AS11 Host-pathogen interactions

NEW INSIGHTS INTO HOST ADAPTATION OF ACTINOBACILLUS PLEUROPNEUMONIAE, THE CAUSATIVE AGENT OF PORCINE PLEUROPNEUMONIA, BY MEANS OF LABEL-FREE LIQUID CHROMATOGRAPHY MASS SPECTROMETRY BASED COMPARATIVE PROTEOMICS

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Background and Aims: Porcine pleuropneumonia caused by Actinobacillus pleuropneumoniae affects pig health status and swine industry worldwide. The clinical manifestation of the acute form of the disease includes respiratory distress, high fever and severe lung lesions. During the chronic phase, some animals become subclinical carriers, harboring the pathogen in the tonsillar crypts, nasal cavities and chronic lung lesions. Despite the extensive number of studies focused on A. pleuropneumoniae pathogenicity and the clinical course of the disease, the bacterial strategies for within-host adaptation during the transition from acute to the chronic form of the disease, are still poorly understood.

Methods: In our previous study, using chemometric assisted Fourier-transform infrared (FTIR) spectroscopy, we demonstrated that A. pleuropneumoniae re-isolated from different organs of experimentally infected pigs, exhibit differences within their metabolic fingerprint (Frömbling & Sassu et al., 2017). To gain further insight into the mechanisms of the host adaptation process we used differential proteomics based on label-free LC-MS/MS protein analysis.

Results: Quantitative mass spectrometry, coupled to comprehensive bioinformatics analysis showed a distinctive regulation of protein expression in host-adapted re-isolates in comparison to the infection strain, indicating a metabolic adaptation of A. pleuropneumoniae to the different sites of the respiratory tract.

Conclusions: Thus, we hypothesize that fine-tuned regulation of metabolic pathways and putative virulence factors is essential for the successful adaptation and persistence of the bacteria within its host. Our work does not only contribute to a better understanding of A. pleuropneumoniae host-pathogen interactions but may also pave the way for novel strategies to prevent and control porcine pleuropneumonia.

EP040 / #485

E-Poster Viewing Topic: AS11 Host-pathogen interactions

HETEROGENEITY OF THE PEPTIDYLARGININE DEIMINASE GENE FROM P. GINGIVALIS CLINICAL STRAINS

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Background and Aims: Background and aims: A major etiopathologic factor of periodontitis (PD) is *P. gingivalis* (*Pg*), an anaerobe equipped with peptidylarginine deminase (PPAD), which catalyzes deimination of arginine to citrulline. Citrullination may affect stability and function of proteins, and therefore have an impact on bacterial virulence. It is, however, unclear whether *Pg* virulence may depend on the variations in the *PPAD* gene sequence. The objective of this study was bioinformatic analysis of the *PPAD* gene from *Pg* clinical strains.

Methods: Gingival crevicular fluid samples were collected from 30 PD and 15 controls (Ctrl). The presence of *Pg* was confirmed in 23 PD and 8 Ctrl by *16S rRNA* and *PPAD* PCR. *PPAD* coding sequences were amplified, sequenced and analyzed against the ATCC 33277 *Pg* strain. Bioinformatics analysis was performed using the NCBI and UniProt databases.

Results: In total, 37 synonymous variants and 16 missense mutations were identified in *PPAD*. In PD group six mutations were classified as polymorphic variants. The most common change in nucleotide sequences was the C->T transition. Specific substitutions at positions g.1168 and g.1170 were observed within codon p.390. First of them constitutes a synonymous variant A390A (2.35%), while the second one a missense mutation A390T (8.69%).

Conclusions: Identified substitutions indicate significant heterogeneity of the *PPAD* gene among *Pg* strains infecting individuals with PD. Their presence in PPAD may affect *Pg* virulence and indirectly the condition of PD periodontium, and need to be confirmed by further *in vitro* and clinical studies. Funding: National Science Centre, Poland (UMO-2018/29/B/NZ2/01930, K.G.).

EP041 / #472

E-Poster Viewing Topic: AS11 Host-pathogen interactions

LEPTOSPIRAL SURFACE PROTEINS THAT COULD MEDIATE THE ATTACHMENT TO HOST CELLS

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Background and Aims: One of the main mechanisms of pathogens during infection is adherence to host tissues. Although adhesion to some host cells was shown, the ligands involved in this adherence are not fully understood. Thus, we aimed to verify the involvement of leptospiral proteins in these interactions.

Methods: Evaluation of the adhesion of pathogenic and saprophytic leptospires to endothelial (EA.hy926, HMEC-1 and HULEC5a), epithelial (293T and MDBK) and fibroblastic (BHK-21 and E derm) by cell-ELISA. Interaction evaluation of OmpL37, OmpL1, LipL21, LipL41 and LipL46 to immobilized cells by ELISA or in suspension.

Results: Pathogenic strain was able to adhere to all cells, however when it was used culture-attenuated, adherence was observed only to 293T, EA.hy926 and HULEC5a. Saprophytic strain adhered only to HULEC5a. OmpL1 showed binding to all immobilized and in suspension cells. LipL41 interaction was also observed to all cells, however it did not show binding to immobilized HULEC5a and to in suspension HMEC-1 cells. LipL46 and OmpL37 showed binding to all cells in suspension; the binding to immobilized cells were observed only to epithelial cells and HULEC5a for LipL46, and 293T cells for OmpL37. LipL21 showed no binding to cells in suspension, but interacted only to immobilized 293T cells.

Conclusions: Leptospira virulent strain binds to mammalian cells more efficiently than cultureattenuated and saprophytic strains. As OmpL1 and LipL41 interacted with all the immobilized or in suspension cells, and both are present in pathogenic strains, it is anticipated a contribution of these proteins during leptospiral infection.

EP042 / #291

E-Poster Viewing Topic: AS11 Host-pathogen interactions

ROLE OF THE CYTOTROPHOBLASTIC CELLS IN THE INVASION OF THE ZIKA VIRUS TO THE PLACENTAL STROMA

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Background and Aims: The Zika virus (ZIKV) is highly teratogenic; however, other phylogenetically related flaviviruses such as dengue virus (DENV) and yellow fever virus (YFV), are not. The mechanisms used by the ZIKV to cross the placental barrier need to be elucidated.

Methods: In this work, the efficiency of infection of two ZIKV lineages (African and Asian), DENV serotype 2 and the vaccine strain YFV-17D, were compared in two placental cell models; cytotrophoblastic cells (CTF) HTR8-SVneo, isolated from trophoblasts from human placenta in the first trimester of gestation, and monocytic cells U937DC-SING, differentiated to M2 macrophages (Hofbauer-like cells).

Results: The results showed that both ZIKV strains are significantly more efficient in replicating in CTF than DENV2 or YFV-17D, as assayed by one-step replication curves, percentage of infected cells, and virus yield/cell. In contrast, no differences were observed among strains in the differentiated macrophages. In addition, infection with the ZIKV strains resulted in a lower chemotactic response (CCL2, CCL3, CCL4) in HTR8 cells, as well as a lower antiviral response (IFN2α, IFN2) in both cell lines, compared with the DENV2 and YFV-17D infected cells. Finally, activation of the mTORC1 and mTORC2 pathways was evaluated in CTF-infected cells. Western blot assays show significantly greater phosphorylation of the fractions S6K1 and AKT in the HTR8-SVneo cells infected with ZIKV, suggesting that activation of the mTOR pathways is related to the enhanced replication of ZIKV in CTF cells.

Conclusions: All these results suggest that CTF cells are a positive modulator of the entry of ZIKV into the placental stroma.

EP043 / #513

E-Poster Viewing Topic: AS12 Plant-microbe interactions

BENEFICIAL MICROORGANISMS DRIVES FOOD SECURITY

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Background and Aims: Food security is a pivotal concern worldwide. The huge gap between global food demand and food production is mainly due to the overpopulation and insufficient cultivated lands. To bridge this gap, farmers tend to use chemical fertilizers to increase plant productivity.Beneficial microorganisms (BMs) comprise diverse groups microbes such as of plant-growth promoting bacteria. Exploitation of BMs is a promising, ecofriendly, cost-effective and efficient strategy sustainable agriculture and food security. The aim of the current study was to isolate and characterize plant growth promoting bacteria from different ecological niches in Al-Ahsa, Kingdom of Saudi Arabia. Furthermore, the effects of inoculation with the bacterial strains on growth of three economically-important crop legumes were also investigated.

Methods: Soil samples from non-agricultural soils were collected from Al-Ahsa city and endophytic bacteria were isolated on Nutrient agar plats. The morphological, plant-growth promotion and biochemical characteristics were determined. The strains were identified using 16S rRNA gene sequencing. The effects of the strains on the growth of three crop legumes were assessed.

Results: Thirty strains were obtained and exhibited diversity with respect to pheotypic and biostimulating traits. Twenty five strains produced IAA solubilized inorganic phosphate, exhibited antimicrobial activities and had positive effects on growth parameters measured for *Lens esculentus*, *Phaseolus vulgaris* and *Pisum sativum*. The strains were identified using 16srDNA sequencing as *Enterobacter cloacae*, *Bacillus megaterium*, *Staphylococcus aureus* and *Sinorhizobium meliloti*.

Conclusions: The strains could be used to develop potential biofertilizers for sustainable agriculture and food security.

EP044 / #773

E-Poster Viewing Topic: AS12 Plant-microbe interactions

POLYCHROME: A BIOINFORMATICS TOOLKIT FOR THE DETECTION AND CLASSIFICATION OF PHYTOPATHOGENS BASED ON NEXT-GENERATION SEQUENCING, GENOMICS AND METAGENOMICS

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Background and Aims: Emerging outbreaks of plant diseases pose enormous threat to agricultural production and global food security. Early detection and identification of plant pathogens using next-generation sequencing (NGS) technology and bioinformatics analysis are important to cope with the increase of international trade. Here, we present the PolyChrome bioinformatics toolkit for the detection and identification of regulated plant diseases.

Methods: The PolyChrome toolkit consists of two programs, PolyChrome Detector (PCD) and PolyChrome Classifier (PCC). The former detects the presence of specific species from metagenomic and meta-transcriptomic data and the latter focus on the classification of closely related microorganisms at species or subspecies levels. In the PCD workflow, adapters and low-quality reads of raw NGS sequences are removed using Atria, an in-house designed trimming program. Clean reads are mapped to individual genomes, and then assembled to larger contigs, which are aligned to databases with taxonomy assignment. At the end of the pipeline, the annotated contigs are filtered with statistics on identity, alignment lengths, and bit scores, and suspected contigs of pathogens are reported.

Results: In PCC platform analysis, we first built curated PCC databases of selected regulated agents, e.g. *Clavibacter, Liberibacter, Dickeya* and *Pectobacter*, containing the genome sequences, annotations and the pre-analysis results, including average nucleotide identity (ANI) values. Testing dataset goes through the similar pipeline as PCD for contig generation and are classified using ANI values.

Conclusions: The PolyChrome with PCD and PCC pipelines have been used to detect and identify plant pathogens, and has great potential in the detection of potato wart pathogen in soil.

EP045 / #326

E-Poster Viewing Topic: AS12 Plant-microbe interactions

USING PLANT GROWTH PROMOTING RHIZOMICROBIOME TO IMPROVE LETTUCE (LACTUCA SATIVA) PLANT PRODUCTIVITY

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Background and Aims: Phyto-microbiomes have been well explored in providing wide range of beneficial services to the plants leading to the enhancement of the plant growth. However, microbiomes of horticultural crops including *Lactuca Sativa* are less explored and must be deciphered to increase the productivity of these important edible plants. This study aimed the isolation and characterization of closely associated bacterial assemblages from lettuce rhizosphere following their use as bioinoculum of lettuce.

Methods: Five closely associated bacterial communities were isolated from lettuce rhizosphere ensuing bacterial purification, morphological identification, and biochemical characterization using QTS-25 kits.

Results: Screening for plant growth promoting traits indicated four isolates positive for protease production, twelve for cellulase, eight for amylase, and five for lipase production. Eleven isolates were capable of producing phytohormone indole-3-acetic acid and three showed production of ammonia. None of the isolates shown the production of volatile hydrogen cyanide and zinc solubilization. Five isolates solubilized phosphate on NBRIP medium (SI= 1.66 ± 0.03), whereas, three isolates could solubilize KCI. Selected bacterial isolates showing promising plant growth promoting traits were used as bioinoculum of wheat. Highest vigor indices were noted for strains LARS19 (*Aeromonas hydrophila*= 1533.65) and LARS9 (*Klebsiella oxytoca* = 1475.57), following the other three strains used for inoculation experiment. Next, the strains were used as bioinoculum of lettuce plants as single-strain inoculum and consortium (microbial community) and plant growth parameters were recorded.

Conclusions: The results demonstrated the potential of these strains to be used as successful biofertilizers of under-pitched lettuce plant suggesting the reduced use of harmful agrochemicals.

EP046 / #588

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

ASSESSMENT OF SOME KEY INDICATORS OF THE ECOLOGICAL STATUS OF AN AFRICAN FRESHWATER LAGOON (LAGOON AGHIEN, IVORY COAST)

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Background and Aims: The supply of drinking water is a vital challenge for the people in Africa due to strong demographic growth and therefore increasing water demands. To meet these needs, surface water resources are becoming increasingly mobilized because underground resources have already been overexploited. This situation is the case in the region of Abidjan. Among the potential resources, local managers have identified a freshwater lagoon, Lagoon Aghien,. Objective: Enhancing knowledge on the ecological functioning of the lagoon and contributing to the assessment of its ability to provide drinking water

Methods: Physical and chemical parameters of the water and the phytoplankton community of the lagoon were monitored for 17 months (December 2016-April 2018) at six sampling stations.

Results: Our findings show that the lagoon is eutrophic, as evidenced by the high concentrations of total phosphorus (>140 µg L-1), nitrogen (1.36 mg L-1) and average chlorophyll-a (26 to 167 µg L-1) concentrations. The phytoplankton community in the lagoon is dominated by genera typical of eutrophic environments such as Peridinium and by cyanobacteria that can potentially produce cyanotoxins. The two rainfall peaks that occur in June and October appeared to be major events in terms of nutrient flows entering the lagoon, and the dynamics of these flows are complex. Overall, these results reveal that the quality of the lagoon's water is already severely degraded.

Conclusions: These results therefore raise questions about the potential use of the lagoon as a source of drinking water if this lagoon aren't protected from increasing eutrophication and other pollution sources.

EP047 / #521

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

PROFILING THE BACTERIAL COMPOSITION AND ANTIBIOTIC RESISTOME FROM WATER SAMPLES IN MANILA BAY SOUTH HARBOR

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Background and Aims: Invasive bacteria and possible antibiotic-resistant forms are carried across the globe and are increasingly being recognized as emerging contaminants in the ships' structure and ballast water; hence, there is an urgency toward early detection to prevent widespread dissemination of these species. In Manila Bay, thousands of ships navigate the South Harbor following international routes however, its microbial diversity remains unstudied. The study's main objective is to provide baseline information on bacterial composition and antibiotic resistome profiles from water samples in Manila Bay South Harbor.

Methods: Shotgun metagenomics sequencing, bioinformatics analysis, and antibiotic susceptibility tests were applied to elucidate the bacterial composition and diversity and identify resistance genes (ARGs) and antibiotic-resistant bacteria (ARB).

Results: A total of 27 bacterial phyla were identified in the sample, and these were further classified into 42 classes and 86 orders, 206 families, and 502 genera. The dominant metagenome predicted function classified using KEGG Orthology Level 1 is Metabolism. At a more specific functional subsystem classification, Level 2, the most prevalent subsystems in the sample are the amino acid metabolism and carbohydrate metabolism. The ARGs encoding multidrug-resistance was the most abundant in the sample. The resistant bacteria identified include ampicillin-resistant *Escherichia coli*, multidrug-resistant *Klebsiella pneumoniae*, ampicillin- and cefuroxime- resistant *Vibrio alginolyticus* and ampicillin resistant *Vibrio furnissi*.

Conclusions: Based on the bacterial composition and antibiotic resistome of the sample, results revealed that Manila Bay contains potentially opportunistic human and animal pathogens. Contaminated water may pose health risks to humans and the environment; thus, water management decisions must be regulated.

EP048 / #212

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

SYMBIOTIC FOLATE DEGRADATION BY VARIOVORAX AND OTHER BACTERIA

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Background and Aims: Folate (vitamin B₉) is an important cofactor to biosynthesize amino- and nucleicacids. The folate biosynthesis and salvage pathway have been well-investigated whereas its biodegradation is not fairly understood. This study aimes to clarify the folate catabolism by *Variovorax*, which was a main folate-utilizing bacterium in the environment soil.

Methods: This study was performed using a field soil prepared from Tsukuba-Plant Innovation Research Center. Folate-utilizing bacteria were isolated from the soil using folate as a sole source of carbon and then identified by partial 16S rRNA gene analysis. Bacterial folate metabolites were analyzed using HPLC and LC/MS/MS. The soil-microbiomes were characterized by meta-16S rRNA gene analysis.

Results: The isolated more than 300 folate-utilizing bacteria were mostly classified into *Burkholderiales*, which was consisted of *Variovorax* (>70%) and the other genera. Meta-16S rRNA gene analysis showed that not only *Variovorax* but also non-folate-utilizing bacteria *Paraburkholderia* are significantly increased in the presence of folate. *Variovorax* sp. strain FA-1, one of *Variovorax* isolates, utilized only L-glutamate cleaved from folate by glutamate carboxypeptidase (GCP) in pure culture but was able to utilize completely folate in co-culture with *Paraburkholderia* bacteria or addition of a nutrient factor. Co-culture with *Escherichia coli* strain BW25113 also enabled strain FA-1 to utilize completely folate but it with the nutrient factor non-production mutants were not.

Conclusions: Our results propose a novel microbial symbiosis on folate degradation that *Variovorax* is received the nutrient factor supply from *Paraburkholderia* bacteria to utilize folate and instead, provides folate-metabolites such as L-glutamate to them.

EP049 / #206

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

DIVERSITY AND ANTIMICROBIAL RESISTANCE OF COAGULASE-NEGATIVE STAPHYLOCOCCUS SPP., INCLUDING MECA POSITIVE STRAINS, FROM SEAWATER IN RIO DE JANEIRO, BRAZIL

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Background and Aims: Despite being less virulent than the coagulase-positive Staphylococcus aureus, coagulase-negative staphylococci (CoNS) cause opportunistic infections and act as resistance gene-reservoirs to more virulent staphylococcal species. These bacteria are widespread, and the environment is a potential source of contamination. In this study, we investigated the presence and antimicrobial resistance of Staphylococcus spp. in the seawater of the Guanabara Bay (Rio de Janeiro, Brazil).

Methods: We isolated 70 CoNS from the Guanabara Bay in selective medium and identified their species by MALDI-TOF/MS and sequencing of the tuf gene. Genetic diversity was assessed by (GTG)₅-PCR, resistance profile was assessed by disc diffusion against eleven antimicrobials, and the presence of the mecA gene was evaluated by PCR.

Results: Eleven CoNS species were identified: Staphylococcus saprophyticus (30%), Staphylococcus warneri (24.3%), Staphylococcus epidermidis (20%), Staphylococcus arlettae (4.3%), Staphylococcus haemolyticus (4.3%), Staphylococcus xylosus (4.3%), Staphylococcus cohnii (2.9%), Staphylococcus kloosii (2.9%), Staphylococcus nepalensis (2.9%), Staphylococcus carnosus (1.4%), Staphylococcus condimenti (1.4%) and Staphylococcus hominis (1.4%). Among these, 35% were pan-susceptible. Strains were mostly non-susceptible to penicillin G (54%), erythromycin (38.5%) and sulfamethoxazole-trimethoprim (10%). All five cefoxitin non-susceptible strains were mecA-positive. Strains of the same species were genetically different, but the presence of some strains with the same genotype, albeit collected from different regions of the bay, indicate that these strains are persistent and widespread.

Conclusions: The presence of antimicrobial resistant Staphylococcus in the recreative waters of the Guanabara bay represents a potential risk for human contamination and their presence should be carefully monitored, as their contamination origin should be investigated.

EP050 / #137

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

MICROBIAL COLONIZATION OF PLASTICS IN CARIBBEAN COASTAL WATERS

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Background and Aims: Marine plastic pollution has increased exponentially since the start of plastic mass production in the 1950's. Typically, plastics are colonized shortly after entering the ocean; however, little is known about the initial colonization of plastic. In particular, interactions between marine plastic and microbes and the influence of photo-oxidation on community succession are unresolved.

Methods: We investigated these parameters during the first 6 days of plastic exposure to shallow Caribbean coastal waters (St. Eustatius). For in situ incubations, we used five important polymer types (PE, PP, PS, PET, Nylon), that were exposed to the Caribbean sun for 12 hours for UV pre-treatment, and controls without pre-treatment.

Results: Multivariate statistical analysis revealed significant differences when comparing the community structures on day 1 and day 6. Polymer type was a relatively minor contributing factor to the differences in community structure. Analysing the communities individually for each timepoint as a subset, revealed that plastic type was a statistically significant factor in community differentiation on day 6, but not on day 1.

Conclusions: Our results indicated that besides temporal effects in community succession, distinct plastics may indeed select for different types of microbes and that plastic colonizers are hence not purely opportunistic. In addition, we were able to identify genera of hydrocarbon degrading bacteria on all polymers. These microbes were previously suggested to potentially degrade plastics and thus to act as a possible sink for marine plastics.

EP051 / #774

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

MICROBIAL COMMUNITIES OF ANADARA TUBERCULOSA, A BIVALVE FROM THE COLOMBIAN PACIFIC COAST

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Background and Aims: Anadara tuberculosa (piangua) is a bivalve that lives in the Colombian Pacific mangroves and is of great economic and social importance to the pacific region human populations. The aim of this study was to characterize the microbial communities associated with pianguas.

Methods: We collected piangua specimens in two regions of the Colombian pacific coast. Each individual was kept in re-sealable bags and transported in a cooler. Later in the laboratory, the animal was removed from the shell and then ground and used to inoculate nutrient agar prepared using seawater from the sample sites. We incubated all media for 24-48 hours at 28°C. Finally, we amplified the 16S rRNA region using 27F/1492R primers for a general identification, and we sequenced the complete genome of the most frequently isolated genre using Oxford Nanopore Sequencing Technology.

Results: We obtained 73 isolates corresponding to eight genera and included potentially pathogenic strains of the *Vibrionales, Pseudomonales,* and *Enterobacteriales* orders. We identified for the first time in Piangua the species *Pseudoalteromonas xiamenensis. Vibrio* spp. was the most frequently isolated genus and preliminary results of the 16S region showed three different species. The complete genome sequences confirmed these results and allowed us to identify other species.

Conclusions: We describe the microbial communities associated with pianguas in Colombia for the first time. The described community composition was similar to microbial communities reported in other marine environments, and we did a first-time report of *P. xiamenensis* in Colombia.

EP052 / #750

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

NOVEL CLADES OF RICKETTSIALES AND HOLOSPORALES INFECTING PROTIST CELLS IN THE TERMITE GUTS AND THEIR METABOLIC CAPACITIES PREDICTED BASED ON GENOME ANALYSIS

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Background and Aims: The orders Rickettsiales and Holosporales (class Alphaproteobacteria) comprise obligate intracellular parasites that infect the cytoplasm and/or nucleus of a variety of eukaryotic hosts. We here report three novel clades belonging to either of the two orders, which are widely distributed in the termite guts, and we predicted their metabolic pathways based on genome sequence analyses.

Methods: We performed sequencing and fluorescence in situ hybridization (FISH) analyses of 16S rRNA to identify their phylogenetic position and cellular localization. Then, we obtained the genome sequences of three endosymbiotic phylotypes to predict their functions.

Results: We discovered two clades in Holosporales and one clade in Rickettsiales, which were consistently detected in the guts of diverse lower termites. FISH analysis specifically targeting several phylotypes in each clade showed that they are facultatively localized in the cytoplasm or nucleoplasm of various protists in species-specific manners. Particularly, one phylotype, RsTu1-89, in Holosporales exhibited a unique localization; each cell is, in most cases, surrounded by "*Candidatus* Desulfovibrio trichonymphae", which are ectosymbionts of the host flagellate. We obtained two complete genomes, each from the two clades in Holosporales, and also a draft genome of the RsTu1-89 phylotype. The genomes showed limited biosynthetic capacity, and instead encoded the gene for ATP/ADP translocase.

Conclusions: The three novel clades of Holosporales or Rickettsiales are widely distributed in the termite guts as cytoplasmic or endonuclear symbionts of the protists. The genomes indicate their obligately parasitic life style, and we will discuss the adaptation and evolution of these alphaproteobacterial clades.

EP053 / #117

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

MICROBIAL COMMUNITY STRUCTURE IN THE MAJOR INFLOW RIVERS OF TAIHU LAKE AND ITS RELATIONSHIP WITH WATER QUALITY AND LAND-USE PATTERNS

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Background and Aims: Taihu Lake is the third-largest freshwater lake in China and it is connected to many inflow rivers, which contribute to the source water and pollution. The water quality and microbial community of some of these inflow rivers were studied previously but not comprehensively. Thus, this study was aimed at assessing the bacterial community structure of ten major inflow rivers in the northwest and southwest regions of the lake and determining the relationship between the bacterial community, water quality and land use.

Methods: Water samples were collected from ten inflow rivers across four seasons in 2019-2020. DNA extracted from the samples was used for 16S rRNA gene-targeted next-generation sequencing to study the bacterial community structures. Thirteen physico-chemical and microbiological parameters were analyzed to assess the water quality. The land-use pattern surrounding each sampling location was also analyzed. Seasonal and spatial differences in the bacterial community and their relationship with water quality and land use were also determined.

Results: The bacterial community showed significant seasonal and spatial variation. *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Cyanobacteria* were the predominant phyla observed. In summer, the community was more influenced by the chlorophyll-*a*, pH and phosphate-P and in winter, electric conductivity, nitrate-N and ammonium-N. The water quality and bacterial community data showed that rivers in the northwest were more nutrient-rich.

Conclusions: Land use, particularly industrial, residential and agricultural categories correlated well with the bacterial community composition and activity, and water quality. The major industrial pollution sources in addition to farmland drainage and untreated domestic wastewater need to be efficiently managed.

EP054 / #313

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

LUMAZINE UTILIZATION PATHWAY IN CUPRIAVIDUS SP. STRAIN LA-1.

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Background and Aims: Pterin compounds are white ~ yellow chemicals, that are also known as pigments included in the wings and exoskeletons of insects such as bees and butterflies. The pterin compounds are frequently converted to lumazine structure by bacterial pterin deaminase. Therefore, lumazine compounds widely present in environment, but their biodegradation pathways are poorly understood. This study aims to isolate a lumazine-utilizing bacteria from environmental soil and identify the bacterial lumazine degradation pathway.

Methods: Lumazine-utilizing bacteria were isolated from soil using minimal media containing lumazine as a sole source of carbon, and their lumazine metabolites were analyzed and identified using LC/MS/MS. Transcriptome profiling of isolate was performed using RNA-seq. Microbiome in the presence or absence of lumazine was characterized by meta-16S rRNA gene analysis.

Results: Meta-16S rRNA gene analysis showed that *Cupriavidus* genus was significantly increased in the presence of lumazine. Lumazine-utilizing bacterium strain LA-1 isolated from the soil was classified into *Cupriavidus*, which agreed with the results of Meta-16S rRNA gene analysis. Gene expression encoding the enzymes for a first and a second step on lumazine utilization process of LA-1 were both lumazine-inducible, and those reaction products were identified as 7-hydroxylumazine (1'st) and 6,7- dihydroxylumazine (2'nd). RNA-seq indicated that a gene cluster (GM003301-3310) containing Xanthine dehydrogenase (Xdh) encoding genes was increased more than 100-folds in the presence of lumazine.

Conclusions: *Cupriavidus* genus are the major lumazine-utilizing bacterium in environmental soil, and the isolated *Cupriavidus* sp. strain LA-1 utilizes lumazine via hydroxylation at position 6- and 7-carbon catalyzed by Xdh-like protein.

EP055 / #150

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

ENHANCEMENT EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM STAPHYLOCOCCUS AUREUS ON VIRULENCE OF PSEUDOMONAS AERUGINOSA

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Background and Aims: Staphylococcus aureus (Sa) and Pseudomonas aeruginosa (Pa) are the most prevalent pathogens isolated from lungs of cystic fibrosis patients and chronic wounds. Synergistic effects on virulence of both bacteria have been reported but their effects mediated by extracellular vesicles (EVs) are unknown. In this study, we investigated whether EVs derived from Sa (SaEVs) promote the virulence of Pa.

Methods: Pa was pretreated with and without purified SaEVs. Human keratinocyte HaCaT cells, lung carcinoma A549 cells, and murine macrophage RAW264.7 cells were used for infection experiments. Differences in protein expression in Pa with and without SaEV treatment were evaluated by quantitative LC-MS/MS analysis. Gene expression in Pa promoted by SaEVs was assessed by real-time PCR.

Results: In comparison with untreated Pa, SaEVs promoted Pa invasion into HaCaT cells and enhanced the cytotoxic effect of Pa after 24 h infection to HaCaT and A549 cells. In addition, SaEVs reduced internalization of Pa into RAW264.7 macrophages. Differential proteomic analysis revealed that PsIE, an exopolysaccharide biosynthesis-related protein, was increased in SaEV-treated Pa. Similarly, the expression of five genes in psI operon (psIA/E/J/K/L) in Pa was increased by SaEV-treatment in a dose-dependent manner.

Conclusions: SaEVs promoted epithelial cell infection and destruction of Pa. On the other hand, SaEVs prevented phagocytosis of Pa by RAW264.7 macrophages. The results suggest that EVs released from Sa are mediators that act on Pa to promote its virulence, and mechanism may be involved in exopolysaccharide biosynthesis.

EP056 / #76

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

BACTERIAL HELPER COMMUNITIES IN ANT FUNGAL GARDENS

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Background and Aims: As ubiquitous microorganisms, bacteria live in interkingdom communities. Those found in fungus-growing ant colonies are a remarkable example, as they support ants' metabolic processes and the maintenance of ant fungus gardens. Little is known about the bacteria associated with the nutrient storage structure of the fungal cultivar (gongylidium). Here, we studied whether bacterial communities are able to influence the biology of the fungus.

Methods: Bacteria isolated from gongylidia surface of the cultivar of Atta sexdens and Acromyrmex coronatus, were screaninig in vitro for metabolic functions able to influence on the mutualistic fungus.

Results: Ten bacterial morphotypes were isolated, corresponding to four Bacillus spp., one Lysinibacillus sp., one Staphylococcus sp., and one Paenibacillus sp. strains, as well as one Actinobacteria and two members of Enterobacteriaceae. These bacteria showed auxiliary metabolic functions useful for the development of the fungus garden such as chitin degradation (Bacillus sp. and the Actinobacteria), siderophore production (Enterobacteriaceae), and cellulose degradation (Bacillus sp.). Likewise, eight bacteria were able to form biofilm on the cultivar hyphae, suggesting they may physically interact with the fungus in the gardens. Two bacterial isolates increased gongylidia production and fungal biomass in vitro, while three had inhibitory effects. Finally, in vitro bacteria-bacteria interaction assays revealed heterogeneous behaviors including synergism, competition and potential parasitism.

Conclusions: Our results suggest that bacteria and the ant mutualistic fungus live in community, within a continuum of positive and negative interactions. These interactions could help the metabolic flow that supports life in the fungus garden and ultimately contribute to the stability of the fungus–ant mutualism.

EP057 / #706

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

FLY A MIDDLE COURSE, ICARUS: EVOLUTION OF MICROBIAL COOPERATION REQUIRES INTERMEDIATE FOUNDER POPULATION SIZES.

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Background and Aims: Cooperation is observed in many domains of life, from human social interactions to cooperation between single-cell bacteria in ecosystems. In the presence of cooperation, cheaters regularly emerge. These cheaters show "selfish" behaviour by taking benefit from the public goods produced by the cooperators, without paying the costs. As natural selection acts on the level of individuals, it is expected to favour this selfish behaviour of cheaters, so why and when is cooperation beneficial?

Methods: To study these questions, we serially propagated a synthetic consortium of cooperating and cheating lactococci in the droplets of a water-in-oil emulsion. Each droplet was randomly inoculated by a small number of bacteria (the founder population), and all consortia were allowed to grow. After growth all groups were mixed, and this mixture was used to re-inoculate new droplets. During these propagations, we measured the abundance of cooperators and cheaters in the mixture.

Results: The results of this competition experiment show that when the growth of cheaters completely depends on cooperators, cooperators outcompete cheaters. However, cheaters outcompete cooperators when they can independently grow to a low cell concentration. This result is the consequence of a probabilistic effect, as low founder population sizes in droplets decrease the frequency of cooperator co-localization.

Conclusions: As natural systems are often spatially structured, these statistical constraints may limit the evolution of cooperation. Cooperators can overcome this constraint by preventing the founder population size to become too high or too low, or by directing part of the benefits of their public good to themselves.

EP058 / #519

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

MICROBIAL SOURCE TRACKING OF FECAL INDICATOR BACTERIA IN URBAN FARMS IN METRO MANILA, PHILIPPINES

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Background and Aims: Fecal indicator bacterium *Escherichia coli* is one of the leading causes of foodborne diseases in the Philippines and its presence has been detected in agricultural crops especially vegetables. Given the risk posed to public health by fecal contamination, measures to mitigate contamination are warranted. However, proper risk assessment and prevention plan cannot be fully implemented without enough knowledge as to the specific sources of fecal contamination. Microbial Source Tracking (MST) is a scientific approach in determining the specific sources of fecal contamination. MST provides data useful in mitigating contamination and its associated health risks, which can potentially positively impact agriculture. Therefore, this study aims to perform source tracking of fecal contamination in selected vegetables in the Philippines.

Methods: A total of 468 vegetables were obtained from randomly selected urban farms and wet markets in Metro Manila. The specific sources of *E. coli* contamination are predicted using detection of host-specific MST markers by PCR amplification and/or detection of MST markers in whole genome sequence of the *E. coli* isolates.

Results: From the samples collected, 21.51% (48 out of 258 samples in dry Season and 48 out of 210 samples from wet season) were positive for presence of thermotolerant *E. coli.* Several sources were found to be causing contamination through molecular analyses, including fecal contamination of animals in the area, soil, and agricultural water.

Conclusions: Thus, MST can be used to mitigate fecal contamination in urban farms and prevent foodborne diseases.

EP059 / #572

E-Poster Viewing Topic: AS13 Microbial communities and microbiomes

LINKING THE LONGITUDINAL DEVELOPMENT OF THE PIGLET NOSTRIL MICROBIOME TO METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS CARRIAGE AS A STRATEGY TO IDENTIFY NASAL PROBIOTIC STRAINS.

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Background and Aims: The reservoir of livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in pig farms forms a potential zoonotic risk for society. We investigated the development of the porcine nasal microbiome and identified bacteria suitable for intervention of LA-MRSA nasal colonization in piglets and tested strains for efficacy against *S. aureus in vivo*.

Methods: Swabs were obtained from 252 piglets from 36 sows from 9 farms in 3 countries (Ireland, Germany, the Netherlands), from birth till ten weeks (n=4032) for species isolation and DNA extraction. DNA was used for *S. aureus* specific qPCR, V3-V4 16S rRNA and *tuf* Illumina sequencing. Amplicon Sequence variants (ASVs) negatively associated with *S. aureus* qPCR counts were identified. ASV assigned species were evaluated on probiotic suitability. Strains from the samples were identified by MALDI-TOF and screened following EFSA guidance, both *in vitro* (phenotypical antimicrobial resistance testing) and *in silico* (whole genome sequencing, taxonomy, antimicrobial resistance genes, virulence factors).

Results: *in silico* we identified 54 species negatively associated with *S. aureus*. Literature safety assessment left 15 candidates, predominantly consisting of lactic acid bacteria (LAB). The isolation effort, *in vitro* safety and efficacy studies yielded three strains, meeting EFSA's Qualified Presumption of Safety (QPS) status. These were consequently utilized in piglets to test safety and efficacy against *S. aureus*/MRSA

Conclusions: Investigation of longitudinal samples from the porcine nasal microbiome resulted in putative probiotic LAB strains currently tested *in vivo*.

EP060 / #758

E-Poster Viewing Topic: AS14 Human microbiome and health

SCREENING FOR CERVICAL CANCER IN BEJAIA, ALGERIA: INVOLVEMENT OF HUMAN PAPILLOMAVIRUS (HPV) AND HUMAN HERPES VIRUS (HHV)

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Background and Aims: Cervical cancer (CC) is the fourth most common malignant neoplasia in women worldwide. Persistent human papillomavirus (HPV) infection is the main factor, but not sufficient for the development of this disease. In addition, the role of the human herpes virus (HHV) also appears to be a subject of debate. Since the main route of transmission of HPV and HHV is sexual, it is fair to assume that co-infection of HPV and HHV is a decisive factor in the development of cervical cancer;

Methods: Cervical cancer screening campaigns were carried out in different regions of Bejaia province, between 2019 and 2021. Cervical smear samples were collected and analyzed to verify the presence of infectious agents and cell abnormalities and lesions.

Results: During this period, 1774 women were screened. 27 women were diagnosed with a cell abnormality (ASC-US, ASC-H, LSIL, HSIL, AGC). HHV was identified in 16 women. However, HPV was identified only in 10 women.

Conclusions: Statistics and data on the presence and even the involvement of HPV and HHV are lacking in Algeria, that is why it is necessary to remedy to the situation.

EP061 / #728

E-Poster Viewing Topic: AS14 Human microbiome and health

VAGINAL MICROBIOME CHANGES IN WOMEN WITH VULVO-VAGINAL ATROPHY AFTER DRUG AND HORMONE TREATMENTS

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Background and Aims: Vulvo-vaginal atrophy (VVA) is a chronic condition that afflicts almost 50% of menopausal women, with many causes that can be associated with several symptoms. Many therapies are available, including systemic hormone therapy (HT) and ospemifene. Up to date, no information about the dynamics of the vaginal environment after ospemifene treatment is available, and little is known for HT. The relationship between VVA microbiome composition, symptoms, and severity is yet to decipher. This study aims to characterize the microbiota of women affected by VVA, and to assess whether the therapies are accompanied by changes in microbial vaginal composition.

Methods: We enrolled 67 women, 28 healthy control ("HC") and 39 with VVA: 20 were prescribed with ospemifene; 19 received systemic HT. Microbiota data was obtained through 16S rRNA gene sequencing from vaginal swabs; vaginal health and maturity index were assessed following clinical evaluation and swab protocols.

Results: Pre-therapy women showed higher biodiversity distributions when compared to HC. Relative abundances were found altered between atrophic and healthy women: *Lactobacillus* reduced in VVA, while *Streptococcus* increased. Both therapies showed reducing effects on alpha-diversity values. Microbiota composition after HT showed high proportions of *Lactobacillus* and a *Prevotella* reduction; ospemifene, contrariwise, reported several significant changes at both family and genus phylogenetic levels, particularly a *Staphylococcus* reduction. VHI and VMI were statistically increased after both treatments, with Post-therapy values comparable to HC. Both index positively correlated with *Lactobacillus* and negatively with *Streptococcus*.

Conclusions: Our results suggest that both therapies, ospemifene particularly, affect the vaginal microbiota and lead to healthier conditions.

EP062 / #502

E-Poster Viewing Topic: AS14 Human microbiome and health

GUT MICROBIOME PROFILE AND INFLAMMATORY BIOMARKERS OF CORONAVIRUS DISEASE – 2019 (COVID-19) PATIENTS FROM COVID-19 REFERRAL CENTERS IN THE PHILIPPINES

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Background and Aims: COVID-19 is primarily a respiratory illness that has affected millions of individuals across the world. Evidence has suggested that a gut-lung axis may play a role in the pathogenesis and severity of this disease. In this study, we characterized the composition of the gut microbiome in SARS-CoV-2 infected individuals with varying severities of disease.

Methods: Patients were recruited from university-based COVID-19 referral centers, where stool samples were obtained from patients admitted for isolation or further management. Stool samples were analyzed using 16S V3-V4 ribosomal RNA sequencing methods and subject to analysis using *mothur* bioinformatics pipeline. Taxonomic characterization and diversity analysis were performed. Clinical and laboratory data were obtained to correlate with identified bacterial biomarkers.

Results: Alterations in the gut microbiota were observed in patients with COVID-19 disease. Increasing fold-changes were noted with Verrucomicrobiales with increasing disease severity. Bifidobacteriales was notably decreased in patients with moderate to severe COVID-19. Lactobacillales was noted to be increased in patients with moderate and severe disease compared to patients who experience mild and asymptomatic disease, suggesting a role in the pathogenesis of COVID-19 severity. No correlation was observed with Bifidobacteriales and Lactobacillales relative abundance with various inflammatory markers in admitted COVID-19 patients.



Figure 1. Jacard Index of Disimilarity Matrix showing beta-diversity between severity groups. High dissimilarity indices are seen in patients with severe disease.



Figure 2. Principal coordinates analysis of samples across different severities of COVID-19. Clustering was observed in asymptomatic to mild COVID-19.


	ASX	MILD	MODERATE	SEVERE
Bacteroidales	-0.07	-0.03	-0.09	-0.06
Lactobacillales	0.00	-0.13	0.16	0.17
Clostridiales	-0.08	-0.05	-0.09	-0.11
Selenomonadales	-0.04	0.06	-0.29	0.22
Aeromonadales	1.68	1.82	1.30	1.61
Enterobacteriales	0.16	-0.06	0.14	0.16
Bifidobacteriales	-0.28	-0.21	-0.59	-0.75
Erysipelotrichiales	-0.03	-0.16	-0.15	-0.06
Fusobacteriales	0.27	0.26	0.02	0.18
Burkholderiales	-0.12	-0.16	-0.26	-0.33
Verrucomicrobiales	0.70	0.37	0.95	1.07

Table 1. Fold-change in the relative abundance of bacterial orders across COVID-19 severity groups

Conclusions: Specific alterations in the gut microbial composition were seen in patients infected with SARS-CoV-2 particularly among low-abundance bacteria with known contributions to host health. However, these changes did not correlate with changes seen in serum inflammatory markers suggesting that modifications in the microbiome may result from immune response or inflammatory response independent modalities.

EP063 / #788

E-Poster Viewing Topic: AS14 Human microbiome and health

BACTERIOCIN DIVERSITY, ANTIMICROBIAL RESISTANCE GENES AND PROPHAGES REPERTOIRE IN LACTOBACILLACEAE FROM THE FEMALE URINARY MICROBIOTA

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Background and Aims: *Lactobacillaceae* are common in female urogenital microbiota (FUM), however bacteriocins, antibiotic resistance genes (ARG) and prophages repertoire is underexplored. We screened the bacteriocin-producing potential, ARG and prophages of *Lactobacillaceae* from urine of healthy women-HW, women diagnosed with overactive bladder-OAB, or recurrent UTI-rUTI.

Methods: Thirty-three isolates (HW-9 *Lactobacillus crispatus*, 6 *Lactobacillus jensenii*, 3 *Lactobacillus paragasseri*, 3 *Lactobacillus mulieris*, 2 *Lactobacillus gasseri*, 1 *Lactobacillus delbrueckii*, 2 *Limosilactobacillus portuensis*, 1 *Limosilactobacillus urinaemulieris/Limosilactobacillus mucosae*, 1 *Lacticaseibacillus* rhamnosus; OAB-2 *Lactobacillus crispatus* and 1 *Lactobacillus paragasseri*; rUTI-1 *Lactobacillus crispatus*) were subjected to WGS (NovaSeq/Illumina). *In silico* analyses were performed to screen putative bacteriocin, ARG, and prophages.

Results: Bacteriocins were detected in 24/33 isolates (72.7%). Fifteen bacteriocins types (class II: penocin A, LS2, acidocin B/LF221B, gassericin T, enterocin X/NKR-5-3D/NKR-5-3A, pediocin, brochocin, carnocin CP52, pentocin, amylovorin; class III: helveticin J, enterolysin A) were identified. Helveticin J was the most common and detected in 4 *Lactobacillus* species, followed by enterolysin A. 10/12 *L. crispatus* encoded \geq 3 bacteriocins (enterolysin+helveticin J+penocyn A/LS2/amylovorin/penocyn A+LS2). Several bacteriocins were identified for the first time in different *Lactobacillaceae (L. delbrueckii*-helveticin J, *L. portuensis*-enterolysin A, *L. paragasseri*-acidocin LF221B/pentocin, *L. mulieris*-enterocin NKR-5-3D/A, *L.rhamnosus*-enterocin X/enterolysin A, *L. crispatus*- amylovorin). ARG [(*erm(B)/Inu(C)/tet(W)/ cat*), and (*tet(L)/tet(M)/tet(W)*] were detected in 2 *L. crispatus* from OAB and rUTI patients. Prophages were only detected in isolates from HW.

Conclusions: This study showed a remarkable bacteriocinogenic potential of *Lactobacillaceae* from FUM and extended the diversity of bacteriocins that might contribute for shaping FUM. UT disease conditions seem to favor isolates carrying ARG.

EP064 / #85

E-Poster Viewing Topic: AS14 Human microbiome and health

IDENTIFICATION OF PERSISTENT CARRIERS AND NON-CARRIERS OF STAPHYLOCOCCUS AUREUS IN THE PHARYNX AND NOSE OF YOUNG ADULTS LIVING IN MEXICO CITY

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Background and Aims: The most studied reservoir of Staphylocuccus aureus in humans is the nose, approximately 30% or more of the population is colonized. S. aureus has been found in the pharynx and has been reported with high variability in different populations from 4 to 64%. The objective of the work was to determine the persistence of S. aureus strains in the pharynx and nose of young adults.

Methods: Pharyngeal and nasal swabs were taken from 134 health science university students from one to three months. The swab samples were seeding in Salt-Mannitol agar. The identification of S. aureus strains was performed by reseeding and isolating mannitol-fermenting and coagulase-positive bacteria. The strains identified as S. aureus underwent the antibiogram and MIC test for oxacillin.

Results: 62% of carriers of S. aureus in the pharynx and 36% in the nose. In addition, 49% are persistent carriers in the pharynx and 19% in the nose, 22% are intermittent carriers in the pharynx and 30% in the nose, and 24% and 50% were non-carriers of S. aureus in the pharynx and nose, respectively. 67% of S. aureus strains are resistant to penicillin, 23% resistant to clindamycin, 20% to erythromycin. And only 6% of the strains isolated from the pharynx and 5% from the nose were MRSA.

Conclusions: More strains of S. aureus were isolated from the pharynx than from the nose in the samples carried out, and more persistent carriers were found in the pharynx than in the nose, and practically the same number of intermittent carriers.

EP065 / #35

E-Poster Viewing Topic: AS14 Human microbiome and health

FROM HARMONY TO HARM: THE ALTERED ACTIVITY OF THE GUT MICROBIOTA IN COLORECTAL CANCER

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Background and Aims: Gut microbiota are critical in driving host inflammation and genetic damage, underpinning the development and progression of colorectal cancer (CRC). Metagenome analyses have revealed both alterations in microbial composition and genetic potential during malignancy, linking key species and their genetic traits to carcinogenesis. However, often gene abundance does not correspond with expression, the levels of which are regulated in response to environmental pressures. Therefore, we employed metatranscriptomics to establish signatures of the expressed microbiome and elucidate the mechanisms underpinning CRC.

Methods: Differential expression of microbial genes/pathways from the faecal metatranscriptomes of non-CRC individuals (n=10) and CRC patients (n=10) was analysed.

Results: We observed numerous changes in expression of crucial microbial activities, reflecting a CRCspecific shift in microbiome function. We found surprisingly high levels of oxidative stress responses in both health and cancer. However, the microbiota responds less to some DNA-damaging reactive oxygen species (ROS), specifically hydrogen peroxide (H₂O₂). Furthermore, microbes colonise the cancerous epithelium and form biofilms therein, facilitating the observed vast genetic exchange, expression of virulence determinants alongside antibiotic and acid resistance.

Conclusions: Our findings argue the microbiota are crucial mediators of ROS levels in health, both protecting the gut from epithelial and DNA damage. Deregulation of this activity may leave the healthy gut epithelium vulnerable to invasion causing inflammation and DNA damage, through accumulation or depletion of H_2O_2 . Simultaneously, the microbiota becomes increasingly pathogenic and resistant to environmental stresses. For the first time we identified specific losses of protective microbial functions and gained virulent features in the pathogenesis of CRC.

EP066 / #793

E-Poster Viewing Topic: AS14 Human microbiome and health

IN-DEPTH INSIGHTS INTO CERVICOVAGINAL MICROBIAL COMMUNITIES AND HRHPV INFECTIONS USING HIGH-RESOLUTION MICROBIOME PROFILING

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Background and Aims: The cervicovaginal microbiome (CVM) correlates with women's cervical health, and variations in its structure are associated with high-risk human papillomavirus (hrHPV)-induced high-grade cervical lesions. The CVM exhibits five community state types (CSTs): I, II, III, IV, and V, based on microbial composition; however, elucidating the impact of CSTs on health and disease is challenging because current sequencing technologies have limited confident discrimination between bacterial species that shape microbial communities. This study aimed to apply high-resolution microbiome profiling to obtain in-depth and unambiguous insights into the composition of the CVM and demonstrate how CSTs associate with hrHPV status and cervical disease.

Methods: Circular probe-based RNA sequencing (ciRNAseq) was used to profile the CVM of a cohort of healthy women from the Dutch population-based screening program (n = 341) and a second cohort consisting of hrHPV positive women with known clinical outcomes (n = 300). CSTs were established and correlated to clinical outcomes.

Results: Based on unsupervised clustering analyses, we define intra-CST differences with respect to the species *Lactobacillus acidophilus, Lactobacillus iners,* and *Megasphaera genomosp type 1*, that arrange CSTs I, III, and IV in novel A and B subgroups. These subgroups further correlate with microbial diversity and abundance. Notably, we describe associations between CST V with hrHPV negative conditions, CST I-A with the absence of cervical abnormalities, and CST IV-A with hrHPV-induced cervical disease.

Conclusions: Overall, we characterize new subdivisions of cervicovaginal CSTs, which will contribute to explaining the microbiome's role in hrHPV-induced cervical cancer and have potential applications for biomarkers discovery and therapy.

EP067 / #734

E-Poster Viewing Topic: AS14 Human microbiome and health

IN VITRO EFFECTS OF BIOENGINEERED WHEAT ARABINOXYLAN ON DEPRESSION-LINKED MICROBES

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Background and Aims: Dietary prebiotic fibres play an essential role in modulating the composition of the gut microbiota via enhancing the abundance of beneficial microorganisms reported to be negatively correlated with anxiety and depressive-like disorders, improving the production of short-chain fatty acids, majorly butyrate, propionate, and acetate. Arabinoxylan (AX) is an important and significant component of dietary fibres and has been receiving attention recently because of its prebiotic potential, ability to improve gut health, and availability. Notably, some bacterial strains have negatively correlated with depression and mental health disorders. Examples of such microbes are *Lactobacillus rhamnosus GG* (LGG) and *Faecalibacterium prausnitzii*. The study aimed to investigate the prebiotic effect of change in the chemical structure of wheat arabinoxylan fibre on the growth and metabolism of selected depression-linked bacteria strains found in the human gut microbiota.

Methods: For this purpose, α -L-Arabinofuranosidase B25 enzyme and α -L-Arabinofuranosidase enzyme were used to modify the structure of AX fibre. The native and modified fibres were subjected to a microbial growth kinetics analysis.

Results: Acetate, propionate, and butyrate were the most abundant short-chain fatty acids (SCFAs) produced. All samples containing AX fibres exhibited faster growth than the control throughout the experiment when inoculated with LGG, while the sample treated with α -L-arabinofuranosidase B25 enzyme showed a more promising result individually. Comparatively, a slightly identical effect was also observed with *F. prausnitzii*.

Conclusions: In conclusion, this warrants attention to modifying the chemical composition and structure of dietary prebiotic fibres to maintain a healthy and stable gut by increasing the availability of beneficial bacterial strains.

EP068 / #516

E-Poster Viewing Topic: AS14 Human microbiome and health

THE EFFECT OF COSMETIC PRODUCTS ON SKIN MICROBIOME: COULD THIS BE POSITIVE AND NEGATIVE EFFECT AT THE SAME TIME?

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Background and Aims: In the light of growing focus on skin microbiome, in our work we have investigated effects of cosmetic products on skin's representative microbiome resident in regard to determine antimicrobial effect which is considered as negative cosmetic effect. Together with this aim we have explored the cosmetics and their actives effect on formation of microbial biofilm - where antibiofilm effect is considered as positive effect of cosmetics.

Methods: The antibiofilm effect of creams and their ingredients - preservatives and plant (plum, hazelnut and pumpkin seed) oils, was investigated against six skin isolates of *Staphylococcus epidermidis* and laboratory control strain *S.epidermidis* ATCC 12228 by a well-established method. Additionally, the potential of creams to inhibit the growth of microbiome constituents was tested against the same bacteria.

Results: Each cream with different preservative systems as well as plant oils had weak to moderate antimicrobial effect against *S.epidermidis*. After incubation of *S.epidermidis* with creams and their individual ingredients, biofilm production was reduced by plant oils (plum, hazelnut and pumpkin seed), preservative, as well as with creams with plant oils. Only sample of placebo cream without plant oil and preservative had no effect on biofilm production.

Conclusions: Obtained results imply that the potential effect of cosmetics on skin microbiome should not be neglected, and that we should look for positive as well as negative effects of cosmetic products. Also, our investigation showed that the choice of cosmetic product ingredients is the most important factor when cosmetics contribution to skin microbiome maintenance is discussed.

EP069 / #193

E-Poster Viewing Topic: AS14 Human microbiome and health

GUT MICROBIOTA CHANGES IN RELATION TO CROHN'S DISEASE ACTIVITY

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Background and Aims: Crohn's disease has been associated with gut microbiota composition, but reported associations with specific microbes vary widely across studies. In addition, the role of the microbiota in disease exacerbation is unclear. Therefore, we investigated the relationship between bacterial abundances and disease activity in a longitudinal cohort of Crohn's disease patients (n = 57) and heathy controls (n = 15).

Methods: We applied quantile regression models to relate bacterial abundances to Crohn's disease activity, which allows for heterogenous responses between patients arising from multi-factorial dependencies. Consequently, possible relationships with bacterial abundance might become apparent outside the mean of the response to Crohn's disease activity.

Results: For example, the families Sutterellaceae and Enterobacteriaceae showed a significant difference in abundance between Crohn's disease patients and healthy controls for the lower and upper quantiles respectively, but no significant difference in the median. We also found different effects for patients experiencing an exacerbation relative to those who remained in remission (patients without self-reported symptoms or measurable inflammation), for example for the families Bacteroidaceae and Pasteurellaceae. Bacterial abundances were no longer significantly associated with a change in disease state after correction for patient characteristics, which might reflect confounding or be due to limited sample size.

Conclusions: To conclude, quantile regression allows identification of associations between bacterial abundances and Crohn's disease that are obscured in methods that focus on the mean response. Our analysis suggests that specific bacterial families might be related to Crohn's disease and disease activity, but larger studies are required to assess their involvement in exacerbation.

EP070 / #505

E-Poster Viewing Topic: AS14 Human microbiome and health

DIET COMPOSITION AND GUT MICROBIOME PROFILE OF NORMAL BMI- AND OVERWEIGHT-OBESE ADULTS IN A RURAL TOWN IN THE PHILIPPINES

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Background and Aims: Urbanization is said to be one of the important drivers of the global rise in obesity. However, in the Philippines, there is an increasing prevalence of obesity even in rural areas without significant urbanization and with a lack of access to urban diet. To explore this, this study aimed to determine the diet composition and gut microbiome and metabolite profile of normal BMI- and overweight-obese adults in a rural town in the Philippines.

Methods: Fifty-five (55) adult participants were recruited from the rural town of Manito, Albay: 25 with normal BMI (healthy group) and 30 with overweight-obese BMI (OWOB group). Their demographic profile, dietary information, and stool samples were collected. Gut microbiome profile was based on sequenced bacterial 16s rRNA gene and the fecal metabolite profile was determined by quantitative NMR and LC-MS/MS.

Results: showed that both groups have essentially similar diet and the same alpha diversity of the gut microbiome. However, the OWOB group has higher relative abundances of Actinobacteria, *Lactobacillaceae*, and *Lactobacillus*, which were positively correlated with bile acid (BA) levels. The healthy group has higher abundances of Bacteroidetes, *Prevotellaceae*, and *Paraprevotellacea* which were positively correlated with butyrate levels. *Succinivibrio* abundance was negatively correlated with BAs.

Conclusions: While diet has been previously shown to facilitate rapid changes in the gut microbiome, OWOB individuals in rural areas have different gut microbiome structure even with similar diet as healthy adults. Further studies may be needed to determine what other factors could explain this observation and the rising obesity in rural areas.

EP071 / #259

E-Poster Viewing Topic: AS14 Human microbiome and health

INFLUENCE OF EXTRACELLULAR SUBSTANCES SECRETED BY LACTOBACILLUS SSP. ON INFECTIOUS UROLITHIASIS CAUSED BY PROTEUS MIRABILIS

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Background and Aims: *Lactobacillus* ssp. secrete molecules such as H₂O₂, organic acids or bacteriocins, which have an antimicrobial effect and impact on the pathogenicity of bacteria. The aim was to demonstrate the influence of extracellular substances secreted by *L.gasseri* and *L.jensenii*, isolated from human urine, on the crystallization caused by *P.mirabilis* in the development of infectious urolithiasis.

Methods: Antibacterial activity of *Lactobacillus* strains against *P.mirabilis* was analysed by well diffusion assay and microdilution method. Components secreted by *Lactobacillus* with these properties were identified: organic acids by HPLC and spectrophotometric methods, H₂O₂ by HRPO-phenol red method. Crystallization assay was performed in synthetic urine using polycarbonate membrane inserts, which allowed for simultaneous cultures of *P.mirabilis* with individual strains of *Lactobacillus*. The amount of ammonia and crystallization intensity were determined. Crystallization was quantified by Ca²⁺ and Mg²⁺ ions levels using AAS and qualitatively using phase contrast microscopy.

Results: Antibacterial assays demonstrated that *L.gasseri* exhibited higher antibacterial activity, mainly acid-dependent, than *L.jensenii*. Both strains produce lactic and succinic acid. However, *L.jensenii* was distinguished by a high level of H_2O_2 release. In crystallization assay, *L.gasseri* reduced the amount of Mg^{2+} and Ca^{2+} ions and the release of ammonia in the tested samples compared to the control (pure culture of *P.mirabilis*).

Conclusions: The results indicate that *Lactobacillus* substances inhibit crystallization of urine, however *L.gasseri* showed higher activity in inhibiting this process. The potential benefits of these results could contribute to a better treatment and prevention of infectious urolithiasis. Project supported by program "Excellence initiative– research university", University of Lodz for years 2020-2026.

EP072 / #115

E-Poster Viewing Topic: AS14 Human microbiome and health

WEAKENED SKIN BARRIER FUNCTION THROUGH DECREASED AHR/OVOL1/FILAGGRIN AXIS BY STAPHYLOCOCCUS AUREUS-SECRETED ALPHA-TOXIN

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Background and Aims: Staphylococcus aureus (S. aureus) is commonly known as one of the main pathogens that cause bacteremia in hemodialysis (HD) patients, yet its effect on their skin has yet to be studied. Thus, our aims consist of the inspection of S. aureus colonization on the skin of HD patients, and the molecular alteration of human keratinocytes by S. aureus-secreted α -toxin.

Methods: Genomic DNA will be extracted from a set of swabs to the inner volar arm of HD patients for the comparison of bacteria relative counts by undergoing real-time PCR. While in vitro co-culture with S. aureus or α -toxin on human keratinocytes was done to inspect regulations on skin barrier proteins such as aryl hydrocarbon receptor (AhR), ovo-like transcriptional repressor 1 (OVOL1), and filaggrin, which helps promote a healthy skin barrier function through the formation of natural moisturizing factors.

Results: In vivo results indicated that HD patients with pruritus had a higher S. aureus relative count compared to Staphylococcus epidermidis (S. epidermidis). Results in vitro showed that α -toxin downregulates filaggrin expression in keratinocytes through AHR/OVOL1 signaling pathways.

Conclusions: HD patients with ongoing pruritus suffer greater from S. aureus than those without pruritus, and S. aureus-secreted α-toxin suppresses FLG expression by AhR/OVOL1/Filaggrin axis in human keratinocytes, in turn weakening the skin barrier function.

EP073 / #151

E-Poster Viewing Topic: AS15 Frontiers in bacterial structural biology

CATALYTIC MECHANISM OF DCSB: ARGINASE FRAMEWORK USED FOR HYDROLYZING ITS INHIBITOR

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Background and Aims: DcsB, an enzyme produced from D-cycloserine biosynthetic gene cluster, displays moderate similarity to arginase in the sequence and three-dimensional structure. Arginase is a ubiquitous enzyme hydrolyzing L-arginine to generate L-ornithine and urea, whereas DcsB hydrolyzes N^{ω}-hydroxy-L-arginine (L-NOHA), an arginase inhibitor, to generate L-ornithine and hydroxyurea. The aim of this study is to clarify the catalytic mechanism of DcsB.

Methods: We determined the crystal structure of DcsB associated with L-ornithine and that with the tetrahedral derivative of 2(S)-amino-6-boronohexanoic acid, whose boron atom forms a covalent bond with an oxygen atom bridging two manganese ions at the active center.

Results: The substrate-binding pocket of DcsB was found to be narrower than that of arginase, suggesting that DcsB is unsuitable for the binding of L-NOHA in an inhibitory manner. The transition state-like structure demonstrated that Asp210 and Glu241 have a role to trap a positively charged ion near the dimanganese cluster. Kinetic analysis using the mutated DcsB showed that the enzyme employs different catalytic mechanisms under the neutral and alkaline pH conditions.

Conclusions: Glu241 in DcsB is likely involved in the recognition of the hydroxyguanidino group of L-NOHA, whereas Asp210, in cooperation with Glu241, seems to contribute to the reactivity toward the protonated L-NOHA, which is a preferable species under the neutral pH conditions. After entering of the protonated L-NOHA to the substrate-binding pocket of DcsB, a hydronium ion may be trapped at the positive ion-binding site. Then, the ion serves as a specific acid catalyst to facilitate the collapse of the tetrahedral intermediate of L-NOHA.

EP074 / #11

E-Poster Viewing Topic: AS15 Frontiers in bacterial structural biology

THE FINE MOLECULAR STRUCTURE OF GLYCOGEN PARTICLES IN ESCHERICHIA COLI

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Background and Aims: Glycogen is a randomly branched glucose polymer and provides energy storage in organisms. Small glycogen β particles in eukaryotes can bind to form composite α particles, which give better glucose release. However, few studies observed glycogen α particles in bacteria. Here we reinvestigated the issue by focusing on the fine molecular structure of Escherichia coli glycogen using four extraction techniques. The objective is to see if glycogen has α particles in E. coli and, if so, whether or not they exhibit any fragility in the H-bond disruptor and protein denaturation agent dimethyl sulfoxide (DMSO).

Methods: Glycogen structures extracted through four different methods were compared, which were TCA-precipitated hot water extraction (TCA-HW), TCA-precipitated cold-water extraction (TCA-CW), hot 30% KOH solution extraction (KOH-HW), and cold-water extraction using sucrose gradient density ultracentrifugation (SGDU-CW). Aqueous SEC was used to examine the molecular density and fragility of glycogen particles, the latter after exposure to DMSO. FACE was used to compare how extraction methods alter glycogen chain-length distributions. TEM was used for morphological characterization.

Results: Based on extraction method comparison and fine molecular structural analysis of glycogen particles, both fragile and stable glycogen α particles were found in Escherichia coli for the first time.

Conclusions: Discovery of co-occurrence of fragile and stable α particles in Escherichia coli opens a novel direction for bacterial glycogen study, such as how glucose concentration influences glycogen structure, how glycogen structure changes in different stages of bacterial life, and what molecular mechanisms are responsible for the formation and fragility of α particles in bacteria, which surely shed new light on bacterial metabolism and physiology.

EP075 / #479

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

CHARACTERIZATION ENDOPHYTIC STREPTOMYCES SP AND ITS EXTRACT AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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Background and Aims: Bioactive compound from endophytic *Streptomyces* sp had been claimed as a source of antibiotics.

Methods: Screening of *Streptomyces* bioactivity was carried out through disc diffusion assay. Characterization of the best isolate was carried out through the morphology observation, 16S rRNA and phylogenetic analysis. Pharmacodynamic characteristic of the extract against MRSA 43300 was determined by time kill assay. Then, the modes of action of the extracts were observed through Biochemical assay and Transmission Electron Microscopy. Cytotoxicity test of the extract against Chang liver cells was also carried out.

Results: Four out of the nine *Streptomyces* sp isolates were showed anti-MRSA activity in which the SUK 25 was the most active isolate. 16S rRNA result showed SUK 25 had 99.9% sequence similarity to *Streptomyces omiyaensis* NBRC 13449^T. Morphology observation showed that both strains had similarities on 4 International *Streptomyces* Project agar but difference on blood agar, starch agar and synthetic agar. The growth temperature and carbon source utilization were different. SUK 25 was isolated from internal layer of plant's root tissues, whereas *Streptomyces omiyaensis* NBRC 13449^T from ground. SUK 25's extracts were bacteriostatic and concentration dependent treatment. A crude extract of SUK 25 caused irregular shapes of cells, cell division, membrane and septal of cells defected, absorbing UV materials released, 'ghost cell' formed against MRSA ATCC 43300. The Extract also was found non-toxic at Chang liver cells with IC₅₀ = 43.32 ± 1.24 µg/ml

Conclusions: SUK 25 had high similarity sequence with *Streptomyces omiyaensis* NBRC 13449^T and could be used as anti-MRSA agent.

EP076 / #708

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

LYSINS AS PROMISING ANTIBACTERIAL AGENTS AGAINST UROPATHOGENIC ESCHERICHIA COLI

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Background and Aims: Uropathogenic *Escherichia coli* (UPEC) is a causative pathogen implicated in vast majority of urinary tract infections (UTIs). Although treatable, the rampant use of antibiotics has led to the emergence of multi-drug resistant (MDR) strains resulting in complications and mortality. Given the prevalence of MDR strains, we are exploring prophage-encoded lysins as potential alternatives to antibiotics. Lysins have an array of interesting properties, making them as putative tools for the treatment of UTIs. Our study involved the identification and *in silico* characterization of distinguished lysin sequences targeting peptidoglycan (PG) in *E. coli* cell wall.

Methods: Distinguished lysin sequences were searched by BLAST homology search and by screening *E. coli* prophages in the database (using PHASTER). The identified lysin sequences were computationally characterized and their domain architecture (using NCBI-CDD, InterProScan) and physicochemical properties (using ProtParam) were examined.

Results: Globular and modular lysin sequences identified had predominantly lysozyme like domain (56%), gluocosaminidase and PG-binding domain composing of three alpha-helices. *In silico* physiochemical analysis predicted eight enzymes to be secretory and also the presence of both positively charged and hydrophobic residues at the C-terminal end and cationic residues in the catalytic domain (known to contribute to the intrinsic bactericidal potential of lysins) was observed. Purification of these lysins as recombinant proteins and optimization of antibacterial assays in the presence of suitable outer membrane permeabilizers is underway.

Conclusions: We believe investigating this bank of distinguished lysins will expand the existing repertoire of lysins against *E. coli* which could potentially lead to the development of potent 'enzybiotics'.

EP077 / #236

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

SUB-MINIMAL INHIBITORY CONCENTRATIONS OF ANTIBIOTICS THAT INHIBIT PEPTIDOGLYCAN SYNTHESIS PROMOTE PLASMID TRANSFORMATION IN ESCHERICHIA COLI

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Background and Aims: Horizontal gene transfer is an important mechanism for bacteria to acquire new properties. We previously reported that DNase-sensitive horizontal transfer of nonconjugative plasmids occurred between two Escherichia coli strains in co-culture, termed "cell-to-cell plasmid transformation (CTC-PT)" [1–3]. Recent studies have reported that sub-minimal inhibitory concentrations (sub-MIC) of antibiotics sometimes influence bacterial cell physiology other than cell growth [4]. We have reported that sub-MIC ampicillin promotes both CTC-PT and transformation with purified plasmid in E. coli [5,6]. In this study, we examined whether other sub-MIC antibiotics that inhibit peptidoglycan synthesis can also promote transformation in E. coli.

Methods: E. coli cells were cultured in solid-air biofilms [1–3] in the absence or presence of sub-MIC antibiotics, and assessed their abilities to perform CTC-PT and transformation with purified plasmid.

Results: We discovered some of the peptidoglycan-synthesis inhibitors at sub-MIC could promote CTC-PT and transformation with purified plasmid, suggesting that weak disturbance of peptidoglycan synthesis permit plasmid DNA traffic through cell surface structures of E. coli.

Conclusions: It was demonstrated that certain sub-MIC peptidoglycan-synthesis inhibitors could facilitate both CTC-PT and transformation with purified plasmid in E. coli. [1] Hasegawa, et al. (2018) Front. Microbiol. 9:2365 [2] Etchuuya, et al. (2011) PLoS One 6 :e16355 [3] Hashimoto, et al. (2019) Biochem. Biophys. Res. Commun. 515:196-200 [4] Andersson & Hughes (2014) Nat. Rev. Microbiol. 12:465 [5] Kasagaki, et al. (2021) World Microbe Forum 2021 [6] Kasagaki, et al. (2022) Curr. Res. Microb. Sci.

EP078 / #746

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

MODELLING THE METABOLIC CONSEQUENCES OF ANTIMICROBIAL EXPOSURE IN ESCHERICHIA COLI

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Background and Aims: Besides genetic mutations, metabolic state of bacterial cells represents another driving factor in the emergence of antibiotic resistance (AR) and in the actual efficacy of antimicrobial treatments. In this direction, studying how bacteria reprogram their metabolism when facing antimicrobial exposure (AE) is crucial to develop new efficient treatments, facilitating the discovery of new resistance mechanisms and thereby enhancing our ability to limit the development and spread of AR. Here, we studied the metabolic consequences of antibiotic exposure in bacteria and whether common metabolic strategies emerge during this exposure, regardless of the kind of antibiotic used or, on the contrary, if antibiotic-specific pathways exist.

Methods: We used an integrated approach that exploits transcriptomics and constraint-based metabolic modeling. To this purpose, we have used a very heterogeneous dataset from six different studies on *Escherichia coli* grown on different media and exposed to different concentrations/types of antimicrobials.

Results: We show that experimental conditions, not AE, is the factor that influences the most the resulting metabolic networks. However, despite condition-dependent metabolic signatures being evident, specific changes in flux distributions by antimicrobial exposed cells could be identified. This suggests the presence of general metabolic strategies to face the stress posed by AE.

Conclusions: Our analysis could predict an overall metabolic rewiring following bacteriostatic vs bactericidal drug exposure, that is in line with the current knowledge about the effects of these two classes of antimicrobials on microbial metabolic phenotypes. These results may represent an untapped resource for the fight against AR.

EP079 / #460

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

PREVALENCE OF QUINOLONE RESISTANCE GENE QNR GENES AMONG CLINICAL ISOLATES OF KLEBSIELLA PNEUMONIAE IN RAS AL KHAIMAH, UNITED ARAB EMIRATES (UAE).

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Background and Aims: *Klebsiella pneumoniae* is a major Gram-negative bacterial pathogen causing a wide variety of infection including pneumonia, urinary trat infection (UTI), meningitis, blood stream infection and wound infections. Resistance of *K. pneumoniae* to different commonly used antibiotics including fluoroquinolines are on the rise globally. Expression of *qnr genes* is one of the three ways the bacteria can exhibit resistance to fluoroquinolone in Enterobacteriaceae.

Methods: A total of 95 recent clinical isolates of *Klebsiella pneumoniae* from different types of infections were screened for resistance to ciprofloxacin by disk diffusion method. Bacterial DNA was isolated from the isolates showing resistance ciprofloxacin and used for PCR detection of *qnrB* and *qnrS* using specific primers. Selected amplicons were analysed by Sanger sequencing.

Results: Out of 95 isolates tested, 47 (49.4%) exhibited resistance to ciprofloxacin in disk diffusion test. Out of these 47 ciprofloxacin resistant isolates, 11 were positive for *qnrS* and 2 isolates were positive for *qnrB*. Sanger sequencing of 2 *qnrS* amplicons revealed perfect match with the published sequence.

Conclusions: The findings indicate prevalence of high level ciprofloxacin resistance and high frequency carriage of *qnrS* among clinical isolates of *Klebsiella pneumoniae* in Ras Al Khaimah. These data stresses the importance of survey of quinolone resistance and molecular analysis of quinolone resistance in *K. pneumoniae* and other members of the family Enterobacteriaceae.

EP080 / #116

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

METAGENOMIC ANALYSIS OF SOIL AND WATER NEAR PIG FARMS SHOW SELECTION FOR SPECIFIC GROUPS AND ANTIBIOTIC RESISTANCE GENES.

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Background and Aims: The world is trying to overcome the ongoing pandemic caused by COVID-19; however, another pandemic, older than the current one, is walking silently: antimicrobial resistance (AMR). There is a wide usage of antibiotics to maintain the health of humans and animals, and as a preventive measure in animal production to promote growth and improve performance indicators. As animals do not metabolize about 70% of the antibiotics administered, many of these substances can pass into animal waste (urine and feces), reaching the environment, favoring the spread of AMR in soil and water. This project aimed to determine the presence of antimicrobial resistance genes in bacteria collected from water, soil, and sediments from effluents in areas close to swine productions in Paraná, Brazil, one of the most prominent swine producers in the country.

Methods: The samples were collected in areas upsteam and downstream surrounding the pig farms and used for microbiological cultivation and metagenome sequencing.

Results: Water samples surrounding pig farms downstream showed the abundance of Pseudomonas genus (61.67%) in the samples, by the metagenomic analysis, and the presence of resistance genes against β -lactam antibiotics (class B and C).

Conclusions: The data suggest the existence of pressure selection from antibiotic use in the whereabouts of industrial pig farming. We must change the model for intensive pig farming to a more sustainable approach to depend less on the suppressive use of antibiotics.

EP081 / #489

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

OCCURRENCE OF ANTIBIOTICS AND ANTIBIOTIC RESISTANCE GENES IN HOSPITAL AND URBAN WASTEWATERS AND THEIR IMPACT ON THE RECEIVING RIVER

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Background and Aims: Antibiotic resistance has become a major health concern; thus, there is a growing interest in exploring the occurrence of antibiotic resistance genes (ARGs) in the environment as well as the factors that contribute to their emergence.

Methods: We investigated, therefore, the pollution level of a broad range of antibiotics and ARGs released from hospital and urban wastewaters, their removal through a wastewater treatment plant (WWTP) and their presence in the receiving river. Several antimicrobial compounds were detected in all water samples collected. Among antibiotic families, fluoroquinolones were detected at the highest concentration, especially in hospital effluent samples.

Results: The results also revealed that copy numbers of ARGs, such as bla_{TEM} (resistance to β lactams), qnrS (reduced susceptibility to fluoroquinolones), ermB (resistance to macrolides), sull(resistance to sulfonamides) and tetW (resistance to tetracyclines), were detected at the highest concentrations in hospital effluent and WWTP influent samples. Although there was a significant reduction in copy numbers of these ARGs in WWTP effluent samples, this reduction was not uniform across analyzed ARGs. Relative concentration of ermB and tetW genes decreased as a result of wastewater treatment, whereas increased in the case of bla_{TEM} , sull and qnrS genes. The incomplete removal of antibiotics and ARGs in WWTP severely affected the receiving river, where both types of emerging pollutants were found at higher concentration.

Conclusions: Taken together, our findings demonstrate a widespread occurrence of antibiotics and ARGs in urban and hospital wastewater to the spread of these emerging pollutants in the aquatic environment.

EP082 / #39

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

ANTAGONISTIC ACTIVITY OF BACTERIOCINS PRODUCED BY LACTOBACILLUS ISOLATES AGAINST MULTIDRUG RESISTANT PATHOGENS

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Background and Aims: Multi drug-resistance pose a great threat to health and are responsible for various life-threatening ailments. There is crucial need to control the outbreaks by finding alternatives to the conventional drugs available. In past, the usage of probiotics, including Lactobacillus spp. and their bacteriocins has gained much attention to ward off various diseases.

Methods: Fifteen Lactobacillus spp. were isolated and identified from Pakistani dairy products (raw milk, cheese, butter milk, pickle and yoghurt). All the isolates were screened by agar well diffusion method, and the bacteriocins were isolated by ammonium sulphate method. For Bacteriocins, the Cell-Free Supernatant Fluid of the best producer strains were tested by agar well diffusion assay. To assess the thermostability of the bacteriocins, they were subjected to temperatures of 40° C, 60° C, 80° C and 100° C.

Results: The study allowed the selection of five bacteriocin producing strains Lactobacillus acidophilus KAL1, Lactobacillus casei KAL3, Lactobacillus plantarum KAL5, Lactobacillus reuteri KAL6 and Lactobacillus delbrukei KAL7, endowed with the strongest and broadest inhibitory ability against both Gram-positive (Methicillin Resistant Staphylococcus aureus) and Gram-negative (Pseudomonas aeruginosa) bacteria. Bacteriocins isolated were significantly thermostable at 80°C (30, 20 min) respectively. Moreover, all the bacteriocins were considerably stable at pH (4–8) but inactive against proteolytic enzyme Proteinase K

Conclusions: It was concluded that bacteriocin extracts from five isolated Lactobacillus can be considered a preferable candidate against MDR pathogens. These partially purified bacteriocins should be further processed to attain purified product that could be useful for further studies for the control of pathogens, food spoilage, etc.

EP083 / #517

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

EPIDEMIOLOGICAL PROFILE AND STATE OF RESISTANCE TO ANTIBACILLARIES IN TUBERCULOSIS IN THE CITY OF MARRAKECH

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Background and Aims: According to the latest WHO data, Tuberculosis (TB) is ranked as the 2nd cause of death due to an infectious agent after COVID19. In Morocco, the evolution of TB cases is on the rise despite the efforts made. In 2020, our country recorded 29018 cases of TB. This epidemiological situation is worsened by the appearance of strains resistant to anti-tuberculosis drugs. The objective of the study: To identify the epidemiological profile and the socio-demographic distribution of TB and to determine the state of resistance in Marrakech.

Methods: This is a retrospective cohort study that took place between January 2019 and December 2020 at the Diagnosis Center for Tuberculosis and Respiratory Diseases in Marrakech. The samples received at the laboratory were analyzed by Gene-Xpert Rif. In case of positivity, the patients started the treatment, In the event that the patients did not improve under treatment, a search for resistance was carried out, using the MTBDRplus/MTBDRsl genotyping methods.

Results: During the study period, 1041 new cases of TB were diagnosed, including 19 HIV+ people, 9 cases of neuromeningeal tuberculosis, and 5 people with resistance to rifampicin. Regarding the state of resistance, we found: 118 cases of resistance to rifampicin, 109 cases of resistance to isoniazid, 95 multi-resistant cases, thus 36 cases of ultra-resistance.

Conclusions: Although the fight against TB is a national health priority, each year the number of resistance increases in a worrying way, which leads us to ask several questions about the factors favoring the spread of resistance to anti-tuberculosis drugs.

EP084 / #605

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

SPORES IN FABRICS: RESISTANCE TO DISINFECTION BY UVC RADIATION AND OZONE

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Background and Aims: The emergence and transmission of the COVID-19 disease in 2019 have led to increased concern about disinfection, including textile clothing. Therefore, the use of disinfection methodologies without resorting to the use of harmful chemicals are under study, such as UV radiation and ozone. Ozone (O₃) has been used for several years as a disinfectant. With an oxidizing power 3,000 times more effective than chlorine, O₃ is the second most powerful oxidant. UVC radiation (200 – 280 nm) has been shown to destroy viruses, bacteria, and fungi. These disinfection tools can be applied in many places, such as nursing homes, hospitals, clinics, clothing stores, hotels, among others. The aim of this study was to investigate the impact of treatments with ozone (30 and 60 ppm) and UVC radiation and their combination at different times (60 and 90 minutes) on the elimination of spores inoculated in different textile substrates.

Methods: These treatments were applied in a prototype of the MTEX PHYS Sterilizer. Spores of *Bacillus atrophaeus* and *Geobacillus stearothermophilus* were used due to their high resistance to different decontamination processes.

Results: The inactivation observed was low, with reductions of only up to 1.4 log cycles, with ozone treatment (60 ppm for 90 minutes).

Conclusions: This demonstrates the resistance of these spores to ozone and UVC treatments and the need for study other combinations and/or treatments to efficiently eliminate them from textile clothing.

EP085 / #607

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY OF KERATINOPHILIC FUNGI FROM SOIL AND BAIT SAMPLES

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Background and Aims: This study was to isolate and characterize keratinophilic fungi from different soil and bait samples in Nsukka metropolis and investigate their antifungal susceptibility.

Methods: The soil samples were collected from 8 areas comprising market areas, school playgrounds, and animal and poultry farms. The defatted bait samples comprise hair from humans and animals, and chicken feather, while pH of each of the soil samples was determined. Isolation by hair bait technique. DNA sequence analysis of the suspected isolates was done using ribosomal ITS1 and ITS4 primers. Thermotolerance of the isolates was examined at different incubation temperatures of 28°C, 37 °C, 40 °C, and 45 °C for 4 days. Susceptibility of the isolates to five antifungal agents was done by disk-diffusion method.

Results: A total of 97 suspected keratinophilic fungi were isolated (46.9% from animal rearing area and 52.9% from different areas). The selected isolates were characterized as *Mucor indicus, Aspergillus terreus, Fusarium oxysporum, Aspergillus tamari, Trichoderma viren and Aspergillus allahabadii.* The pH of the soil samples ranged between 4.95 and 6.92. The thermotolerance of the isolates decreased with temperature. All the isolates were sensitive to itraconazole, while there were variations in resistance against other antifungal agents (clotrimazole 10µg, miconazole 30µg, ketoconazole 30µg and fluconazole 10µg).

Conclusions: This study highlights the abundance and distribution of keratinophilic fungi among different species in soil samples within the study area. It also raises concern on the emergence of antifungal resistant keratinophilic fungi.

EP086 / #70

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

ANTIBIOTIC RESISTANCE OF ENTEROCOCCUS SPECIES IN RETAIL RABBIT MEAT

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Background and Aims: Rabbit meat can be a carrier of enterococci which intrinsically have resistance to many antimicrobial agents and ability to transfer it, which turns out to be a major concern worldwide. The objective of this study was to evaluate the antibiotic resistance of Enterococcus spp. present in retail rabbit meat.

Methods: Enterococcus spp. isolates were recovered from 17 retail rabbit meat samples purchased in Spain and identified by MALDI-TOF/MS. After identification, the isolates were screened for resistance to a panel of 16 antibiotics using disk-diffusion method.

Results: A total of 29 isolates were identified as Enterococcus spp.: E. faecalis (22), E. hirae (5) and E. gilvus (2). Overall, 27 isolates (93.1%) were resistant to at least one of the antibiotics tested and the highest percentage of resistance was observed for tetracycline (26 isolates, 89.65%), followed by norfloxacin (6 isolates, 20.69%), ciprofloxacin (5 isolates, 17.24%), and enrofloxacin (5 isolates, 17.24%). Only one E. faecalis isolate showed resistance to vancomycin, as well to 6 other antibiotics, nevertheless it was susceptible to tetracycline. Finally, 7 isolates of E. faecalis (31.82%) and 1 isolate of E. hirae (20.00%) were resistant to 3 or more antibiotics.

Conclusions: This report suggests that rabbit meat can be a source of resistant Enterococcus spp. and alerts on the cautious use of tetracycline as an antibiotic. This work has received funding from the European Union's H2020 research and innovation program under Marie Sklodowska-Curie grant agreement No 801586, Pre-doctoral UR-CAR fellowship and POCTEFA Project (INTERREG Program) TESTACOS EFA 152/16.

EP087 / #524

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

ANTIMICROBIAL AND ANTIBIOFILM PROPERTIES OF ENGINEERED HISTATIN 8 PEPTIDES AGAINST NON-RESISTANT AND ANTIBIOTIC-RESISTANT PSEUDOMONAS AERUGINOSA

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Background and Aims: The unregulated use of antibiotics triggered the rapid spread of antimicrobial resistance. Nosocomial and gut-related infections are usually associated to antibiotic-resistant (AR) *Pseudomonas aeruginosa*. Histatin 8 (HS8), a promising antibiotic alternative and antimicrobial peptide (AMP), is a suitable candidate for engineering studies to significantly enhance its properties. The study aimed to determine the effect of amino acid (X) substitutions on the antimicrobial, antibiofilm, and hemolytic properties of HS8 against non-resistant (NR) and AR *P. aeruginosa*.

Methods: Various programs were utilized to evaluate the biofunctional propensities of the peptides. The minimum inhibitory concentration (MBC) and minimum bactericidal concentration (MBC) values of the peptides were determined by microdilution broth method. The minimum biofilm inhibitory concentration (MBIC) values were determined by crystal violet assay. Finally, a hemolysis assay was performed to assess the hemolytic potential of the peptides.

Results: Using the test peptides HS8-1 (Y12X), HS8-2 (S8X and Y12X), and HS8-3 (G11X), HS8-1 (MIC=270.83 μ g/mL, p<0.001; MBC=140.63 μ g/mL; p<0.0001) was a more potent antimicrobial peptide against NR *P. aeruginosa* compared to HS8-2 and HS8-3, displaying 3.0-fold and 3.5-fold decrease in MIC and MBC, respectively, compared to HS8. Furthermore, HS8-1 (MIC=450.78 μ g/mL, p<0.001) and HS8-2 (MBC=380.89 μ g/mL) displayed significant antimicrobial values, respectively, against the AR *P. aeruginosa*. HS8-2 (MBIC=15.63 μ g/mL) and HS8-1 (MBIC=250 μ g/mL) were potent antibiofilm peptides against NR and AR *P. aeruginosa*, respectively. The hemolytic activities of the peptides were then assessed.

Conclusions: Results suggested that the observed property improvements were achieved by performing specific amino acid substitution(s) in the peptide at specified positions.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

STUDY OF ANTIMICROBIAL EFFICACY OF NOVEL BIOMATERIALS AGAINST CLINICAL MULTIDRUG RESISTANT STRAINS OF WOUND INFECTIONS PATHOGENS

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Background and Aims: The global surge of antibiotic resistance of bacteria is a major concern for public health and proving to be a key challenge in modern wound care and prevention of infectious complications. However, the development of biomaterials with antimicrobial properties is the most relevant. The aim was to study the effect of calcium alginate based antimicrobial biomaterials, as a polymer system of local prolonged delivery of quaternary ammonium compounds, on reference and clinical multidrug resistant strains of microorganisms.

Methods: The susceptibility of reference and clinical multidrug resistant strains of Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Enterobacter cloacae, Citrobacter freundii, Stenotrophomonas maltophilia to samples of antimicrobial biomaterials (6 mm in diameter) containing decamethoxin (0.03-0.07 wt%) and polymers (polyvinyl alcohol and calcium alginate) was determined by the disk-diffusion method. Zones of inhibition (ZOI) were registered.

Results: The high antimicrobial properties of the studied samples of antimicrobial biomaterials have been established. The most susceptible strains were S. aureus (ZOI were in the range of 12.1–17.6 mm), A. baumannii (ZOI: from 9.2 mm to 20.8 mm), S. maltophilia (ZOI: from 10.1 mm to 14.9 mm), E. cloacae (ZOI: from 10.8 mm to 15.6 mm), C. freundii (ZOI: from 10.4 mm to 16.03 mm).

Conclusions: Novel biomaterials based on the quaternary ammonium compound decamethoxin and natural polymers, which inhibit the growth of multidrug-resistant microorganisms, may be a promising effective topical antimicrobial agent for wound healing.

EP089 / #386

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

A PROMISING RESULT OF PHAGE THERAPY IN THE TREATMENT OF CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE INFECTION

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Background and Aims: *Klebsiella pneumoniae* is a well-known opportunistic human pathogen and is often multi-drug resistant. Thus, because of the difficulty to treat this superbug, an alternative approach is prudent. Among many options, phage-mediated control and/or treatment is considered one of the promising alternatives. This study aimed to isolate, characterize and evaluate the therapeutic efficacy of phage in the treatment of carbapenem-resistant *K. pneumoniae* infection.

Methods: A novel lytic bacteriophage (phage) Kp_Pokalde_002 was isolated and characterized against carbapenem-resistant *K. pneumoniae* (Kp56), which was isolated from the patient's urine sample. A group of mice were infected with a lethal dose ($\sim 1.10^7$ CFU/mouse) of the bacteria and treated with the phage (Kp_Pokalde_002) via both oral and intraperitoneal (IP) routes.

Results: The phage Kp_Pokalde_002 successfully rescued mice infected with a lethal dose of carbapenem-resistant *K. pneumoniae* (Kp56) without any deleterious side effects. All of the infected mice were survived with the phage treatment through the IP route, while the survivability was decreased to 40% via the oral route. Phage therapy significantly reduced the blood bacterial load (5-7 log₁₀ CFU/mL), decreased the mRNA expression levels of pro-inflammatory cytokine markers (TNF- α and IL-6), and lesser extent of lung inflammation as compared to the control group.

Conclusions: The result provides evidence of successful phage therapy against carbapenemresistant *K. pneumoniae* infected mouse model using locally isolated phage. The phage Kp_Pokalde_002 can be considered an excellent candidate for future phage therapy.

EP090 / #52

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

SYNTHETIC AND NATURAL ANTIMICROBIALS AS A CONTROL AGAINST FOOD BORNE PATHOGENS

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Background and Aims: Food borne pathogens are one of the most common yet concerning cause of illnesses around the globe. These microbes invade the body via food items through numerous mediums of contamination. It is impossible to completely eradicate these organisms from food. Extensive research has been made regarding their treatment. Unfortunately, the only available treatment currently is by antibiotics. Recent exponential increase in antibiotic resistance and the side effect of synthetic compounds have established a need for alternate therapies that could be utilized either on their own or along with antibiotics to provide protection against food-borne diseases. The aim of this study is to provide information regarding common food borne diseases, their current and possible natural treatment. In the study, species of Listeria and Campylobacter have been isolated and identified. Then antibiotics and bacteriocins isolated from the Enterococcus species have been tested against Listeria. It has been found that the Bacteriocins serve as better antimicrobials against the commonly found food pathogens.

Methods: Cultures of Listeria and Campylobacter were isolated and identified. Antibiotics Susceptibillity testing was done by Kirby Baeur Method. Bacteriocin isolated from the Enterococcus species were tested for antimicrobial activity against the Listeria and Campylobacter species.

Results: Bacteriocins gave greater zone of inhibition against the Listeria and Campylobacter species as compared to the antibiotics used.

Conclusions: Natural Antimicrobials can be used as better options for treatment against food borne pathogens as compared to the synthetic antimicrobials.

EP091 / #690

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

MODE OF ACTION STUDY OF A POTENT ANTIMALARIAL COMPOUND, DIHYDROLUCILACTAENE

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Background and Aims: Malaria is a mosquito-borne tropical disease responsible for more than 200 million clinical cases, with a half-million death annually. The recent emergence of drug-resistance strains against the current frontline antimalarial drug, artemisinin, further complicates the problem. Hence, there is an urgent need to develop antimalarial compounds with new chemical structures and mechanisms of action. As we have found a potent antimalarial agent, dihydrolucilactaene (DHLC), we will report the mode of action of the compound.

Methods: DHLC, a new lucilactaene derivative, was isolated from *Fusarium* sp. RK97-94. The antimalarial activity was evaluated against the wildtype (3D7) and chloroquine (CQ) resistant (K1) *Plasmodium falciparum* strains. To understand the mode of action of DHLC, the erythrocytic stage-specific activity profile was investigated based on a time-specific drug exposure/washout assay against a sorbitol-synchronized *P. falciparum* 3D7 culture.

Results: DHLC inhibited the growth of *P. falciparum* 3D7 (wild type) and K1 (resistant) strains at similar IC₅₀ values (ca. 1.5 nM). Microscopic observation showed that DHLC and CQ caused distinctive morphological changes in the parasites. DHLC reduced the egression rate of the parasites from the host cell, while CQ induced shrunken morphology. DHLC mainly targets trophozoites of *P. falciparum*, followed by schizont and ring stage. It has a different stage-specific inhibitory profile than both CQ and artemisinin, implying a distinct mode of action.

Conclusions: DHLC is an exciting hit compound that may provide a new drug regimen to combat the widespread of drug-resistant strain. <Acknowledgement> We thank Drs Islam A. Abdelhakim, Takayuki Motoyama, and Shunji Takahashi (RIKEN) for providing DHLC.

EP092 / #409

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

EXCHANGE OF PANDEMIC LINEAGES AND GENETIC PLATFORMS OF BLAKPC-2 IN AN INPATIENT

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Background and Aims: Culture-based methods for detecting carbapenem-

resistant *Enterobacterales* (CRE) are routinely used for infection control practice. Since prospective studies investigating the clonal relationships between colonization and infection by KPC carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) strains are unfrequent, our aim was to perform whole genome sequencing (WGS) of three CRE strains isolated from an inpatient in order to identify genetic features.

Methods: The colonizing strain was isolated on the first day of hospitalization from a surveillance rectal swab (HAp3Kpn, a KPC-Kp strain), and the other two strains were isolated at 23 (HA4Ec, a KPC-*E.coli* strain), and 70 (HA15Kpn, another KPC-Kp strain) days after hospitalization, were from infections. All 3 strains showed resistance to imipenem, meropenem, ceftazidime, cefotaxime, and chloramphenicol. WGS was performed with Illumina MiSeq-I and de novo assembly with SPAdes v.3.11.Genomic analyses, gene prediction and annotation, multilocus sequence typing (MLST), resistome, plasmids, ISs, and integrons were investigated.

Results: HAp3Kpn belonged to ST258, HA4Ec to ST730 (Pasteur), ST224 (Achtman), and HA15Kpn to ST11. This last lineage was identified in several KPC-Kp strains from our hospital. HAp3Kpn had 20 transferable antimicrobial resistance genes (ARG), HA4Ec had 2 and HA15Kpn had 5, being only *bla*_{KPC-2}, located on different genetic platforms, the ARG shared by these strains.

Conclusions: The change of KPC-Kp lineages was evidenced in our study during hospitalization, as well as the transmission of the same genetic platform between two species. These findings showed the capacity of transmission of ST11, suggesting that molecular surveillance of ST11 KPC-Kp should be performed in our region to prevent further spread.

EP093 / #287

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

INDEPENDENT EMERGENCE OF MULTIPLE MULTIDRUG-RESISTANT SERRATIA MARCESCENS CLONES IN HOSPITALS FROM ARGENTINA

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Background and Aims: The emergence of multidrug-resistant (MDR) bacteria is an increasing danger to public health. The raise of *S. marcescens* after administration of colistin to patients infected with Gram-Negative Bacilli harbouring carbapenemases is common in some hospitals from Argentina. The aim of this study was to perform an in-depth analysis of both population structure and antimicrobial resistance genes (ARG) of *S. marcescens* from Argentina, which could contribute to design effective means to control its dissemination.

Methods: 152 MDR *S. marcescens* isolates associated with infections recovered from 14 hospitals across Argentina between 1997 and 2018 were sequenced. Sequences were assembled with SPAdes 3.9.0, annotated with Prodigal, RefSeq database and curated with BLAST. Plasmids were identified with PlasmidFinder and ARG with RESfinder, CARD and Blastn. A phylogenetic tree was constructed with RAxML.

Results: showed a high level of genomic diversity, with a great variety of novel genetic findings including not yet reported conjugative IncM1a-v1 and IncC plasmids harboring *bla*_{KPC-2} and *mcr-9*, and a new chromosomally located genomic island harboring *bla*_{CTX-M-2}. Twelve acquired β -lactamase families were found including novel alleles of the carbapenemase *bla*_{SPR-1}. Also, polyclonal dissemination of most relevant ARG was found.

Conclusions: *S. marcescens* possesses intrinsic MDR phenotype and can persist on inanimate surfaces of the hospital. The expansion of MDR *S. marcescens* that can disseminate a pool of novel plasmids and ARG suggests that this species should be monitored to prevent a greater burden of hospital acquired antibiotic resistance.

EP094 / #290

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

"THE TROJAN HORSE: SERRATIA MARCESCENS". IMPORTANCE IN THE DISSEMINATION, UPTAKE AND MAINTENANCE OF GENES ASSOCIATED WITH ANTIMICROBIAL RESISTANCE IN THE HOSPITAL ENVIRONMENT

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Background and Aims: Antimicrobial resistance (AMR) is a global public health problem. Carbapenemase New-Delhi (NDM-1) causes therapeutic failures with currently available antibiotics. In addition, *Serratia marcescens* is naturally resistant to several antibiotics, including colistin. Our aim was to analyze the genetic platforms involved in the dissemination of *bla*_{NDM-1} in a *S. marcescens* multidrug-resistant (MDR) strain.

Methods: *S. marcescens* SM938 was isolated in 2018 and sequenced by Miseq. The contigs were assembled with SPAdes 3.9.0. The genome was annotated with Prodigal, RefSeq database and Blast. Insertion sequences were searched by ISfinder. Antimicrobial resistance genes (ARG) were identified using RESfinder, CARD and Blastn. The resistance phenotype was assessed by disk diffusion and MIC. Plasmid conjugation and maintenance assays were performed with both *E. coli* J53 and *S. marcescens* SCH909 as recipient strains.

Results: Genetic platforms related to the dissemination of ARG were found including the IncC-type pDCASG6-NDM plasmid (137.269 pb, G+C: 52%), which contains a genomic resistance island (17.833 pb) with *bla*_{NDM-1}. Maintenance studies showed that only in the transconjugants obtained with *S. marcescens* SCH909, pDCASG6-NDM was maintained over ten days without antimicrobial pressure. The transconjugants obtained with *E. coli* J53, showed that the 80% of pDCASG6-NDM was lost at the day 10.

Conclusions: These findings, and the fact that *S. marcescens* is considered a relevant nosocomial pathogen that can have a wide range of niches –human, plant, animal, soil, and inanimate surfaces, showed the ability of this species to capture, maintain and spread a broad variety of AMR platforms and ARG, including *bla*_{NDM-1}.

EP095 / #775

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

CARBONYL-CYANIDE-3-CHLOROPHENYLHYDRAZONE EFFLUX PUMP INHIBITOR TO RESCUE COLISTIN SUSCEPTIBILITY

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Background and Aims: Global trend is showing an increasing rate of infections caused by multidrugresistant Gram-negative bacteria. This precarious situation makes colistin as the last bullet in the antimicrobial armamentarium. Apart from plasmid-mediated *mcr* genes, mutations involving *mgrB*, *phoP/phoQ*, *pmrA/pmrB* and *arnBCADTEF* genes, are leading causes of colistin resistance. Efflux pumps such as *KpnEF* and *AcrAB-TolC* complex also contributes to antibiotic resistance including colistin. The effect of Carbonyl-Cyanide-3-chlorophenylhydrazone (CCCP) on the reversal of colistin resistance was studied in extensively drug-resistant (XDR) *Acinetobacter baumannii* isolates.

Methods: A total of 127 *Acinetobacter baumannii* isolates were tested for colistin susceptibility by broth microdilution (BMD). The presence of the *mcr1* gene was determined by PCR. The effect of CCCP on colistin MIC was analysed in XDR *Acinetobacter baumannii* isolates at a concentration of 10 µg/mL.

Results: Out of 127 Acinetobacter baumannii isolates, 89.8% (114/127) were multidrug-resistant strains followed by 10.2% (13/127) as XDR isolates. Of the 13 XDR Acinetobacter baumannii strains tested, 8 Acinetobacter baumannii isolates were colistin resistant and rest 5 were colistin susceptible strains by BMD. MIC change of \geq 8-fold was observed after addition of CCCP in 8 XDR colistin resistant Acinetobacter baumannii strains, confirming the presence of efflux pump. The mean fold change in MIC was seen highest in *mcr-1* positive colistin resistant strains.

Conclusions: Efflux pumps inhibitors (EPI) like CCCP reversed colistin resistance in both *mcr-1* positive and *mcr-1* negative *XDR Acinetobacter baumannii* strains. EPIs could be an alternative for restoring colistin activity in MDR and XDR GNBs specially XDR *Acinetobacter baumanii*.

EP096 / #454

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

INFLUENCE OF FOOD MICROBES ON HORIZONTAL TRANSFER OF B-LACTAM RESISTANCE GENES BETWEEN SALMONELLA STRAINS IN THE MOUSE GUT

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Background and Aims: Consumption of food contaminated by antibiotic-resistant (AR) bacteria may lead to transmission of AR genes in the gut microbiota and cause AR bacterial infection, a significant public health concern. However, information is limited on if and how background microbes from the food matrix (food microbes) may influence resistance transmission. Thus, we assessed the colonization of a β -lactam resistant *Salmonella* Heidelberg strain (donor) and a β -lactam susceptible *S*. Typhimurium strain (recipient) and the transfer of the resistance genes in the mouse gut in the presence or absence of food microbes that were derived from washing freshly-harvested carrots.

Methods: Mice were pre-treated with streptomycin and then inoculated with both donor and recipient bacteria in the presence or absence of food microbes. Fecal shedding of the donor, recipient and transconjugant bacteria were enumerated using selective culture techniques. Transfer of AR genes was confirmed by whole genome sequencing. Gut microbial composition was determined by 16s rRNA amplicon sequencing.

Results: Significantly lower numbers of donor and recipient were shed from mice that were inoculated with food microbes compared to those without food microbe inoculation. *S.* Typhimurium transconjugants were only recovered from mice without inoculation of food microbes.

Conclusions: The results suggest that the food microbes may compete with both the donor and recipient *Salmonella*, limit their growth and reduce transmission of the β -lactam resistance gene in the mouse gut.

EP097 / #748

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

ACRAB EFFLUX PUMP CONFERS INTRINSIC MULTIDRUG-RESISTANCE AND SELF-PROTECTION IN PHOTORHABDUS LAUMONDII

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Background and Aims: *Photorhabdus laumondii* TT01 is an excellent model to study host-microbe interactions due to its dual lifestyle as an insect pathogen and mutualist of *Heterorhabditidae* nematodes. During insect infection, *Photorhabdus* synthesize diverse antimicrobial molecules including the polyketides stilbenes and anthraquinone pigments that are gradually accumulated in the cadaver. Resistance-nodulation-division (RND)-type efflux pumps comprising the prototype AcrAB-ToIC are major contributors to multidrug-resistance (MDR) in Gram-negative bacteria. Here, we uncovered the roles of the three and only RND pumps MdtABC, AcrAB and AcrAB-like identified in *P. laumondii* TT01.

Methods: Genetic and biochemical analyses were performed to study the phenotypic traits of RNDmutated strains.

Results: We showed that AcrAB is the major RND efflux pump that confers bacterial MDR since only $\Delta acrA$ and $\Delta mdtA\Delta acrA$ mutants were multidrug sensitive. Moreover, AcrAB was expressed *in vitro*, *in vivo* and post-mortem within insect cadavers, suggesting that AcrAB may be involved in bacterial resistance against different environmental stresses prevailing in these microhabitats. Interestingly, we observed that the wild-type (WT) strain exhibits an antimicrobial activity against $\Delta acrA$. $\Delta acrA$ was more sensitive than the WT to two synthetic stilbene derivative and that less native stilbenes were detected in its supernatant. Furthermore, $\Delta acrA$ developed defectively pigmented colonies while the WT showed yellow pigmented colonies which we correlated with a significant reduction of anthraquinone derivatives in $\Delta acrA$ supernatants compared to those of the WT.

Conclusions: Taken together, these results suggest that AcrAB confers self-resistance against stilbenes and contributes to anthraquinone-mediated pigmentation in *P. laumondii* TT01. LH was granted a doctoral fellowship from CNRS-Lebanon/University of Montpellier.
EP098 / #301

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

EMERGENCE OF MULTIDRUG RESISTANCE HELICOBACTER PYLORI STRAINS ISOLATED FROM PATIENTS WITH HISTORY OF FAILURE ERADICATION THERAPY: A FIVE-YEAR SURVEILLANCE STUDY IN KLANG VALLEY, MALAYSIA

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Background and Aims: The emergence of multidrug-resistant *Helicobacter pylori* has complicated eradication strategy to prevent development of gastric cancer. This study was conducted to determine the prevalence of secondary antibiotic resistance of *H. pylori* at urban multicultural area in Malaysia.

Methods: From January 2017 to December 2021, gastric biopsies from 222 (mean age= 43.86 ± 12.69 years) patients in Klang Valley with history of *H. pylori* eradication failure were sent to our laboratory for antibiotic susceptibility testing. Minimal inhibitory concentration was determined in six antibiotics, namely metronidazole, clarithromycin, levofloxacin, amoxicillin, tetracycline, and rifampicin using E-test.

Results: Majority of the samples were obtained from Malaysian Chinese (42.3%) while the lowest number of samples were obtained from Malays (16.2%). *H. pylori* were successfully isolated from 51 patients (23%). Overall, antibiotic resistance rates of *H. pylori* to metronidazole, clarithromycin, levofloxacin, and amoxicillin were 82.4% (42/51), 72.5% (37/51), 52.9% (27/51) and 3.9% (2/51), respectively. Resistance to tetracycline and rifampicin were not observed during the study period. Resistance to more than one antibiotic was observed in 82.35% (42/51) of *H. pylori* isolates, of which 45.23% (19/42) were resistant to three antibiotics classes. Resistance to both clarithromycin and metronidazole were most frequently observed in isolates with dual resistance (13/23; 56.5%).

Conclusions: Our results reveal the emergence of multidrug resistance in secondary isolates of *H. pylori* and for the first time detection of amoxicillin resistance in secondary isolates of *H. pylori* isolated from Malaysian patients. Continuous surveillance of antibiotic resistance in *H. pylori* is pertinent for antibiotic better eradication strategy for Malaysian patients.

EP099 / #379

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

FIRST REPORT OF THE DETECTION OF THE MOBILE COLISTIN RESISTANCE GENE (MCR-9.1) IN MORGANELLA MORGANII ISOLATED FROM SEWAGE

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Background and Aims: The emergence and global dissemination of the plasmid-borne mobile colistin resistance genes (*mcr*) have substantially impacted the efficacy of colistin, a last-resort antibiotic. Evidence suggests that *mcr* is being introduced to the USA via different routes. However, in-depth investigations are required to assess *mcr* emergence in a variety of overlooked matrices in the USA. Here, we investigated the occurrence of *mcr* in raw sewage samples collected from an urban wastewater treatment plant (WWTP).

Methods: Sewage samples (1-liter) were collected from a WWTP. An aliquot (100 μ l) was spread onto the selective RAPID'*E.coli* 2 agar supplemented with 4 μ g/ml colistin. Putative colonies were purified and tested for resistance to colistin and other antibiotics using the broth microdilution and disc diffusion assays, respectively. Selected isolates were then subjected to Whole-genome sequencing (WGS) analysis. Transformation (heat-shock assay) and biofilm assays were performed to assess if the detected *mcr* was plasmid-borne and could persist in biofilms, respectively.

Results: *mcr-9.1* was detected in an isolate that was identified as *Morganella morganii*. The isolate was highly resistant to colistin (MIC> 640 µg/ml) and was classified as multi-drug resistant. WGS analysis showed that the isolate carried an additional 19 acquired antibiotic resistance genes. *mcr-9.1* was localized on an IncF plasmid, which was successfully transferred and conveyed colistin-resistance to naive *E. coli* JM109. Furthermore, *mcr-9.1* persisted in the 6-day old *M. morganii* biofilms

Conclusions: The detection of *mcr* in intrinsically colistin-resistant bacteria suggests that *mcr* dissemination might have been overlooked in the USA, highlighting an urgent need for robust investigations.

EP100 / #59

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

SUB-MINIMAL INHIBITORY CONCENTRATIONS OF ANTIBIOTICS THAT INHIBIT PROTEIN-SYNTHESIS PROMOTE PLASMID TRANSFORMATION IN ESCHERICHIA COLI

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Background and Aims: Horizontal gene transfer is a strong tool of bacteria to evolve for their better survival. We previously reported that DNase-sensitive horizontal transfer of non-conjugative plasmids occurred between two Escherichia coli strains in co-culture, termed "cell-to-cell plasmid transformation (CTC-PT)" [1-3]. Recent studies have reported that the antibiotics sometimes function as modulators of bacterial cell physiology, particularly at their sub-minimal inhibitory concentration (sub-MIC) [4]. We have recently revealed that sub-MIC ampicillin facilitate CTC-PT [5,6]. In this study, we investigated the effects of sub-MIC protein-synthesis inhibitors on CTC-PT in E. coli.

Methods: CTC-PT experiments were performed in the absence or presence of sub-MIC protein-synthesis inhibitors in air-solid biofilms, where E. coli strains, comprising one strain and two kinds of compatible plasmids, were co-cultured. Transformation experiments were also performed using purified plasmid DNA and biofilm cells consisted of a plasmid-free strain.

Results: We found that certain protein synthesis inhibitors promoted CTC-PT at sub-MIC. A similar promoting effect was also observed with purified plasmid DNA instead of plasmid-harboring donor cells, confirming a transformation mechanism.

Conclusions: This is the first demonstration of the promoting effect of certain sub-MIC protein-synthesis inhibitors on horizontal plasmid transfer between E. coli cells by transformation mechanism. [1] Hasegawa, et al. (2018) Front. Microbiol. 9:2365 [2] Etchuuya, et al. (2011) PLoS One 6 :e16355 [3] Hashimoto, et al. (2019) Biochem. Biophys. Res. Commun. 515:196-200[4] Andersson & Hughes (2014) Nat. Rev. Microbiol. 12:465 [5] Kasagaki, et al. (2021) World Microbe Forum 2021 [6] Kasagaki, et al. (2022) Curr. Res. Microb. Sci.

EP101 / #512

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

UNDERSTANDING THE THERAPEUTIC POTENTIAL OF HYDROGEN SULFIDE FOR TREATING MICROBIAL SKIN INFECTIONS

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Background and Aims: Skin infections are very common, e.g. dermatophytosis which is caused by fungi, affecting 20-25% of the world population (Havlickova et al., 2018). The administration of antimicrobials is challenging due to the properties of skin and nails as protective tissues of the body. Topical treatments can be lengthy and often fail due to poor penetration of antimicrobials. However, we hypothesise that hydrogen sulfide (H₂S), which is small and has antimicrobial activity, provides an alternative treatment strategy as this may penetrate much better into skin or nails as compared to conventional antimicrobial agents. This study is to understand the effectiveness and mechanism of actions of H₂S to pathogens that cause skin and nail infections, including fungal dermatophytes and *S. aureus*.

Methods: To study H₂S' activities on different species, fungicidal, serial dilution spot, DCHF-DA fluorescence assays were used.

Results: H₂S generated using the H₂S donor NaHS was shown to be fungicidal against *Trichophyton rubrum* and *interdigitale*. H₂S was fungistatic against other fungi that cause nail infections, including *Neoscytalidium dimidiatum*, *Fusarium oxysporum*, and *Candida albicans*. Early confocal studies showed that H₂S kills dermatophytes in a time- and fungal life cycle-dependent manner. It also has a strong bactericidal activity against *S. aureus*, even at high bacterial density levels. The mechanism of action is not clear yet, but one hypothesis is that H₂S leads to the production of reactive oxygen species and its effectiveness would be affected by the environmental pH.

Conclusions: The study demonstrated the antimicrobial activity of H₂S produced by NaHS to several dermatophytes and MRSA.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

YEASTICIDAL EFFECTS OF CHEMICAL DISINFECTANTS ON CLINICALLY IMPORTANT CANDIDA SPECIES.

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Background and Aims: Infections by *Candida species* are increasingly recognized as a cause of nosocomial infections. In particular, *Candida species* were reported to demonstrate persistence on plastic and steel. This poses unique challenges in choosing the appropriate disinfection protocol. This study aimed to assess the yeasticidal effects of chemical disinfectants against different *Candida* species, under both clean and soiled conditions, and on different surface materials.

Methods: Type strains and clinical strains of *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida auris* were used. Effects of six disinfectants were studied by the quantitative suspension tests and on non-porous surfaces (EN13624 & EN17387). These were sodium hypochlorite (at 1000ppm chlorine), chloroxylenol, benzalkonium chloride, prophylene glycol, potassium peroxymonosulfate, and didecyldimethylammonium chloride (DDAC). Three surface materials were tested: stainless steel (304), acrylate, and silicone. Clean and dirty conditions were simulated.

Results: In the quantitative suspension tests and in both clean and dirty conditions, all disinfectants except benzalkonium chloride, prophylene glycol and DDAC achieved 4 log reductions at 5 minute exposure time. When organic soiling was applied on stainless steel (304), acrylate and silicone, only sodium hypochlorite achieved > 4 log reductions at 5 minute exposure time. No significant difference was observed among the *Candida* species in both suspension and surface tests.

Conclusions: The yeasticidal effects of prophylene glycol and quaternary ammonium compounds were inadequate in both suspension and surface tests. On soiled surfaces, only sodium hypochlorite demonstrated consistent disinfectant effects. The soiled surface tests is probably more clinically relevant, as healthcare environment are often frequently re-contaminated.

EP103 / #322

E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

THE CYCLIC PEPTIDE RUFOMYCIN ATTENUATES EXCESSIVE INFLAMMATION AND INHIBITS P38 MAPK SIGNALING IN MACROPHAGES DURING INFECTION WITH MYCOBACTERIUM ABSCESSUS

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Background and Aims: The rufomycins 4/5/6/7 (Rufomycin), which targets ClpC1 as a subunit of caseinolytic protein complex ClpC1/ClpP1/ClpP2 of mycobacteria, showed antimicrobial responses against nontuberculous mycobacterial infection. In this study, we examined whether Rufomycin regulates the inflammatory responses during *Mycobacterium abscessus* (Mabc) infection.

Methods: To explore whether Rufomycin ameliorates inflammatory responses and signaling, relative expression of cytokines and chemokines were examined in the Mabc-infected bone marrow-derived macrophages (BMDMs).

Results: Rufomycin treatment significantly decreased the Mabc-induced gene expression of various proinflammatory cytokines and chemokines in BMDMs. However, the mRNA expression levels of Mabc-induced *Cxcl2* and *II12p40* were significantly increased in BMDMs, suggesting that Rufomycin may increase the induction of Th1 and Th17 responses. In addition, we found that the phospho-p38-mitogen-activated protein kinase was significantly downregulated with Rufomycin treatment. However, Rufomycin did not modulate the activation of other signaling pathways such as phospho-NF-KB, phospho-extracellular signal-regulated kinase 1/2, phospho-c-Jun N-terminal kinase or phospho-Akt/protein kinase B in BMDMs during Mabs infection.

Conclusions: These results suggest that Rufomycin selectively inhibits the Mabs-induced inflammatory cytokine generation and the inflammatory p-p38-MAPK signaling, which may principally contribute to inflammatory responses, in BMDMs

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DISCREPANCIES IN THE DETECTION OF METHICILLIN-RESISTANT COAGULASE NEGATIVE STAPHYLOCOCCI

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Background and Aims: The most important mechanism of antibiotic resistance generated by *Staphylococcus* spp. is methicillin resistance (MR). MR determination in coagulase-negative staphylococci (CNS) is routinely based on the disc diffusion method with cefoxitin. The aim of the study was to assess the efficacy of cefoxitin disc diffusion test in the detection of methicillin-resistant CNS and compare it with other methods.

Methods: A total of 103 CNS strains were isolated from the oral cavity, identified by the MALDI-TOF. Methicillin resistance of CNS (MRCNS) was preliminarily tested by the disc diffusion method with cefoxitin (30 μ g) and oxacillin (1 μ g), and further was verified with the detection of PBP2a protein and *mecA* and *mecC* genes, and agar dilution methods.

Results: Among 103 CNS, 16 (15.5%) MRCNS were detected based on the presence of the PBP2a protein and the *mecA* gene. The detected MRCNS belonged to *S. haemolyticus* (5/16), *S. saprophyticus* (5/16), *S. epidermidis* (2/16), *S. equorum* (1/16), *S. hominis* (1/16), *S. pasteuri* (1/16) and *S. warneri* (1/16). Among 11/ 16 (68.8%) MRCNS discrepancies appeared between the phenotypic disc diffusion method with cefoxitin and other methods. Cefoxitin-sensitive strains were resistant to oxacillin confirmed by high MIC values in the oxacillin dilution method and the presence of the PBP2a protein and the *mecA* gene.

Conclusions: The results showed that the detection of MRCNS strains using the recommended disc diffusion method with cefoxitin ($30 \ \mu g$) may not always give reliable results, which may lead to incorrect treatment and failure in the therapy of these infections.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

NOVEL SEQUENCING TYPE OF AN ENTEROBACTER CLOACAE COMPLEX STRAIN WITH THE POTENTIAL TO BECOME A HIGH-RISK CLONE

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Background and Aims: Carbapenem resistant *Enterobacterales* (CRE) belong to the highest priority group of the WHO. In particular, *Enterobacter cloacae* complex (ECC) has lately awoken interest due to their increasing resistance to carbapenems codified by several genes all over the globe. Outbreaks of ECC are particularly dangerous for children and newborns, especially in low- and middle-income countries. Even though there are some sequence types (ST) which represent high-risk clones, there is a substantial clonal diversity in the ECC.

Methods: Here, we analyzed the genome of a CRE multidrug resistant (MDR) ECC strain isolated from urine from a patient in a hospital in Argentina. The whole-genome sequencing was done by Illumina MiSeq-I. The genome was assembled with SPAdes 3.9.0, and annotated with PROKKA, RAST and Blast. Plasmids were identified with PlasmidFinder. Antibiotic resistance genes were detected using RESfinder, CARD and Blastn. ST were identified with pubMLST.

Results: No ST could be assigned because the *pyrG* gene could not be classified as any known allele. All other alleles of the genes used for the multilocus sequence typing (MLST) of ECC were the same as for ST66, which is a high-risk clone. We found multiple metal and antibiotic resistance genes including the carbapenem resistance gene bla_{KPC-2} , several plasmids and secretion system VI, which can favor the prevalence of ECC strains while competing with other bacteria.

Conclusions: Due to its MLST almost identical to ST66, the acquired multiple resistance and the presence of the secretion systems, the potential of this strain for becoming a new high-risk clone cannot be discarded.

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IMPACT OF GLYPHOSATE HERBICIDES ON PREVALENCE OF ANTIMICROBIAL RESISTANCE IN SOILS FROM ARGENTINA

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Background and Aims: To unveil the mechanisms that affect antimicrobial resistance (AMR) in the environment is crucial because environmental bacterial communities are the source of antibiotic resistance genes subsequently detected in hospitals. Glyphosate inhibits the shikimate pathway, which is necessary for the synthesis of aromatic amino acids in plants but also in bacteria. Glyphosate could exert a selective pressure on bacteria, favouring tolerant/resistant strains in the environment. Because one microorganism can carry several AMR genes (ARG), our working hypothesis is that the intensive use of glyphosate herbicides is a driving factor for the development and spread of AMR in the environment. Our aim was to analyse glyphosate and AMR strains in environmental samples and compare them with clinical isolates.

Methods: We isolated bacterial strains from one site at the Paraná river (Argentina) in an area with high concentrations of glyphosate and tested them for their tolerance towards glyphosate, a glyphosate-containing herbicide, and antibiotics. Isolates were identified by sequencing of their 16S rRNA gene. Same procedure was carried out with clinical isolates.

Results: Bacteria showed concentration-dependent and differential susceptibility to glyphosate and its commercial formulation. The isolates that were tolerant to the highest glyphosate concentrations were also resistant to several classes of antimicrobials. *Enterobacter cloacae* Complex and *Serratia marcescens* which are critical in the local hospital environment tolerated the highest concentration of herbicide.

Conclusions: Understanding the mechanisms of the interrelationship between herbicide resistance and AMR, as well as the epidemiology of AMR related to herbicide use, is fundamental to develop guidelines for the rational use of herbicides.

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NOVEL INCM1 PLASMID HARBOURING BLAKPC-2 DISSEMINATING IN KLEBSIELLA PNEUMONIAE AND ENTEROBACTER HORMAECHEI WITHIN A PATIENT

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Background and Aims: According to the World Health Organization, carbapenem resistant *Enterobacterales* (CRE) bacteria belong to the highest priority group. WHONET data from Argentina showed that Gram-negative resistance levels to imipenem have been increasing since 2010 mostly in two CRE species: *Klebsiella pneumoniae* and *Enterobacter cloacae* Complex. Our aim was to analyze the *bla_{KPC-2} genetic platform* of two strains belonging to these species colonizing a patient during hospitalization.

Methods: We isolated two CRE strains from one patient in a hospital from Argentina and sequenced their whole genome by Illumina MiSeq-I. The genome was assembled with SPAdes 3.9.0, and annotated with PROKKA, RAST and Blastn. Plasmids were identified with PlasmidFinder. Antibiotic resistance genes were detected using RESfinder, CARD and Blastn. Sequence types (ST) were identified with pubMLST. *In vitro* competition assay was done.

Results: The first isolated strain was a *Klebsiella pneumoniae* strain which belonged to ST18. The second one was an *Enterobacter hormaechei* strain that belonged to the pandemic lineage ST45. Both strains were multidrug resistant and carried the same conjugative lncM1 plasmid (77.2 Kb) with *bla_{KPC-2}* embedded in the genetic element IS*Kpn27-blaKPC-2*-IS*Kpn6*-HP-Tn*3*, previously detected in IncP and R plasmids. *In vitro* competition did not show a clear advantage of a strain over the other nor over *K*. *pneumoniae* ST258. Secretion system IV present in *Enterobacter* and not in *Klebsiella* could account for prevalence of the latter *in the patient*.

Conclusions: This study describes a novel plasmid and further confirms that *bla_{KPC-2}* is highly mobilizable and prone to horizontal genetic transfer events between species of *Enterobacteriaceae*.

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THE ANTIMICROBIAL ACTIVITY OF NEW OXAZOLINE-SUBSTITUTED BENZOSILOXABOROLES AND THEIR DERIVATIVES.

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Background and Aims: Benzosiloxaboroles are organoboron compounds with promising antibacterial and antifungal activity. We investigated the antimicrobial activity of new benzosiloxaboroles comprising 4,4-dimethyl-2-oxazoline ring: agent **A** (6-(4,4-dimethyl-2-oxazolin-2-yl)-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), and its **5** derivatives: **A1** (6-(3,4,4-Trimethyl-2-oxazolinium-2-yl)-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole trifluoromethanesulfonate), **A2** (6-[2-methyl-2-(N-methylpyrazinamido)propoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), **A3** (6-[2-methyl-2-(N-methylphenylsufonamido)propoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), **A3** (6-[2-methyl-2-(N-methylphenylsufonamido)propoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), **A4** (6-{2-methyl-2-[N-methyl-(4-chlorophenyl)sufonamido]propoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), and **A5** (6-[2-methyl-2-ammoniumpropoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), and **A5** (6-[2-methyl-2-ammoniumpropoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), and **A5** (6-[2-methyl-2-ammoniumpropoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), and **A5** (6-[2-methyl-2-ammoniumpropoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole), and **A5** (6-[2-methyl-2-ammoniumpropoxycarbonyl]-7-fluoro-1,1-dimethyl-3-hydroxybenzo[1,2,3]siloxaborole chloride).

Methods: Antimicrobial activity, expressed as minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC), was determined against the reference strains of Grampositive (n=4) and Gram-negative (n=12) bacteria and yeasts (n=7). Two compounds were also tested against 5 clinical methicillin-resistant *S. aureus* (MRSA) strains. For Gram-negative rods, MICs of new agents in the presence of the efflux pumps inhibitor Phe-Arg- β -naphthylamide (PA β N) were also determined.

Results: Two sulfonamide derivatives **A4** and **A3** showed the highest activity against MRSA (MICs 3.12-6.25 mg/l) and enterococci (MICs 25-100 mg/l). Compound **A** showed only moderate activity against staphylococci (MICs 50-100 mg/l) and no activity against enterococci. Pyrazinamide derivative **A2** exhibited only low activity against staphylococci (MICs 100-400 mg/l) and *E. faecium* ATCC 6057 (MIC=200 mg/l). Agents **A1** and **A5** weren't active against Gram-positive cocci. None of the tested compounds was active against Gram-negative rods, and no new agent MIC's decrease in the presence of PAβN was observed. Among yeasts, only *S. cerevisiae* ATCC 9763 was sensitive to compounds **A** (MIC=50 mg/l) and **A4** (MIC=100 mg/l).

Conclusions: Oxazoline-substituted benzosiloxaboroles possess promising activity against staphylococci, also clinical MRSA strains, and can be considered a potential source of antibacterial drugs. This work was supported by the National Science Centre (Poland) within the framework of the project DEC-UMO-2018/31/B/ST5/00210.

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GENOMIC ANALYSIS AND IDENTIFICATION OF GENES INVOLVED IN ANTIBIOTIC RESISTANCE OF SALMONELLA ENTERICA ISOLATED FROM A CHICKEN FARM

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Background and Aims: Foodborne diseases are a public health and food industry concern. One of the most frequently transmitted foodborne bacteria belongs to the species *Salmonella enterica*. Outbreaks associated with *Salmonella* are characterized by the presence of emerging serovars that are multiresistant to antimicrobials. This work focuses on the search for the genetic determinants of antimicrobial resistance of *Salmonella enterica* strains isolated from a chicken farm located in the Metropolitan Region of Santiago de Chile.

Methods: Ninety strains were sequenced (Illumina), genoserotyped, and their genomes were assembled and annotated. Using the ABRicate software we found 18 antimicrobial resistance elements, such as genes for resistance to aminoglycosides, beta-lactams, quinolones, sulfonamides, and tetracyclines, among others. Furthermore, we determined that 70% of the genetic profiles shows resistance to 2 or more antimicrobials. Moreover, we performed antibiograms to correlate the genotypic and physiological information.

Results: We found plasmid replicons belonging to the type Col, Col3M, IncFIB, IncI1-I and IncH1A, of which draft sequences were created to analyze them. The Infantis serotype stood out for presenting an IncFIB megaplasmid that also presents other mobile elements such as transposons and integrons that are associated with antimicrobial resistance elements. Consequently, the *Salmonella* Infantis serotypes presented the broadest multiresistance profiles with more than 11 resistance genes per strain.

Conclusions: From this study it is possible to identify a correlation between the mobile elements present in the bacterial genome and its phenotypic resistance profile. **Acknowledgments** This project is funded by the ANID-FONDECY Regular project 1210633 and ANID-FONDECYT postdoctoral 3210156

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TEMPORAL REGULATION OF ANTIBIOTIC RESISTANCE AGAINST COLISTIN IN KLEBSIELLA PNEUMONIA

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Background and Aims: Antibiotic resistance is an ongoing public health threat. *Klebsiella pneumonia* (KP) is a virulent pathogen that quickly generates antibiotic resistance. Colistin, or polymyxin E, is the last-line antibiotic used to treat multidrug-resistant gram-negative bacterial infections, but the resistance to colistin also happens frequently. Although clinical evidence links certain mutations to colistin resistance (COL-R) in KP, the origination and association of the mutations remain unclear. We hypothesize that specific early mutations predispose other COL-R mutation generations and are critical to COL-R development.

Methods: We performed bacterial evolution studies using KP (ATCC 43816) under the selection pressure of colistin with increasing concentrations every three days. The MICs were monitored throughout the duration, and bacterial DNA was extracted for Illumina whole-genome sequencing.

Results: The MIC values continued to rise under the selection pressure of colistin, reaching a 2048-fold increase after 36 days. We have detected multiple gene mutations that are known to be associated with resistance to colistin, such as *rmpA*, *phoQ*, *nadA*, *mscL*, and *pmrB*. Mutations in the mucoid phenotype A regulator *rmpA* were detected early during the evolution and existed in all three independently evolved populations. Mutations in *phoQ* and *pmrB* occurred at different positions throughout evolution in each population prior to fixed mutation at day 36, which may contribute to the exceedingly high MIC to colistin.

Conclusions: A colistin-sensitive KP strain rapidly generates mutations and elicits resistance to increased concentrations of colistin treatment. These results will be useful in developing novel therapeutics to treat multidrug-resistant KP infections in the clinical setting.

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CANDIDA ALBICANS INCREASES THE MEROPENEM TOLERANCE OF ESCHERICHIA COLI IN CROSS-KINGDOM POLYMICROBIAL BIOFILMS

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Background and Aims: Biofilms are three-dimensional microbial populations that form on the surfaces of medical devices such as catheters, and exhibit resistance to antimicrobial agents. *Escherichia coli* is the taxon most frequently isolated from patients with bloodstream infections. *Candida albicans* is the fourth most frequent etiological agent of catheter-related bloodstream infections. We explored the effect on antimicrobial tolerance of the *E. coli–C. albicans* interaction in polymicrobial biofilms.

Methods: Fifty-two *E. coli* isolates from blood cultures of patients at Toshiba-Rinkan Hospital, Japan, between 2013 and 2020 were classified according to their biofilm-formation ability. Meropenem (MEPM) was added to biofilms of high biofilm-forming *E. coli* strains treated or not with *C. albicans* and survival was evaluated by enumerating colony-forming units (CFUs). MEPM was added to *E. coli* biofilms with or without *C. albicans* biofilm supernatant and *E. coli* survival was evaluated by enumerating CFUs.

Results: Fourteen of the fifty-two (26.9%) *E. coli* strains formed biofilms, among which four (7.7%, 4/52) possessed high biofilm-formation ability. The survival of *E. coli* was increased in polymicrobial biofilms (*E. coli/C. albicans*) compared with single-species biofilms (*E. coli*). Also, the survival of *E. coli* was increased in biofilms treated with *C. albicans* biofilm supernatant.

Conclusions: *Candida albicans* induced MEPM tolerance in *E. coli* in polymicrobial biofilms in a manner independent of the presence of *C. albicans* cells. Therefore, *C. albicans* secretes a factor that induces MEPM tolerance in *E. coli* biofilms.

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ANALYSIS OF THE BINDING AND NICKING-CLOSING ACTIVITIES OF A MOBT RELAXASE REVEALS UNUSUAL FEATURES

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Background and Aims: Bacterial genomes mainly evolve via horizontal gene transfer which are responsible for the emergence antibiotic resistant bacteria.Conjugation can be mediated by integrative and conjugative elements (ICEs).The initiation of conjugation requires a key protein, relaxase which nicks the DNA to be transferred at the origin of transfer. My work focuses on uncanonical MOB_T relaxases encoded by the Tn*916*/ICE*St3* superfamily. My research aims at understanding the DNA processing steps of conjugation orchestrated by MOB_T relaxases.

Methods: Binding site identification was performed by electrophoretic mobility shift experiments (EMSA). Nicking-closing assays were done using labeled oligonucleotides in the presence of the cationic cofactor of RelSt3, Mn²⁺.

Results: We determined the minimal sequence on the *oriT* recognized by RelSt3. It includes the conserved nicking site and a downstream region containing inverted repeats. We showed that this binding is mediated by the HTH domain of RelSt3. This binding is important for a full nicking activity *in vitro*, and is essential *in vivo*, as demonstrated by the absence of transconjugants using a variant of ICE *St3* depleted for this HTH domain. We also further characterized the enzymatic activities of RelSt3, demonstrating its closing activity using oligonucleotides, and revealing the existence of a covalent intermediate for the first time for a MOB_T family.

Conclusions: in this work, we characterized the interaction between RelSt3 and its original binding site in the oriT through its HTH domain. We showed that this HTH binding is required for effective nicking activity. We also demonstrated the biochemicals activities of RelSt3 for the first time for the MOBT family

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A MULTIDRUG RESISTANT KLEBSIELLA OXYTOCA ST2 STRAIN HARBORING A BLAKPC-2 -PLASMID DISSEMINATES AMONGST COLONIZED PATIENTS

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Background and Aims: Within the genus *Klebsiella, Klebsiella oxytoca* species Complex (KOSC) is the second most found in hospitals after *K. pneumoniae*. It has been shown that KOSC can exchange antimicrobial resistance genes (ARGs) with other bacteria, even class A and B carbapenemases, leading to hospital outbreaks. Surveillance, diagnosis and treatment of carbapenem resistant *Enterobacterales* belong to the highest priority group of the World Health Organization. The aim of this work was to determine ARGs, genetic platforms and plasmids in three carbapenem resistant *K. oxytoca* colonizing isolates obtained from three inpatients during hospitalization from our hospital.

Methods: Whole-Genome sequencing was performed using Illumina MiSeq-I. Genomic assembly and annotation were done using SPAdes and RAST, respectively. In silico detection of multi locus sequence typing, core genomes' SNPs, plasmids, ARGs and their genetic platforms was carried out using pubMLST, Snippy-core, PlasmidFinder, resFinder, BLASTn, etc.

Results: The three isolates were identified as a single *K. oxytoca* sequence type ST2 strain, from the international clonal complex 2 (CC2) with the same multiple acquired ARGs including bla_{KPC-2} gene which was part of a Tn3-like element. The three isolates also shared predicted plasmids that belonged to IncFII(K) with the bla_{KPC-2} gene, Col(pHAD28) and IncM1 incompatibility groups.

Conclusions: Our results evidence the presence of *K. oxytoca* ST2 in Argentina and not only the global spread of multidrug resistant KPC-producing KOSC CC2 but also the silent dissemination of this lineage amongst inpatients that highlight the importance of continuous surveillance programs to identify *bla*_{KPC-2} reservoirs as well as prevent eventual outbreaks due to KPC-producing strains.

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ANALYSIS OF CATB2 PSEUDO-GENE CASSETTE RECOMBINATION USING INTI1 AND INTI2 INTEGRON INTEGRASES

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Background and Aims: Mobile integrons captures, spreads and expresses gene cassettes (GCs) with the hallmark of being associated to lateral genetic transfer events. GCs that confer resistance to antibiotics can be inserted and excised through a site-specific recombination mechanism, by an Intl integrase. Class 1 and 2 integrons are the most relevant in the antimicrobial resistance (AMR) dissemination. Although the most common *intl2* is a pseudogene, there are functional alleles. Our aim was to evaluate the mobility of the pseudo-GC *catB2* located in the variable region of class 2 integron In2-8 with Intl1 and Intl2, and to analyze the prevalence of the *intl2* alleles.

Methods: The *in vivo* recombination assays were done in *E. coli* TOP10 with plasmids pCATB2S containing *catB*2 in the *attC_{aadB}-catB2-\Deltaattl2* architecture from In2-8 and pMI1-1 carrying *intl1*, or pCI2-2 harboring *intl2fun*. Selection was done with antibiotics and the recombination frequencies were obtained by colony PCR. An Entrez database was done to analyze the *intl2* alleles detected in the NCBI Ref_Seq Genomes database.

Results: From the 3185 *intl*2 detected, 12 alleles were functional and 30 non-functional, with that used here being the most prevalent behind the canonical non-functional. The excision frequency for *catB*2 with Intl1 was 49%, but no recombination was observed with Intl2.

Conclusions: Our results suggest not only that the widespread of pseudo-GC could be underestimated but also that even though this Intl2 allele recombined other GC, there is a specificity for each integrase and GC in different architectures, that can play a pivotal role in the AMR dissemination and reservoir.

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GENOMIC DATA REVEALS THE EMERGENCE OF BLAKPC-2 AND BLACTX-M-15 IN E. COLI ST648 FROM RECTAL SWAB ISOLATES IN THE CONTEXT OF HOSPITAL SURVEILLANCE

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Background and Aims: The epidemiological success of pandemic KPC carbapenemases was linked to pandemic lineages capable of disseminating plasmids carrying the *bla_{KPC-2}*gene on the mobile genetic element platform Tn*4401*. The most frequent plasmids are of the IncF type, followed by IncN, IncX, IncA/C, IncP, IncL/M, IncR, IncH, IncI, IncU and Col, which spread rapidly, especially among the international clones of *Escherichia coli* and *Klebsiella pneumoniae*. Our aim was to analyze a carbapenemase producing *Escherichia coli* strain isolated from hospital surveillance.

Methods: The strain HA25pEc was isolated from a rectal swab sample taken on the first day of hospitalization as part of the hospital epidemiological surveillance protocol. WGS was performed by Illumina MiSeq. Bioinformatic analysis was carried out with SPAdes 3.9.0, Prodigal, RefSeq, Blast, PlasmidFinder, pubmlst, ResFinder and CARD. Conjugation assays were also performed.

Results: HA25pEc corresponds to the first report of *E. coli* ST648 carrying *bla_{KPC-2}* and *bla_{CTX-M-15}*, in Latin America. It possesses 17 antibiotic resistance genes (ARGs) apart from *bla_{KPC-2}* and *bla_{CTX-M-15}*. This strain possesses the replicons Col(MG828), IncFIA, IncFIB, IncFII and IncR. *bla_{KPC-2}* was identified on the Tn4401(*tnpR*)-Tn3(*tnpA9*-IS21(*TnpA*)-IS*Kpn7*(*TnpA*)-IS*Kpn7*(*TnpA*)-*bla_{KPC-2}* gene among *E. coli* strains belonging to ST648 has been described to be mediated by large plasmids IncN and IncHI2. Since these replicons were not found in HA25pEc, it is possible that another plasmid or mechanism is disseminating the *bla_{KPC-2}* gene in Argentina.

Conclusions: Our results reinforce the importance of molecular surveillance to identify reservoirs and to warn about the spread of new international clones in patients carrying carbapenemases.

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THE COMPLEX ROLE OF NIM PROTEINS IN METRONIDAZOLE RESISTANCE

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Background and Aims: *Bacteroides fragilis* is an anaerobic gut commensal but can also cause severe disease. Metronidazole, a 5-nitroimidazole drug, is an effective treatment but metronidazole resistance does occur. The so-called Nim proteins have been described as resistance determinants which reduce the nitro group of metronidazole to a non-toxic aminoimidazole. Recent research, however, showed that expression levels of *nim* genes are independent of the degree of resistance. In the search for an alternative model for *nim*-mediated metronidazole resistance we screened strains carrying episomal *nim* genes and their parental strain 638R without a *nim* gene for physiological differences.

Methods: We performed multiple enzyme assays, oxygen exposure assays and RT-qPCR to monitor physiological changes conferred by *nim* genes to recipient *B. fragilis* strains and evaluated their relevance for resistance.

Results: Recipient strains with a *nim* gene had a far higher pyruvate-ferredoxin oxidoreductase (PFOR) activity than the respective parental strain. Strains carrying a *nim* gene fully retained PFOR activity and other enzyme activities after higher levels of resistance had been induced. In the parental strain 638R these were lost or very strongly down-regulated during the development of resistance. After induction of high-level metronidazole resistance parental strain 638R was highly susceptible to oxygen whereas the daughter strains with *nim* genes were not. RT-qPCR measurements showed that hemin uptake is downregulated in resistant 638R but not in the resistant daughter cell lines with a *nim* gene.

Conclusions: We propose that nim genes prime *B. fragilis* towards an alternative pathway of resistance by enabling the preservation of normal iron levels in the cell.

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BACTERIAL ACQUISITION OF ANTIBIOTIC RESISTANCE BY NATURAL TRANSFORMATION ON LETTUCE LEAVES

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Background and Aims: Antibiotic resistance diffusion in agri-food systems is an issue of increasing concern worldwide, and it could be amplified by water reuse in agriculture since wastewater treatment plants are not designed for the removal of emerging contaminants, like pharmaceuticals and antibiotic resistance genes (ARGs). Phyllosphere bacteria can represent transient hosts capable to transfer ARGs to human pathogenic bacteria by Horizontal Gene Transfer (HGT) mechanisms. For instance, lettuce and ready-to-eat fruits have been reported as a reservoir of multidrug resistant *Acinetobacter* spp., isolated from both plant samples and produces. Here, we investigated the bacterial acquisition of an ARG on lettuce leaves by natural transformation.

Methods: Experiments were conducted using the model bacterium *Acinetobacter baylyi* BD413 and the pZR80(gfp) plasmid carrying the kanamycin resistance gene aphA-3 and the GFP marker.

Results: Firstly, *A. baylyi* BD413 was demonstrated to colonize lettuce leaves. *A. baylyi* BD413 was able to naturally acquire exogenous DNA on leaf surface (i.e., leaf discs and *in planta*), showing transformation frequencies comparable to those detected *in vitro* under optimal conditions. Furthermore, *A. baylyi* BD413 transformant colonies were isolated from the leaf endosphere 24 hours after bacteria and exogenous DNA administration to the plants. The influence of a surfactant molecule on DNA acquisition and bacterial penetration into leaf tissues was also tested.

Conclusions: Our results showed that natural transformation and ARG acquisition by bacteria can occur on edible plant portions, highlighting the urgency to collect more data of interest for risk assessment procedure. Acknowledgments: Funded by the Cariplo Foundation project "WARFARE" (GA n° 2018-0995).

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ENTEROCOCCUS SPP. RESISTANCE ISOLATED FROM RETAIL CHICKEN MEAT

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Background and Aims: Resistance to antibiotics is an increasingly problem. The objective of this work was to evaluate resistance of Enterococcus spp. strains from retail chicken meat to different antibiotics.

Methods: Thirteen samples of retail chicken meat were analyzed and presumed colonies of Enterococcus spp. were isolated and identified by MALDI-TOF/MS. After that, antimicrobial resistant was evaluated using disk-diffusion test with 16 different antibiotics: ampicillin (10 μ g), chloramphenicol (30 μ g), ciprofloxacin (5 μ g), doxycycline (30 μ g), enrofloxacin (5 μ g), gentamicin (120 μ g), imipenem (10 μ g), levofloxacin (5 μ g), linezolid (30 μ g), minocycline (30 μ g), nitrofurantoin (300 μ g), norfloxacin (10 μ g), teicoplanin (30 μ g), tetracycline (30 μ g), tigecycline (15 μ g) and vancomycin (30 μ g). The results were classified as resistant, intermediate and sensitive.

Results: Twenty-three isolates of Enterococcus spp. from six different species were obtained, being these: E. cecorum (1), E. faecalis (15), E. faecium (4), E. gallinarum (1), E. gilvus (1) and E. phoeniculicola (1). Of the 23 isolates, one isolate presented resistance to 5 antibiotics: ciprofloxacin, enrofloxacin, tetracycline, tigecycline and norfloxacin. Additionally, 3 of the enterococci presented resistance to 4 antibiotics. On the other hand, 14 of the 23 isolates (60.87%) showed resistance to tetracycline. Three isolates of E. faecalis and one of E. gilvus were susceptible to all antibiotics tested.

Conclusions: This work suggests that chicken meat can be a source of multidrug-resistant enterococci. This work has received funding from POCTEFA Project (INTERREG Program) TESTACOS EFA 152/16, Pre-doctoral UR-CAR fellowship and European Union's H2020 research and innovation program under Marie Sklodowska-Curie grant agreement No.801586.

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GENOMIC ISLANDS DISCOVERED IN HIGHLY RESISTANT SERRATIA SP. HRI: A PATHWAY TO DISCOVER NEW BIOCIDE RESISTANCE MECHANISMS

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Background and Aims: In the fight against antimicrobial resistance very little is known about an emerging problem, microbial resistance to disinfectants and biocides. Molecular insights into the mechanisms of resistance to disinfectants are severely limited, and the role of various mobile genetic elements, such as genomic islands, is largely unknown. Serratia sp. HRI is an isolate with high disinfectant resistance capabilities. It, therefore, provides a unique opportunity to add to the knowledge of resistance to disinfectants and uncover previously undescribed resistance mechanisms.

Methods: Whole-genome sequencing and bioinformatic analysis by IslandViewer4 and RAST were used to identify and annotate a total of 11 resistance islands within the genome of Serratia sp. HRI.

Results: Resistance genes active against several antimicrobials were annotated in these islands, most of which are multidrug efflux pumps belonging to the MFS, ABC and DMT efflux families. Antibiotic resistance islands containing genes encoding for multidrug resistance proteins ErmB (macrolide and erythromycin resistance) and biclomycin were also found. A metal fitness island harbouring 13 resistance and response genes to copper, silver, lead, cadmium, zinc, and mercury was identified. In the search for disinfectant resistance islands, two genomic islands harbouring smr genes, notorious for conferring disinfectant resistance, were found.

Conclusions: These resistance islands add to the evidence pointing towards a new mechanism of disinfectant and biocide resistance. In a field like disinfectant resistance, where knowledge of mechanisms is minimal, the vast number of hypothetical proteins within these resistance islands are attractive targets in searching for novel mechanisms of disinfectant resistance.

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DRUG SUSCEPTIBILITY PATTERNS OF FULMINANT GBS INFECTION AS A RE-EMERGING INFECTIOUS DISEASE IN JAPAN

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Background and Aims: Severe streptococcal infections are invasive, reemerging infections that rapidly worsen and lead to death. It is not well known that not only group A streptococcus (GAS) but also group B streptococcus (GBS) is the causative agent of this infection. GBS produces hemolytic toxins, and other toxins like GAS. Furthermore, as with other pathogenic bacteria, drug-resistant Streptococcus are on the rise worldwide, but drug resistance has not been studied extensively in GBS. We investigated the drug susceptibility of clinical isolates of GBS, which are closely related to fullminant streptococcal infections.

Methods: We investigated GBS strains isolated from sterile sites of invasive infections at a hospital in Nagoya city, Japan from 2017 to 2021. Bacterial identification and drug susceptibility testing was performed by VIETEK2 system.

Results: Sixty-six strains were included in the study. As a result, four antibiotics were found to be resistant to GBS. Seventeen patients (25.8%) were resistant to erythromycin, 9 (13.6%) to clindamycin, 25 (37.9%) to minocycline, and 26 (39.4%) to ciprofloxacin. Six (9.1%) isolates were two-drug resistant, seven (10.6%) were three-drug resistant, and one (1.5%) was four-drug resistant.

Conclusions: GBS is not only drug resistant to individual antibiotics, but also to multiple antibiotics, suggesting that GBS is becoming increasingly multidrug-resistant. Continued surveillance of drug susceptibility of GBS as a potential cause of fulminant streptococcal infections was considered necessary in the future.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

DECIPHERING THE ROLE OF LYTIC TRANSGLYCOSYLASES AND ITS INTERACTING PARTNERS TOWARDS BACTERIAL MORPHOGENESIS AND ANTIBIOTIC SUSCEPTIBILITY IN CAULOBACTER CRESCENTUS.

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Background and Aims: Bacterial antibiotic resistance is serious health problem, it is necessary to search for new antibiotic targets. β -lactam antibiotics inhibits cell wall modifying enzymes which play a major part in bacterial morphogenesis. Till now the role of these enzymes in bacterial antibiotic resistance were less focused on. Much studies have been done on enzymatic functions and localization of these PG hydrolases but little is known about their spatiotemporal regulation and their definite role in cell morphogenesis. The focus of our research is to study the enzymes (Lytic Transglycosylases) involved in cytokinesis and their major role in antibiotic susceptibility of α -proteobacterium *Caulobacter crescentus*.

Methods: In the present study, overexpression of LTs genes were characterized and the cross talk between LTs and its interacting genes like *ftsN* and *ftsZ* were evaluated via confocal imaging and fluorescence microscopy. Hypersensitivity of LTs knockout mutants towards different β -lactam drug and the underlying mechanism was also investigated by performing spot assay along with UPLC (Ultra performance liquid chromatography) and LC-MS (Liquid chromatography-mass spectrometry).

Results: In *LTs* deleted strains, β -lactam resistance was significantly decreased compared with that of wild-type strains along with morphological defects. Overexpression of LTs leads to morphological defects. UPLC and LC-MS data showed changes in cytoplasmic and periplasmic metabolites abundance in different LTs mutant strains.

Conclusions: In strains of several antibiotics-resistant *C. crescentus*, LTs contribute differently to β -lactam resistance. Besides, characterization of overexpression of LT further proved their importance in regulating the cell shape of *C. crescentus* and its interdependency on its interacting partners.

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INVESTIGATING BACTERIAL FITNESS IN ST239-SCCMEC TYPE III-SCCMERCURY TO ST22-SCCMEC TYPE IV MRSA CLONAL REPLACEMENT: A PRELIMINARY STUDY

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Background and Aims: Methicillin-resistant *Staphylococcus aureus* (MRSA) clonal replacement has been documented in many countries including in Malaysia, where the dominant clone ST239-SCC*mec* type III-SCC*mercury* has been replaced by ST22-SCC*mec* type IV presumably between 2013 – 2017. In this study, we determined bacterial fitness of ST239-SCC*mec* type III-SCC*mercury* MRSAs isolated in 2005 (strain M57/05) and 2009 (strain M222/09) compared to ST22-SCC*mec* type IV MRSAs isolated in 2017 (strains M181/17 and M080/17) from Hospital Canselor Tuanku Muhriz (HCTM) located at Kuala Lumpur, Malaysia.

Methods: Growth curves of tested strains in pure culture and their tolerance to desiccation in 6, 24 and 48 hours were determined. Co-cultures of tested strains were performed up to 72 hours and subsequently plated on BHI agar with and without chloramphenicol (20ug/mL), where only the ST239 strains were resistant to the antibiotic. Numbers of viable colonies were enumerated; colony morphologies were recorded. SCC*mec* typing of representative purified colonies was also performed.

Results: During single bacterial culture, ST22 MRSAs grew faster and survived desiccation better than ST239 MRSAs. Nevertheless, intriguingly, during co-culture experiments, a mixture of >1000 bright yellow smaller-sized colonies and 4~18 light yellow larger-sized colonies were observed on drug-free BHI agar plates. The smaller and larger colonies were later identified as SCC*mec* types III and IV, respectively.

Conclusions: This preliminary experiment showed that while ST22-SCC*mec* type IV MRSAs were fitter in single bacterial cultures, the strains did not outcompete ST239-SCC*mec* type III-SCC*mercury* MRSA in co-cultures. Further studies are required to confirm if these findings are associated with clonal replacement.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

INHIBITION OF HIGH-FREQUENCY ANTIMICROBIAL RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS THROUGH PHYTOCHEMICALS OF THE MEDICINAL PLANTS FROM INDIA

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Background and Aims: Phytochemicals derived from time-tested medicinal plants can revolutionize antimicrobial development. *Mycobacterium tuberculosis* is one of the most prevalent infections throughout the world and is declared a global public health emergency by the World Health Organization. This study aims to identify phytochemicals from the medicinal plants from India against the mechanisms of drug resistance in *M.tuberculosis*.

Methods: The phytochemicals from more than 1700 medicinal plants from India are screened against the high-frequency antimicrobial-resistant genotype identified from more than 8600 drug-resistant samples of *M.tuberculosis* through an insilico approach. The drug-like phytochemicals are extracted using a seven-layered drug-likeness filter before performing the molecular docking and molecular dynamics simulation analysis to identify potential inhibitors of drug resistance in *M.tuberculosis*. The physiology-based-pharmacokinetic (PBPK) analysis of the potential phytochemicals was performed to identify their concentrations in various body tissues using Ribostamycin as a reference.

Results: The genotype analysis shows that in over 99% of samples, aminoglycoside 2'-Nacetyltransferase is responsible for drug resistance in *M.tuberculosis*. The molecular docking and molecular dynamics simulation studies identify that N-Deacetyl-thiocolchicine can suppress the inactivation of aminoglycoside antibiotics by the aminoglycoside 2'-N-acetyltransferase. The PBPK analysis reveals that the concentration of the N-Deacetyl-thiocolchicine is relatively higher than the Ribostamycin in the lung tissues for over 24 hours after oral administration.

Conclusions: N-Deacetyl-thiocolchicine can inhibit drug resistance in *M.tuberculosis* by suppressing the acetylation activity of the aminoglycoside 2'-N-acetyltransferase that is responsible for enzymatic degradation of various antibiotics. The PBPK simulations reveal that competitive enzyme inhibition kinetics of N-Deacetyl-thiocolchicine aids the aminoglycoside antibiotics to act against drug-resistant *M.tuberculosis*.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

INSIGHTS INTO THE FUNCTION OF A STRESS-INDUCED FITNESS FACTOR OF E. COLI

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Background and Aims: The β -barrel assembly machinery (BAM) complex promotes biogenesis of integral Outer Membrane proteins (OMPs) in gram-negative bacteria. Perturbations in biogenesis of OMPs trigger sigmaE stress response, regulating a number of genes such as subunits of *bam*. Another gene, *doIP*, is upregulated and encodes for a division septum-enriched lipoprotein that promotes OM integrity and promotes activation of amidases during cell division. The precise role of this protein was uncharacterized and this work aimed at understanding the role of DoIP during envelope stress response.

Methods: Approaches used in this project include microbiology techniques, genetics, protein biochemistry and cell imaging.

Results: A proteomic approach aiming at defining the BAM interactome in the envelope of *E. coli* identified DoIP as a putative partner of the BAM complex. We demonstrated that DoIP interacts directly with OM-assembled BamA. Upon sigmaE activation, overproduction of BAM is initially beneficial, however prolonged overproduction can be detrimental by the accumulation of a non-properly folded state of BamA. This effect is coped by the concomitant overproduction of DoIP, which was shown to promote proper folding of BamA. We demonstrated that localization of DoIP is impaired upon sigmaE stress response, suggesting an unprecedented link between OM biogenesis and regulation of cell division.

Conclusions: Our work suggests DoIP is a fitness factor involved in the regulation of the activity of the BAM complex. We speculate that during sigmaE stress response, DoIP could be a factor that enhances BAM activity by promoting its proper assembly, reducing its mid-cell localization to regulate cell division.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

SYNERGISTIC EFFECT OF CURCUMIN AND IMIPENEM ON CARBAPENEM-RESISTANT P.AERUGINOSA WITH GALLERIA MELLONELLA LARVAE MODEL.

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Background and Aims: Antimicrobial resistance is an important problem that is increasing rapidly and restricting the treatment options. Synergistic effects of natural products with antibiotics and researching new treatment options have come to the fore. *Pseudomonas aeruginosa (P.aeruginosa)* is an opportunistic pathogen and infection with multi-drug resistant (MDR) *P. aeruginosa* is a major treatment challenge. Curcumin has taken its place in the literature as one of the promising natural ingredients with its strong antimicrobial activity. We aimed to investigate the synergistic effect of curcumin with imipenem (IMP) in vitro and in vivo activity on *Galleria mellonella* larvae.

Methods: In this study, three clinical isolates of MDR *P. aeruginosa*, which were determined to be phenotypically resistant to carbapenems, were used and KPC and OXA48 resistance genes were determined by PCR method. The synergistic effect of curcumin with IMP was investigated using the checkerboard method. Efficacy was tested in vivo and larval survival and bacterial load were compared.

Results: IMP, MIC values decreased significantly (2-8 fold) in the presence of curcumin and partial synergy was observed. Bacterial load was evaluated to investigate the effect of antimicrobials during infection. While CFU values in untreated larvae increased day by day, bacterial load decreased significantly in the curcumin and IMP-treated groups compared to the untreated group (p<0.05).

Conclusions: It was concluded that curcumin-antibiotic combinations could provide an alternative approach in the treatment of infections with multi-drug resistant (MDR) bacteria. **Acknowledgments** I would like to thank Prof.Dr.Meryem Akpolat Ferah, for Curcumin and Dr.Fürüzan Köktürk, who provided the control of statistics.

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E-Poster Viewing Topic: AS16 Antimicrobials and antimicrobial resistance

APPLICATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES AGAINST THE EMERGING DRUG-RESISTING NOSOCOMIAL PATHOGEN ACINETOBACTER BAUMANNII

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Background and Aims: In recent years, A.baumannii has emerged as a major nosocomial pathogen that causes various serious illnesses and contributed an extensive range of mortality and morbidity. Nanoparticles exhibit unique characteristics in possessing a surface-to-volume ratio that makes them a potential antibacterial agent. To combat the infection caused by this multi-drug resisting nosocomial pathogen, green synthesized silver nanoparticles(AgNPs) were prepared from the leaf extract of Syzygium cumini.

Methods: UV-Vis spectroscopy, Transmission electron microscopy (TEM), and FTIR were performed to characterize the nanoparticles. After characterization the nanoparticles were tested for various antibacterial, anti-motility, and anti-biofilm activity using different biochemical assays. Structural and morphological reduction in biofilm formation was also visualized through Light, fluorescent and confocal microscopy

Results: Characterization of nanoparticles revealed that they were spherical in shape, and exhibit phenolic, aromatic and alkynes groups. Also, these NPs showed significant antibacterial, anti-motility, and anti-biofilm activity. MIC against A. baumannii was achieved at 15 μ g/mL. A marked reduction in twitching and surface-associated motility were also observed beyond 50 μ g/mL concentration of AgNPs. Green synthesized AgNPs significantly inhibited biofilm mass at different concentrations.

Conclusions: Conclusion: The present study displayed the efficacy of green synthesized silver nanoparticles at a very low concentration. Therefore, a greener route of silver nanoparticles synthesis can provide considerable assistance in eliminating infection caused by this multi-drug resistant nosocomial pathogen

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MOLECULAR CHARACTERIZATION OF METALLO-B-LACTAMASE GENES AND INTEGRONS IN IMIPENEM -RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES FROM IN-PATIENTS AT A UNIVERSITY HOSPITAL

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Background and Aims: Pseudomonas aeruginosa is a pathogen associated with a broad spectrum of infections in human. P.a is intrinsically resistant to many antibiotics and acquires novel resistance genes via horizontal gene transfer. The increase in the prevalence of carbapenem-resistant P.a isolates, particularly caused by the metallo-ß-lactamase is of great concern in the clinical settings worldwide. That is why, this study was focused on the detection and identification of MBL producing and the characterization of gene cassette-containing integrons in imipenem-resistant P. a isolates.

Methods: In this study, 100 non-replicate imipenem-resistant P. aeruginosa (IRPA) clinical isolates were subjected to a screening test for detection of MBL using the IP/IPI E-test, combined IPM-EDTA disk test (CDT), and double-disk synergy test (DDST). The three phenotypic methods, were evaluated in comparison to PCR detection of MBL genes as the gold standard. The different variants of MBL genes present among IRPA clinical isolates from an University Hospital in Fatih Turkey, were also determined using PCR. Integrons and their associated gene cassettes were characterized by PCR/RFLP and the genetic relatedness of the isolates was investigated using random amplification of polymorphic DNA (RAPD) analysis.

Results: This study highlights the resistance to imipenem due to IMP- and VIM-producing P. aeruginosa and their associated class 1 integrons. The genes detected from MBL-positive isolates were blaIMP-7, aacC1; blaVIM-11; aacA7, blaVIM-2, attl, and aacA7.

Conclusions: Horizontal dissemination of the class 1 integron-associated MBL genes may contribute to the further emergence of carbapenem resistance in other Gram-negative bacteria as well as the dissemination of antimicrobial resistance in the clinical settings.

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EFFECT OF CHEMICAL ANTIMICROBIAL DISINFECTANTS ON RESISTANT EIMERIA SPECIES OOCYSTS

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Background and Aims: Eimeria are a species of parasitic protozoans that cause a disease called coccidiosis in a number of vertebrates. Coccidiosis causes bloody lesions in the gut which reduce growth rate and may lead to death. Coccidiosis cost the poultry industry billions of dollars annually. Current treatments such as anti-coccidial drugs are grouped with antibiotics which are being phased out due to the build- up of resistance by Eimeria species to antimicrobial drugs. An alternative combat strategy is the development of chemical antimicrobials capable of destroying the resilient exogenous stage of the parasites life cycle (oocyst).

Methods: Experimental work was done to evaluate the effects of commercial antimicrobials; Virukill, Saniwash, Prontosan and Hypercide on resistant Eimeria species oocysts. This study used oocysts of E. acervulina (strain VND-A10), E. maxima (strain VND-M27) and E. tenella (strain LPRL-49) obtained from ADVENT coccidiosis vaccine. The oocysts were exposed for a contact time of 30 minutes at concentrations of 20, 40, 60, 80 and 90% after which the mixture was suspended on a slide and observed under a light microscope. Efficacy of the antimicrobials were determined by identifying any morphological damage to the oocysts

Results: Hypercide was found to have no effect on the oocysts, whilst the other three antimicrobials (Virukill, Saniwash, Prontosan) showed limited success at all concentrations, in affecting the morphological structure of the oocyst. However, majority of the oocysts appeared undamaged

Conclusions: Future studies are needed in order to determine the efficacy of this strategy to combat coccidiosis. Without anti-coccidial drugs, treatment options are severely limited.

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ANTIMICROBIAL RESISTANCE AND CLONAL RELATIONSHIPS OF STAPHYLOCOCCUS PSEUDINTERMEDIUS ISOLATED FROM CANINE PYODERMA

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Background and Aims: *Staphylococcus pseudintermedius* is an important cause of canine pyoderma. Methicillin-resistance in *S. pseudintermedius* isolates (MRSP) is predominantly due to the *mecA* gene, which encodes a protein (PBP2a) with low affinity for β -lactam antibiotics. *S. pseudintermedius* has emerged as an important problem due to multi-drug resistance (MDR), recent increased incidence, and the risk for zoonotic transmission.

Methods: Twenty-nine isolates recovered from canine pyoderma cases in Argentina were sequenced and genotyped and antimicrobial susceptibility testing was performed using Sensititre COMPGP1F plates. The presence of antimicrobial resistance (AMR) genes was detected using AMRFinder Plus and Abricate.

Results: All MRSP isolates (n=23) showed MDR (resistance to \geq 1 agent in \geq 3 antimicrobial classes). All were susceptible to nitrofurantoin, imipenem, and vancomycin. Among methicillin-susceptible isolates (n=6), only one was MDR, presenting resistance to 8 antimicrobials. Twenty-one MRSP isolates were resistant to penicillin and oxacillin, two were resistant to penicillin but oxacillin susceptible. MRSP showed 91.6% resistance to trimethoprim/sulfamethoxazole, erythromycin and clindamycin, 82.6% to fluoroquinolones, 39.1% to chloramphenicol, 21.7% to tetracycline, 8.7% to gentamicin and rifampin. The main AMR genes identified were *mecA*, *blaZ*, *dfrG*, *ermB*, *catA*, *tet*M, *aac*(6')-*aph*(2''). Sequence types (STs) identified were ST339 (n=7), ST1412 (n=3), ST71 (n=2), ST45 (n=1) and ST313 (n=1) and 15 STs previously undescribed. Two isolates with ST71 showed resistance to 7 antimicrobial classes. ST339 was the most prevalent, presenting resistance to 5 antimicrobial classes and suggests a locally evolved clone.

Conclusions: *Staphylococcus pseudintermedius* should be included in surveillance programs to track the emergence of clonal populations with increased AMR.

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CATIONIC PHENOSAFRANIN PHOTOSENSITIZERS BASED ON POLYHEDRAL OLIGOMERIC SILSESQUIOXANES FOR INACTIVATION OF GRAM-POSITIVEAND GRAM-NEGATIVE BACTERIA

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Background and Aims: An increasing resistance of bacteria to available antibiotics is a serious problem globally and requires the development of new, safe for humans, specific nanoparticles, which can interact with various cellular structures of pathogens. A potential alternative for antibiotics is an antimicrobial photodynamic inactivation (APDI). The aim of the work was the synthesis of new cationic light-activated phenosafranin polyhedral oligomeric silsesquioxane (POSS) conjugates (POSSPSF, POSSPSFDAU, iBuPOSSPSF) and the study of photodynamic properties against of the three strains of bacteria, like: the Newman strain of the *S. aureus* and of clinical strains of *S. aureus* MRSA 12673 and *E. coli* 12519.

Methods: The chemical structure of conjugates was confirmed by ¹H, ¹³C NMR, HRMS, IR, fluorescence spectroscopy and UV-VIS analyzes. Microbiological studies in vitro included photodynamic inactivation assay, the singlet oxygen detection studies and microscopic analyzes.

Results: We showed the high photodynamic effect of silsesquioxanes in low concentrations (0.38 μ M) after light irradiation ($\lambda_{em. max} = 522$ nm, 10.6 mW/cm²) for 5 min. TEM studies showed ruptured *S. aureus* cells with leaking cytosolic mass and distorted cells of *E. coli*. The results from confocal microscopy studies indicated that the POSSPSF accumulated inside the cells whereas iBuPOSSPSF and POSSPSFDAU accumulated in the cell wall or cell membrane. The singlet oxygen detection studies supported the hypothesis that bacterial inactivation may be caused by the generation of reactive oxygen species (ROS).

Conclusions: We constructed light-triggered ROS-responsive phenosafranin-polyhedral oligomeric silsesquioxane conjugates for photodynamic antimicrobial therapy with high antibacterial activity.

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GENOMIC ANALYSIS OF VANCOMYCIN-INTERMEDIATE ST81-MRSA-IV STRAIN FROM PACEMAKER-ASSOCIATED SEPTICEMIA, HOKKAIDO, JAPAN

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Background and Aims: The emergence of vancomycin resistant strains limits treatment options. High level vancomycin resistance (MIC \geq 16 µg/ml) in *S. aureus* is conferred by the *vanA* operon, while the molecular basis of intermediate-resistant *S. aureus* (VISA, MIC = 4-8 µg/mL) is polygenic, and involves stepwise mutations in regulatory genes. We investigated the genomic changes related to the reduced susceptibility against vancomycin in an *S. aureus* strain belonging to sequence type 81, with type IV SCC*mec* (ST81-MRSA-IV) obtained in Hokkaido, Japan.

Methods: An ST81-MRSA-IV exhibiting VISA phenotype (strain HV2019-1) was isolated from a patient with pacemaker-associated septicemia (Sakurada M et al., 2020). The whole genome sequence of the strain was determined by next-generation sequencing, and compared with that from vancomycin susceptible ST81-MRSA-IV.

Results: HV2019-1 displayed a small-colony-variant (SCV) -like phenotype on agar plates: formation of small colonies, decreased pigmentation, decreased hemolysis. Comparative analysis detected amino acid substitutions in following molecules: WalR (Ala38Val); WalK (Ile304Val); GraS (Ile18Leu). The regulatory genes, *walKR* and *graSR*, are most frequently implicated in VISA phenotype, and the identical substitution in GraS is reported from the US (Hafer C et al., 2012). In addition, nonsense mutation occurred in *yjbH*, resulting in deletion of its C-terminal region. Contribution of YjbH to reduced pigment production, and VISA phenotype (in combination with mutations both in *vraS* and *stp1*) are previously demonstrated in laboratory strains (Paudel A et al., 2021; Renzoni A et al., 2011).

Conclusions: Genetic alterations in *walKR*, *graS*, and *yjbH* of Japanese VISA strain (HV2019-1) can have some impact on its VISA/SCV-like phenotype.

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HIGH CLONAL DIVERSITY OF CARBAPENEM-RESISTANT ESCHERICHIA COLI IN TAIWAN

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Background and Aims: The rise of carbapenem resistance E. coli poses a great public health threat and genomic analysis provided insight into the spreading. This study aims to use whole-genome sequencing (WGS) to analyze the the clonal relatedness and related resistance mechanisms among carbapenem-resistance E. coli (CREc).

Methods: This study was conducted at a tertiary hospital in Taichung, Taiwan. E. coli isolated from blood culture and resistant to carbapenem were enrolled. These CREc were subjective to WGS using Nanopore Gridlon and the assembled genomes were uploaded into the Center for Genomic Epidemiology for analysis (https://www.genomicepidemiology.org/). The MLST, serotype, fimH type and phylogroup were determined using the following programs: MLST (version 2.0.4), SerotypeFinder (version 2.0.1), FimTyper (version 1.0), and ClermonTyping (version 1.4.0), respectively.

Results: Eight ST types were identified among E. coli bacteremic isolates (including ST 44, 58, 95, 167, 131, 349, 405 and 1485). Three of the CREc had carbapenemase, including two OXA-48 and one NDM-1. Each of the carbapenemase-producing E. coli, belongs to a different ST type. Five phylogenic groups were detected (including A, B1, B2, D and F). FimH Type and serotype were also highly heterogeneous. Clonality-relatedness was not identified regarding the source of bacteremia or the acquisition of CREc from the hospital or community.

Conclusions: Our data used WGS to characterize the molecular resistance mechanisms and phylogenetic relatedness of CREc. These findings suggest the polyclonal and highly genetically diverse nature of bacteremic carbepenem-resistant E. coli in Taiwan.

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THE EFFECTIVENESS OF PHOTOCATALYTIC NANOPARTICLES AS ANTIMICROBIAL AGENTS ON PERSONAL PROTECTION EQUIPMENT

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Background and Aims: Healthcare-associated infections cause high mortality and morbidity worldwide. Wearing personal protective equipment (PPE) is pertinent to prevent transmission of healthcareassociated infections from healthcare workers to patients in healthcare settings. Recent pandemic of severe acute respiratory coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) has led to global economic shutdown. Therefore, designing reusable PPE coated with antimicrobial agent is necessary to prevent transmission of healthcare-associated infections. In this study, nanoparticles contained "metal oxides", and use photocatalytic activity against microbes has been evaluated.

Methods: Antimicrobial testing using photocatalytic nanoparticles coated on mask that inoculated with Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were conducted at different exposure time, which were 10, 30, 50, and 60 seconds. The effectiveness of photocatalytic nanoparticles will be compared with Ultraviolet type C (UVC) radiation.

Results: Exposure of mask toward UVC radiation resulting in 99.9% of reduction tested with 50 and 60 seconds exposure to E. coli. Simulation of using UVC radiation on used mask resulting on more than 80% of bacterial reduction. Antibacterial testing of mask that coated with photocatalytic nanoparticles showed 99.99% reduction of E. coli ATCC 25922 and 100% reduction of S. aureus ATCC 25923.

Conclusions: This study showed that photocatalytic nanoparticles was a better option in killing bacteria as compared to UVC radiation and can be used as antimicrobial agents. The direct output of this product is to designing PPE with antimicrobial property that can be reused for future supply shortage as well as to avoid healthcare-associated infections.
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E-Poster Viewing Topic: AS17 Microbial evolution and diversity

A NOVEL STREPTOMYCES STRAIN ISOLATED FROM A MAPLE LEAF ENCODES AN ALCOHOL DEHYDROGENASE WITH A PUTATIVE LANTHANIDE-BINDING SITE

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Background and Aims: *Streptomyces* are a genus of filamentous bacteria within the phylum Actinobacteria, that mainly live as saprophytes. They have been primarily studied as (rhizo)soil organisms, but in recent years it has been shown that they can colonize plant tissue as well. During a third-year practical centred on the cultivation and sequencing of plant leaf-associated methylotrophs, we noticed a filamentous bacterium growing on agar plates containing a mineral salt medium supplemented with methanol and lanthanide salts. Lanthanides are a group of transition metals that are of great importance to e.g. modern technology. They were long thought to have little biological relevance, until the discovery of a lanthanide-dependent methanol dehydrogenase (XoxF, a PQQ-dependent beta propeller enzyme). Since then, XoxF-type dehydrogenases have been shown to be ubiquitous among the known methylotrophs within the proteobacteria and verrucomicrobia.

Methods: We isolated the filamentous *Streptomyces* strain and used a combination of Nanopore and Illumina Miseq sequencing to fully resolve its genome.

Results: Annotation revealed five alcohol dehydrogenases, including a polyvinyl alcohol dehydrogenase (PVAdh). This PVAdh contains a PQQ-binding motif as well as a metal-binding site that contains the aspartate residues that in XoxF are essential for binding lanthanides. A BlastP search against the non-redundant protein database revealed close to 100 highly similar sequences with these features within the Actinobacteria, Acidobacteria, Deltaproteobacteria and Cyanobacteria.

Conclusions: This hints at a much richer diversity of lanthanide biochemistry than thus far considered. Current research focuses on the expression and purification of the *Streptomyces* PVAdh, to study its biochemical properties, including the ability to bind lanthanides.

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E-Poster Viewing Topic: AS17 Microbial evolution and diversity

GENETIC DIVERSITY OF THE EMERGING HUMAN FUNGAL PATHOGEN PICHIA NORVEGENSIS

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Background and Aims: Background and aims: *Pichia norvegensis* (=*Candida norvegensis*) is increasingly isolated from hospital settings, especially from immunocompromised patients. Understanding this otherwise rare pathogen, including its emergence and distribution, is crucial for accurate diagnosis and infection prevention. We studied the genetic diversity of a large collection of clinical *P. norvegensis* isolates obtained from Dutch hospitals along a set of non-Dutch clinical and environmental isolates.

Methods: Methods: Clinical (n=236; 90.8%) and environmental (n=24; 9.2%) *P. norvegensis* isolates were subjected to Amplified Fragment Length Polymorphism (AFLP) fingerprinting and a novel six-loci microsatellite typing panel. Data was analyzed with BioNumerics and STRUCTURE. We applied a novel mating-type assay to determine the *MAT* α locus presence/absence.

Results: Results: AFLP fingerprinting separated the *P. norvegensis* isolates into three main clusters. Two clusters fully consist of clinical isolates, the third represented a mix of clinical and environmental isolates. By microsatellite typing the overall genetic diversity was low (Simpson's D=0.90), due to the large number of Dutch clinical isolates with similar genotypes. Minimum spanning tree-analysis showed that Dutch clinical isolates fell into two clusters. Environmental and non-Dutch isolates were more distantly related. STRUCTURE analysis showed the presence of four genotypes, with signs of genetic admixture between geographic locations and environmental/clinical isolates. Nearly all isolates harbor the *MAT* α mating-type allele.

Conclusions: Conclusions: The *P. norvegensis* isolates obtained from Dutch hospitals appeared to be largely clonal, independent of geographic origin and isolation date. The observed clonality is supported by the extensive number of $MAT\alpha$ isolates. Microsatellite typing indicated potential admixture between clinical and environmental isolates.

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E-Poster Viewing Topic: AS17 Microbial evolution and diversity

GENETIC DIVERSITY IN THE FNBA, HLA AND SSTD VIRULENCE GENES IN CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS CAUSING BACTEREMIA

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Background and Aims: Staphylococcus aureus (SA) is the main cause of bacteremia. Fatality rate is up to 20-40% of people with BSI (bloodstream infections). Most of virulence factors are genes whose expression is related to infection severity. Differences in the regulation of virulence factors expression among SA isolates have been associated with SA genetic diversity.

Methods: Next-Generation Sequencing (NGS) was used to study the relationship of the genetic variability of the aforementioned virulence factors to: gene expression regulation data obtained in a previous study, and to the genetic context of each isolate. Multilocus sequence typing (MLST) and Core Genome Single Nucleotide Polymorphisms (cgSNPs) were used to investigate the genetic clonality.

Results: There was no relationship found between gene expression patterns and genetic variants of virulence genes. These genetic variants seem to be related to clonal evolution of the clinical isolates. We identified, for the first time in Ecuador, the SA ST2625 SCCmec IVa clone, which has been linked to outbreaks in European pediatric units

Conclusions: The gene variants described provide valuable information for a better understanding of the pathogenicity of the clinical isolates studied. Interestingly, the variant genetic information might also be of value for the development of novel vaccines or targeted therapeutics. Information derived from whole genome analysis such as the one presented here, will become the foundation for future evidence-based decision making in clinical practice.

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E-Poster Viewing Topic: AS17 Microbial evolution and diversity

MOLECULAR EPIDEMIOLOGY OF STREPTOCOCCUS PNEUMONIAE ISOLATES CAUSING INVASIVE AND NON-INVASIVE INFECTION IN ETHIOPIA

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Background and Aims: Streptococcus pneumoniae, a bacterial commensal of the upper respiratory tract, is a major public health concern and ranging from invasive to non-invasive pneumococcal disease. It is responsible for substantial global morbidity and mortality due to pneumonia, meningitis sepsis and acute otitis media. The main goal of this study is to assess the molecular epidemiology of S. pneumoniae strains causing invasive and non-invasive infection in Ethiopia

Methods: A total of 54 S. pneumoniae isolates were collected from patients in Addis Ababa city and Amhara National Region State hospitals, 33 invasive and 21 from non-invasive infections. All isolates were analyzed by whole genome sequencing. Serotypes and multilocus sequence types were extracted from the genomic data. Antimicrobial susceptibility testing was performed using the E-test.

Results: Using the multilocus sequence typing scheme, 42 sequence types (STs) were identified, including 21 new ones. The predominant STs among the invasive isolates were ST3500, ST5368, ST11161, ST15425, ST15555, ST15559, and ST15561 (2/33, 6% each). These STs were associated with serotypes 8, 7C, 15B/C, 16F, 10A, 15B and 6A, respectively. Among the non-invasive isolates only ST15432 associated with serotype 23A was represented by multiple isolates (4/21, 19%). Serotypes 14 was the predominant penicillin G resistant whilst 3, 8, 7C and 10A isolates were resistant for erythromycin. Importantly, all 6A serotype isolates showed both erythromycin and penicillin G resistance.

Conclusions: Such analysis provides increased resolution on the circulating variants of S. pneumoniae. Continued genomic surveillance of pneumococcal population' dynamics post-vaccination, with increased geographical representation, is necessary for optimizing future vaccine design.

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E-Poster Viewing Topic: AS17 Microbial evolution and diversity

MOLECULAR PHYLOGENETIC RELATIONS BETWEEN DOMESTIC AND FOREIGN BRUCELLA ABORTUS STRAINS USING MLSA

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Background and Aims: Brucellosis is one of the important zoonotic diseases worldwide and still shows a high incidence in developing countries. Considering this status, tracking back the infection causes through molecular phylogenetic analysis is an important effort to prevent spreading the disease.

Methods: We analyzed the phylogenetic features using multi-locus sequence assay (MLSA) to clarify the genetic correlation of 301 *Brucella* (*B.*) *abortus* strains; 9 reference strains, 6 vaccine strains, 8 Mongol strains, 6 Thailand strains, and 272 Korea strains from domestic cattle in 8 regions. MLSA investigated with 18 specific single nucleotide polymorphisms(SNPs) of 14 genes that could display phylogenetic characteristics.

Results: All strains were divided into 14 sequence types(STs) which well displayed a phylogenetic tree constructed with all SNPs. The domestic strains were classified into ST1 to ST8, among them ST1 and ST3 account for 33.8% and ST1 is distributed nationwide over long periods. The strains from Mongol were included in ST11 to ST13, and those from Thailand in ST1 and ST11. A strain from Thailand was genetically correlated with certain strains from Korea, classifying into the same ST1, but the genetic correlation between Korea and Mongol strains was low.

Conclusions: Accordingly, we revealed genetic correlations between domestic and foreign *B. abortus* strains. These data can demonstrate *B. abortus* strains distribute across different countries in various types, being possible to widespread through all boundaries, as well trace back infection sources from overseas. In the future, we will verify the genetic relationship with strains from other countries and provide the database about additional infection sources.

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GENETIC CORRELATIONS OF BRUCELLA CANIS STRAINS ISOLATED IN SOUTH KOREA

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Background and Aims: Canine brucellosis, caused by *Brucella (B.) canis*, has been drawing attention as a public health risk by increasing the number of pet dogs, which are in close contact with owners. The molecular epidemiological analysis is indispensable to unearth reliable etiological evidence according to the genetic relationships of them.

Methods: To investigate genetic characteristics and relatedness among regional groups of *B. canis* isolates, multilocus sequence typing(MLST) analysis was conducted with 8 specific single nucleotide polymorphisms(SNPs).

Results: A total of 195 *B. canis* strains isolated from domestic dogs, confirmed to be sero-positive, in 12 regions of South Korea were divided into 11 different sequence types(STs). Most of them were consist of two main STs, ST1 and ST2. ST1 included 70 strains(35.9%) in five regions and ST2 included 64 strains(32.8%) in six regions. In Gyeonggi region, particularly, were distributed most of STs, which revealed these strains were related with most of strains in all parts of the country. The most strains isolated in Chungbuk region were in ST2(88.7%), correspondingly we estimated that the resident strains were circulating in this region and were even spreading to adjacent regions, including Gyeonggi and Chungnam, which having the same type.

Conclusions: Considerably, the current study revealed the genetic correlations among regional groups of *B. canis* isolates in South Korea. Furthermore, this molecular epidemiological approach can provide trace-back evidence to be a step forward for control of canine brucellosis.

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E-Poster Viewing Topic: AS17 Microbial evolution and diversity

A UNIQUE SUBFAMILY OF FUNGAL SELF-SUFFICIENT IN-CHAIN HYDROXYLATING CYTOCHROME P450 MONOOXYGENASES FROM THE ASPERGILLACEAE

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Background and Aims: The cytochrome P450 monooxygenases (CYP450s) are abundant in eukaryotes and specifically in stationary organisms like plants and fungi. In these organisms they play important roles in the synthesis and degradation of secondary metabolites making them important for the discovery and synthesis of new pharmaceuticals and agrochemicals. In eukaryotes the best studied "self-sufficient" CYP450s, with a fused redox partner, belong to the CYP505 family. We recently described a CYP505 from *Aspergillus terreus*, named CYP505E3, which gives remarkable in-chain hydroxylation of alkanes and fatty alcohols and showed that another six CYP505Es, sharing at least 75 % amino acid identity with CYP505E3, displayed the same unique activity. The aim of this study was to further explore the occurrence and possible function of the CYP505Es.

Methods: BLASTP searches were performed of the Uniprot and NCBI databases using CYP505E3. Multiple sequence alignments and phylogenetic analysis were done of 206 CYP505 sequences using MEGAX. Searches of the antiSMASH database (https://fungismash.secondarymetabolites.org/#!/start) were performed to link the CYP505E gene clusters to secondary metabolite biosynthetic gene clusters.

Results: A total of thirty-one CYP505Es were identified, all from the Aspergillaceae. Many *Aspergillus* and *Penicillium* spp have two CYP505s, but only a few have CYP505Es. These CYP505Es all occur in very distinct genetic environments which include a specific ABC transporter, but they could not be linked to a known secondary metabolite gene cluster.

Conclusions: The CYP505Es are unique in-chain hydroxylases that are limited to a few species of the Aspergillaceae where they are most likely involved in synthesis or degradation of a secondary metabolite.

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E-Poster Viewing Topic: AS18 New Insights into phage bacteria interactions

NEMATODE MEDIATION OF HOST-PHAGE INTERACTIONS IN SOIL

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Background and Aims: Bacteriophages, viruses that infect bacteria, are ubiquitous in the environment, with phage particles outnumbering bacterial hosts in many systems. Phages are also known to influence ecosystem dynamics at multiple levels through processes like top-down control of disease bacteria. Thus, knowledge about their ecology is useful for understanding biological phenomena at every scale. Yet, little is known about soil phage ecology. The porous structure of soil presents a challenge to phages, which must overcome adsorption, lack of pore connectivity, and relatively large distances between susceptible host populations to survive and replicate.

Methods: Here, we tested the hypothesis that bacterivorous nematodes are important vectors for phages, overcoming these barriers to provide active transport between patches of host bacteria while foraging. To test this hypothesis, we inoculated agar and soil microcosms with nematodes (*C. elegans*), host (*P. putida*), and phage (Phi Ppu-W11). We subsequently tracked the dispersal and abundance patterns of host and phage in a spatiotemporally explicit manner.

Results: Our results show nematode vectoring of phages and impacts on host-phage interactions as well as influence on resistance development in the host population.

Conclusions: Overall, this study demonstrates a new approach for testing spatiotemporally explicit hypotheses about host-phage interactions in soil, and suggests that third-party vectors may be critical for mediating such interactions.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

IMPROVEMENT AND EVALUATION OF PULLULAN BIOPOLYMER PRODUCED BY AUREOBASIDIUM MANGROVEI CBS 142331 IN ENHANCEMENT OF OIL RECOVERY

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Background and Aims: The world economy depends heavily on crude oil. With a conventional oil recovery process, only one-third of crude oil is extracted. Various technologies have been developed to maximize the recovery of oil resources from natural reservoirs. One of these is polymer technology that has been used in many oil fields around the world. The biopolymer pullulan, produced by A. mangrovei CBS 142331 showed high potential for EOR with the ability to increase oil recovery to a degree comparable to that achieved with many polymers used in oil fields around the world. However, pullulan is produced by the fungus simultaneously with a melanin pigment that is strongly attached to the polymer and considered an impurity for polymer flooding.

Methods: The aim of this study is to improve and optimize the yield and purity of pullulan biopolymer and investigate the effect of different environmental factors on the expression of the genes responsible for pullulan and melanin synthesis.

Results: Under optimized conditions, that is, sucrose as the carbon source in the medium, a pH of 9, incubation at 25 °C, and 250 rpm agitation, the fungus was able to produce 10 g/L of pullulan, which ultimately showed the ability to recover 36.7% of heavy crude oil. Melanin synthesis was strongly suppressed under nitrogen starvation where all the melanised cells transformed into transparent cells at the concentration of 30 g/L (NH₄)₂SO₄

Conclusions: It was concluded that pullulan production by A. mangrovei CBS 142331 with limited melanin synthesis can be achieved by controlling the nitrogen concentration in the

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

ANTIMICROBIAL ACTIVITY OF THE METABOLITES PRODUCED BY ENDOPHYTIC FUNGI ISOLATED FROM ARRABIDAEA CHICA

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Background and Aims: Endophytic fungi are microorganisms that live inside plant tissues without causing damage to the host. These organisms and have earned more space in the pharmacological field since they are sources of bioactive compounds. Therefore, the search for new substances with biological activities, such as antimicrobial ones, has increased, since the indiscriminate use of drugs has intensified the resistance of fungi and bacteria to the current drugs available on the market. In this sense, the objective of this study was to investigate the antimicrobial activity of metabolites of endophytic fungi isolated from the medicinal plant *Arrabidaea chica*.

Methods: The fungi are deposited in the Central Collection of Microorganisms from the Amazonas State University (CCM/UEA). The fungal metabolites were obtained by submerged culture, extracted with ethyl acetate and resuspended with 10% DMSO. The microdilution technique was used to verify the antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*.

Results: The fungal extracts did not show activity against the bacteria strains evaluated here. However, three of the isolated fungi showed action against the yeast *C. albicans*. The extracts obtained from the fungi CF1-8, CF1-23 and CF1-29 presented fungistatic activity at a concentration of 5 mg/mL. Using TLC technique, we found the presence of phenolic compounds and flavonoids in the active extracts.

Conclusions: The endophytic fungi isolated from *A. chica* have phenolic compound that may be responsible for the activity agaisnt *C. albicans.* These results are in acordance with the fact that this plant species has been used as an agent for the treatment of vaginal candidiasis.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

PIGMENT PRODUCTION BY HYPOXYLON INVESTIENS ISOLATED FROM THE MEDICINAL PLANT ARRABIDAEA CHICA

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Background and Aims: The demand for natural products has increased the global market for natural pigments. This is due to the proven harmful effect of synthetic pigments, in addition to their disposal, which present negative effects on the environment. Endophytic fungi can serve as a source of pigments. Fungi present relatively rapid growth and produce a high yield of pigments if the cultivation conditions are optimized. Another advantage of using endophytic fungi is the fact that the production of fungal pigments has a lower production cost and does not depend on climatic conditions and seasonality. In this sense, the objective of this study was to investigate the production of extracellular pigment by the endophytic fungus *Hypoxylon investiens* isolated from the leaf of the medicinal plant *Arrabidaea chica* (Bignoniaceae).

Methods: Submerged fermentation was performed in two culture media: (1) potato, dextrose, yeast extract and NaCl, pH 5.0; and (2) potato, dextrose, peptone, pH 5.0. The fungus was cultivated at 30 °C, in stationary condition, with no light for 14 days. The culture was filtered and the absorbance was verified (350 to 700 nm).

Results: It was observed that the fungus *H. investiens* produced extracellular pigment in green color only in the medium that contained peptone, thus showing that peptone may be the adequate nitrogen source to produce extracellular pigment in this fungal species.

Conclusions: To our best knowledge there are no reports in the literature concerning the production of extracellular pigment by *H. investiens*. Therefore, the cultivation conditions must be optimized and the pigment fully characterized.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

DEVELOPMENT OF MICROBIAL FORMULATIONS FOR THE BIOREMEDIATION OF SOIL CONTAMINATED WITH PLASTICS

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Background and Aims: The massive production of petroleum-based plastics and the lack of efficient treatments at their end-of-life, has led to their accumulation in the natural environment, causing serious imbalances in ecosystems. By extension, agricultural land is not exempted from this problem, in this case, aggravated by the practices adopted in intensive agricultural models. In this work, different carriers were evaluated to harbor plastic-degrading microorganisms to be applied in soils and composting materials contaminated with plastics.

Methods: Three types of carriers were used, including vermicompost, biochar and alginate beads, to which pure and mixed cultures of plastic degrading *Bacillus* and *Pseudomonas* were added. Cell viability and enzymatic activities related to plastic degradation of the inoculated carrier were assessed after storage at room temperature for 30, 60 and 90 days. Finally, the efficacy of the formulations for plastic mineralization was validated at lab-scale by measuring the CO₂ emission after incubation for 2 months.

Results: The results showed that alginate beads ensured a higher survival rate and maintenance of plastic degrading capabilities in both consortium and independent strains; while in compost and biochar important variations were observed depending on the strain and time.

Conclusions: The results obtained in this study lay the groundwork for future field-scale trials, and provide new technologies for the decontamination of plastic waste in the context of the circular economy. This project received funding from the Bio based Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 887648.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

ENGINEERING THE OUTER MEMBRANE PORIN OMPF FOR DRAINING EXTRACELLULAR AMYLOIDS

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Background and Aims: Protein amyloid aggregates are ubiquitous in natural environments. They typically originate from microbial secretions (1) or spillages from mammals infected by prions (2), raising concerns about their infectivity and toxicity in contexts such as gut microbiota or soils. Therefore, the development of tools to monitor their presence for enabling bioremediation is of utmost interest.

Methods: *Escherichia coli* outer membrane protein engineering (mutagenesis, 3D modeling). Binding of peptide probes to bacterial cells and bacteria to proteins on arrays. Fluorescence microscopy.

Results: Exploiting the self-assembly potential of amyloids for their scavenging, here we report, on the abundant *Escherichia coli* outer membrane porin OmpF (3), the insertion in one of its extracellular loops (L5) of an amyloidogenic sequence stretch from a model bacterial prion-like protein (RepA-WH1) (4). The expression of this grafted porin enables bacterial cells to trap on their envelopes the same amyloidogenic sequence when provided as an extracellular free peptide. Conversely, when immobilized as bait on a surface, the full-length prion-like protein including the amyloidogenic peptide can catch bacteria displaying the L5-grafted OmpF. Polyphenolic molecules known to inhibit amyloid assembly interfere with peptide recognition by the engineered OmpF, indicating that this is compatible with the kind of homotypic interaction expected for amyloid assembly.

Conclusions: Our study (5) suggests that synthetic porins may provide suitable scaffolds for engineering prion biosensor and clearance devices. (1) *Nat. Rev. Microbiol.* **19**:241 (2021) (2) *Microbiol. Mol. Biol. Rev.* **81**:e00001-17 (2017) (3) *J. Biol. Chem.* **295**:10340 (2020) (4) *mSystems* **5**:e00553-20 (2020) (5) *ACS Synth. Biol.* **11**:655 (2022)

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

PRELIMINARY RESULTS FROM THE ASSESSMENT OF PHOTOSYNTHETIC ORGANISMS IN PHOTOBIOREACTORS FOR IMPROVING RESOURCES RECOVERY AND WASTEWATER REUSE

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Background and Aims: Membrane photobioreactors (MPBR) combine the development of phototrophic and photosynthetic microorganisms with membrane ultrafiltration with the objective of improving wastewater reclamation.

Methods: Two laboratory-sized MPBR (A and B) were operated at different photoperiods (on/off): 9/15 and 12/12, respectively. They were inoculated with autochthonous microalgae-bacteria consortia from a pilot PMBR and fed with an anaerobic effluent. Three samples collected from each MPBR after 11, 41 and 55 days, were analyzed. Samples were visualized in a Leica DM750 microscope and images were obtained using LAS EZ software (Leica). Identification was performed using Berk and Gunderson Atlas. Quantification was obtained as the average from 20 fields counts and expressed as microorganisms/ml.

Results: All three groups of microorganisms analyzed increased their concentration from the first sample to the second one, showing a significant development and growth. However, in the last samples, concentrations remained stable or slightly decreased, suggesting a decline in the system evolution. Within the Green Algae group, the genera *Scenedesmus* and *Chlorella* were predominant, and in the last sample, *Chlorella* showed a higher concentration. Diatoms were mostly represented by *Nitzschia*genus. And among the Cyanobacteria, the order Oscillatoriales and the genus *Microcoelus* were identified.

Conclusions: Photosynthetic groups are able to reach a stable community improving the quality of the effluents and resources recovery. Conditions for the growth of the photosynthetic group need to be monitored to keep the optimal conditions for their maintenance. Acknowledgements: This work is part of the RTI2018-B-100-093736 project, funded by the Spanish Ministry of Science and Innovation (MCI), National Agency for Research (AEI) and European Regional Development Fund (ERDF).

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

ELUCIDATION OF THE ROLE OF A VEGETATIVE MYCELIUM SPECIFIC HYDROPHOBIN VMH3 FOUND IN WHITE-ROT FUNGUS PLEUROTUS OSTREATUS

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Background and Aims: Hydrophobins, which are small-secreted proteins with both hydrophobic and hydrophilic parts, can self-assemble into an amphiphilic film. In Schizophyllum commune, hydrophobin SC3 secreted by emerging aerial hyphae self-assembles at the cell wall–air interface and allows the formation of aerial mycelia. In white rot fungus Pleurotus ostreatus, one hydrophobin family 'Vegetative Mycelium specific Hydrophobin (Vmh)' has been characterized with similar expression pattern to SC3. The vmh3 has been reported with high expression level in vegetative growth stage of monokaryon or dikaryon. No research has been done about the function of hydrophobin to saprophytic patterns in agaricomycete. Therefore, we try to elucidate function of Vmh3.

Methods: The vmh3 was disrupted in the 20b strain (ku80 disruptant) by homologous recombination. The Δ vmh3 and 20b strain was cross with #64 (vmh3⁺) strain for fruiting body formation.

Results: The Δ vmh3 was shown decreased lignin degradation on 20 and 30 days compared with 20b on beech wood sawdust medium. Deletion of vmh3 reduced the hydrophobicity of mycelia. As a result of fruiting body formation, Δ vmh3×#64 displayed slow fruiting comparing with 20b×#64.

Conclusions: These data may provide new insights into the relationship between hydrophobins and wood decay mechanism in Agaricomycetes. Additionally, the Vmh3 might be required for fruiting body formation in P. ostreatus.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

FATTY ACIDS SECRETION BY WHITE ROT FUNGUS, TRAMETES VERSICOLOR

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Background and Aims: Fungi can acquire and store nutrients through decomposing and converting organic matter into fatty acids. This research demonstrates for the first time that the white-rot fungus Trametes versicolor has the ability to secrete extracellular droplets which can contain a high concentration of long chain fatty acids and unsaturated fatty acids as well as monosaccharides and polysaccharides.

Methods: Ref: Hao, G., & Barker, G. C. (2021). Fatty acid secretion by the white rot fungus, Trametes versicolor. Journal of Industrial Microbiology and Biotechnology.

Results: Ref: Hao, G., & Barker, G. C. (2021). Fatty acid secretion by the white rot fungus, Trametes versicolor. Journal of Industrial Microbiology and Biotechnology.

Conclusions: Our research shows for the first time that the white-rot fungus, Trametes versicolor has the ability to secrete short lived extracellular droplets that contain long chain fatty acids with health benefit and also monosaccharides. The composition of these droplets varied according to their age and the feedstock used for growth of the fungi. Our findings would suggest that the mycelium is synthesising fatty acids which are then transported via the mycelium and secreted, forming these droplets. Although the exact reason for the droplet formation is unclear, the utility of their constituents in improving health makes further investigation important.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

CRISPR-CAS9-MEDIATED DISRUPTION OF THE QDR2 MULTIDRUG TRANSPORTER GENES OF MUCOR LUSITANICUS

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Background and Aims: Mucor lusitanicus is an opportunistic human pathogenic fungus causing a frequently fatal infection, mucormycosis, in immunocompromised patients. It is resistant to most clinical antifungals. One possible mechanism of antifungal resistance could be the drug extrusion from the cytoplasm to the outer medium by multidrug transporters. Qdr2 is a multidrug transporter of the major facilitator superfamily that can efflux the antimalarial drug quinidine and the herbicide barban in Saccharomyces cerevisiae. Our research is focused on the clarification of function of the qdr2 genes in Mucor.

Methods: CRISPR-Cas9 system was used to create qdr2a, qdr2b, qdr2c and qdr2d single knock out mutants of M. lusitanicus. The double-strand break caused by the Cas9 enzyme was repaired by the non-homologous end-joining.

Results: Transcription analyses revealed that qdr2b and qdr2d were up-regulated in \triangle qdr2c mutant; qdr2c was up-regulated in \triangle qdr2b and \triangle qdr2d. The loss of qdr2d caused upregulation of qdr2c and qdr2c disruption led to qdr2d upregulation. The loss of qdr2b, qdr2c and qdr2d genes may be compensated by each other since they have overlapping expression patterns. Disruption of all four QDR2 genes has no effect on the growth in the presence of cell wall stressors. Spore production ability of the QDR2 deletion mutants was significantly lower than that of the control strain. In the Galleria non-vertebrate model, qdr2c and qdr2d deletion mutants had significantly decreased virulence.

Conclusions: These results suggest that QDR2 genes may have a role in the pathogenicity of M. lusitanicus. The study was supported by the grants NKFI K131796 and ITM NKFIA TKP-2021-EGA-28.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

SECRETION SIGNAL OF PEROXIDASE FROM MOSS, PHYSCOMITRIUM PATENS, FUNCTIONS IN ESCHERICHIA COLI.

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Background and Aims: Secretion techniques for proteins are important in simplifying the purification of the products using heterogeneous host systems. Here we present a peroxidase (Prx34, XP_024372504) from a moss, Physcomitrium patens, has a secretion signal sequence functioning also in Escherichia coli. The Prx34 was found as a peroxidase released from chitosan-treated P. patens (Lehtonen et al., New Phytologist 2009).

Methods: The whole and N-terminus deleted Prx34 sequences, with and without His-tag, were expressed in E. coli (Rosetta DE3). The putative signal sequence, 32 amino acids of the N-terminus, was also connected to EGFP and the expression was observed .

Results: High peroxidase activity was only detected in the medium of E. coli expressing the whole sequence of Prx34. The putative signal region could be cut during secretion, since the product was not bound to cobalt column when the N-terminus was labeled with His-tag. Fluorescence was observed in the medium of E. coli expressing the signal connected to EGFP. The GFP signal was also detected at the periplasmic fraction.

Conclusions: These results indicate that the signal sequence functions as a secretion signal for E. coli. This is the first report to our best knowledge that a plant signal peptide also functions in E. coli, whereas several sequences of yeast and animals have been reported to act for secretion in E. coli. The signal peptides of Prx34 will contribute to the study of heterogeneous protein production using E. coli system. We are grateful to Dr. Jari P.T. Valkonen for his collaboration.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

ESTIMATION OF THE POTENTIAL PRODUCTION OF VALUABLE COMPOUNDS WITH ANTIOXIDANT PROPERTIES BY DIFFERENT MICROALGAE

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Background and Aims: Background: Microalgae are widely known for their nutritional and therapeutic applications due to the richness in nutrients and bioactive elements. The aim of this research was to investigate the growth and production of bioactive compounds with antioxidant properties by different microalgal strains: Chlorella vulgaris R-06/2; Scenedesmus acutus M Tomaselli 8, Scenedesmus obliquus BGP, Porphyridium aerugineum and Porphyridium cruentum (Chlorophyta and Rhodophyta). Most of them are freshwater species, with only one marine microalga P. cruentum.

Methods: Monoalgal, non-axenic cultures of the investigated strains were grown autotrophically in 200 ml flasks, CO2 - 2% at 132 µmol m-2 s-1 photon flux density and T 25°C. Algal biomass concentration was measured daily by the dry weight. The content of polyphenols, flavonoids and ferric reducing ability of algae were measured at the end of the cultivation process, when stationary phase of growth was reached

Results: The highest biomass yield was achieved by Scenedesmus obliquus BGP- (6.6 g/L) after 144 hours of cultivation. Ch. vulgaris showed much higher levels of polyphenols and ferric reducing ability of plasma (FRAP), showing the best antioxidant properties from the assessed strains. The red microalga Porphyridium aerugineum also exhibits promising reducing antioxidant power.

Conclusions: Conclusion: This study confirmed the view that microalgae are promising producers of food supplements and pharmaceuticals. Acknowledgement: This work was supported by the Bulgarian National Science Fund, Ministry of Education and Science, grant number KII-06-OIIP04/1

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

BIOMASS PRODUCTION AND ANTIBIOTIC SENSITIVITY OF CHLORELLA SOROKINIANA UNDER HETEROTROPHIC CULTIVATION

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Background and Aims: Normally, microalgae are considered as photoautotrophic organisms, whereas heterotrophic cultivation has also been used to obtain high-value products. Microalgal hosts have been continually developed for expression. The research about heterotrophic microalgal host is still required. In this study, fast-growing microalga, Chlorella sorokiniana AARL G015 was observed for a growth in different mediums to enhance biomass. The antibiotic sensitivity was tested under dark cultivation. These assessments were tested in order to develope this strain for being heterotrophic microalgal host and efficient screening and selection of transformants.





Methods: Growth optimization of three medium including mJM, mBG11, and ISP2 were determined by measuring growth under dark mode through optical density, cells count, and dry cell weight. The sensitivity of the microalga was tested under 9 antibiotics, which are commonly used as a selectable marker assessed through liquid cultivation on suitable mBG-11 under dark cultivation.

Results: Among these media, the best growth of this strain was achieved under mBG-11 given maximum biomass at 5.5 g/L. The mBG-11 was selected as a heterotrophic medium. The highest sensitivity on growth was G418 which showed maximum suppression at 95% inhibition, following with 92% inhibition of hygromycin, and 92% inhibition of streptomycin at 1 mg/mL.

Conclusions: This study found that mBG-11 enhanced biomass of Chlorella sorokiniana AARL G015. All three antibiotics can suppress the growth in heterotrophic cultivation; thus, these antibiotics could be used as selectable markers for this strain. The characterized properties of this organism seem to have better potential as a heterotrophic host for gene expression or industrial production.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

ECO-FRIENDLY APPROACH FOR THE CONTROL OF FOLIAR PHYTOPATHOGENIC FUNGI VIA THE APPLICATION OF AQUEOUS COMPOST EXTRACTS

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Background and Aims: Numerous studies highlight the ecological importance of using compost extracts for the control of phytopathogenic fungi. In fact, this type of product is considered today an interesting alternative to the intensive use of chemical fungicides. This work was focused on the physicochemical and biological characterization of aqueous extracts obtained from different compost using several procedures, as well as on determining their capability to inhibit the growth of *Alternaria sp.* BIO-175, a phytopathogenic fungus.

Methods: For this, compost samples were subjected to different extraction protocols (CEP1, CEP2, CEP3, CEP4). CEP1 and CEP4 were incubated at room temperature for 48h and 14 days, respectively; CEP2 was incubated for 24h at 40 °C, and CEP3 was incubated for 12h at 70 °C. Stirring was applied at 120 rpm in all cases, except in the CEP4 protocol (static). The extracts obtained were used to determine pH, electrical conductivity, phenolic content, and the enzymatic profile related to the capacity to suppress plant pathogens. Finally, the ability of the extracts to inhibit the *in vitro* growth of *Alternaria sp* was also determined.

Results: CEP1 and CEP4 extracts showed the most diverse enzyme profiles, whereas the CEP3 protocol resulted in extracts with less functional diversity. In turn, CEP2 and CEP4 extracts significantly inhibited the growth of *Alternaria sp.* compared to the other extracts.

Conclusions: Raw material and extraction protocol significantly influenced the pH and phenolic content of the extracts, as well as the ability to inhibit the *in vitro* growth of *Alternaria sp.* Work funded by the project FEDER-UAL2020-BIO-B1964.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

PROTEIN FUNCTIONAL ELUCIDATION IN BACTERIA _ PROTEIN FUNCTIONAL CHARACTERIZATION IN VIVO

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Background and Aims: Recently, much attention is being paid to approaches for protein functional characterization performed within the native bacterium but they are hindered by the difficulty to manipulate the host bacterium or to perform analyses *in vivo*. In this study, we used cell-penetrating peptides (CPPs) in combination with peptide nucleic acids (PNA) as a simple and effective approach to overcome these challenges.

Methods: Here, *Paenibacillus* sp. strain YYML68, was used as the model. Prior to employing CPP-PNA in protein function elucidation, its applicability was evaluated by designing a CPP-PNA probe designed to target the mRNA of the acyl carrier protein (AcpP) within strain YYML68. Next, two candidate iota-carrageenases, CgiA and CgiB, were identified from the draft genome and CPP-PNA probes designed and synthesized were used to verify iota-carrageenan degradation. Finally, CgiA and CgiB were 3D structurally modelled and compared with reported enzymes to elucidate its characteristics.

Results: In our initial evaluation on the applicability of CPP-PNA, we showed that CPP-PNA inhibited the growth of strain YYML68 in a concentration dependent manner with concentrations >6 μ M being the most effective. Subsequently, when 6 μ M was used to inhibit the degradation ability of CgiA and CgiB, only CgiB showed the ability to degrade iota-carrageenan. Interestingly, our results from the functional analysis of CgiA and CgiB, together with the 3D predicted structure showed new insights to the function of iota-carrageenases.

Conclusions: In conclusion, we showed that CPP-PNA was an effective tool to perform protein functional characterization *in vivo* and was applicable with hard-to-transform strains such as *Paenibacillus*.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF ALPHA-1,3-GLUCAN SYNTHASE GENE IN PLEUROTUS OSTREATUS

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Background and Aims: The cell wall of filamentous fungi is an essential structure for hyphal growth. The cell wall polysaccharides in Agaricales have been reported several beneficial characters as pharmacological agents and for the material production. However, the regulation and biosynthesis of cell wall in Agaricales has not been elucidated well. In this study, we analyzed cell wall contents and a gene for α -1,3-glucan (α -glucan) synthesis in Pleurotus ostreatus. The biosynthetic gene was identified, and its function was analyzed.

Methods: From a result of BLAST search using α -glucan synthase (ags) genes from Aspergillus oryzae as a query, a homolog named ags1 was identified in P. ostreatus genome. This gene was disrupted in the 20b strain (ku80 disruptant) by homologous recombination. The scanning electron microscope and the transmission electron microscope was used for electron microscopy observation. Cell wall polysaccharides were analyzed by HPAEC-PAD system after fractionation of the cell wall.

Results: Analysis of the monosaccharides that make up the cell wall revealed that the distribution of cell wall polysaccharides in P. ostreatus is quite different from that of the Ascomycota. The α -glucan content of the Δ ags1 strain was significantly reduced. Electron microscopy observation of the Δ ags1 strain showed thinner cell wall and lower hyphal density. In addition, increased sensitivity against chitin and β -glucan synthesis inhibitors was observed in the Δ ags1 strain.

Conclusions: The ags1 is essential for cell wall integrity of P. ostreatus through the formation of a-glucan.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

APPLICATION OF CELL-PENETRATING PEPTIDES IN DIVERSE BACTERIAL STRAINS FOR BIOMOLECULE DELIVERY

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Background and Aims: Technology for delivery of biomolecules into cells is essential for the effective utilization of bacteria. In this work, we focused on the application of cell-penetrating peptides (CPPs) to develop a simple, efficient, and versatile biomolecule delivery system directed towards both gramnegative and positive strains.

Methods: To achieve our goal, we first evaluated and optimized the conditions for CPP permeation based on the abiotic factors affecting its uptake and the design of the CPP itself. Next, based on the results from this evaluation, we designed CPPs comprised of amphipathic and cationic properties and evaluated their permeation efficiency and applicability against diverse strains of gram-negative and positive bacteria.

Results: Using *E. coli* and bacterial strains from the Enterobacteriaceae family as models, we showed that temperature and solution tonicity played a major role in promoting CPP permeation. In our evaluation on the design of CPPs, we observed that altering the side chains of the amino acid residues also influenced the permeation efficiency and versatility of CPP. Finally, using our optimized conditions, we found that permeation efficiency between gram-negative and positive bacteria differed where higher permeation efficiency was attained from gram-negative bacteria when amphipathic CPP were used while cationic CPP was more efficient with gram-positive strains. Furthermore, in both cases, CPP permeation did not show cytotoxic effects to the test strains.

Conclusions: In summary, we found that CPP can be applied to diverse bacterial strains and that it has the potential to serve as an effective tool for biomolecule delivery.

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ISOLATION OF A GENE ESSENTIAL FOR BASIDIOSPORE PRODUCTION IN PLEUROTUS OSTREATUS BY A COMBINATION OF TRANSCRIPTOME ANALYSIS AND CRISPR/CAS9

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Background and Aims: A sporeless strain is an important breeding target in the mushroom industry. However, to our knowledge, no gene in which mutation completely impairs basidiospore production has been identified in cultivated mushrooms to date. In this study, we propose a strategy for efficient isolation of genes essential for basidiospore production in the oyster mushroom *Pleurotus ostreatus*.

Methods: Total RNAs were extracted from each tissue (gills, stipes, and pilei) of fruiting bodies of *P. ostreatus* strain N001 (PC9×PC15), followed by RNA-seq analysis to find genes specifically expressed in gill where basidiospores are formed/produced. Plasmid containing expression cassettes for the hygromycin B-resistance gene, Cas9, and gRNA targeting a candidate gene was introduced into the dikaryotic PC9×#64 to introduce a mutation into both alleles in the paired nuclei using CRISPR/Cas9.

Results: Based on the results of RNA-seq, a gene encoding a putative protein related to basidiospore formation was selected as a target. After formation of a fruitbody, no basidiospore was observed in three out of 13 obtained transformants, while the number of basidiospores produced by the remaining ten transformants and the parental control PC9×#64 was $3.6 \times 10^8 - 2.6 \times 10^9$. Genomic PCR and DNA sequencing analyses indicated that the gene mutations had been introduced in both nuclei of the three transformants without spore production.

Conclusions: The results suggest that the gene is essential for basidiospore production and a good target for breeding of sporeless strain in *P. ostreatus*. The demonstrated strategy would be useful in efficient/smart isolation of genes that are essential for basidiospore production.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

MARKER-FREE GENOME EDITING IN THE EDIBLE MUSHROOM, PLEUROTUS OSTREATUS, USING TRANSIENT EXPRESSION OF GENES REQUIRED FOR CRISPR/CAS9 AND FOR SELECTION

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Background and Aims: Genetic transformation is a basic tool for the functional analysis of the genes of interest, as well as for molecular breeding. In a previous study, we reported a transient transformation system using repeated screening for hygromycin B (Hyg) resistance in the basidiomycete Ceriporiopsis subvermispora. In the present study, by combining this technique with CRISPR/Cas9, we demonstrated successful marker-free genome editing in Pleurotus ostreatus, which is one of the most economically important cultivated mushrooms as well as a model white-rot fungus.

Methods: The possibility of selecting transformants by transient expression of marker genes was tested using a plasmid carrying the Hyg resistance gene (hph) in P. ostreatus. Genome editing of fcy1, of which disruption confers 5-fluorocytosine (5-FC) resistance to the host cell, was tried by the transient expression of Cas9, gRNA, and hph. Genome editing of fcy1 in these strains was confirmed by Sanger sequencing.

Results: Transient expression of hph can be used to select the transformants. Then, marker-free genome editing was achieved by the transient expression of Cas9, gRNA, and hph.

Conclusions: To our knowledge, this is the first report of marker-free genome editing through the transient expression of Cas9, gRNA, and hph in agaricomycetes, which opens the door for repeated genome editing in these fungi. Our new genome-editing system is expected to acquire fewer off-target effects by CRISPR/Cas9 and allow the repeated use of the selection marker to investigate the functions of multi-copy genes.

EP160 / #365

E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

AQUEOUS COMPOST EXTRACTS AS A SUSTAINABLE ALTERNATIVE TO THE ABUSIVE USE OF CHEMICAL FERTILIZERS

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Background and Aims: The continuous raise of the world's population has required an intensive use of agrochemicals to provide food resources. The exacerbated application of these chemicals has had direct negative effects on the environment, soils and even human health. In order to deal with this situation, organic fertilizers and humic amendments such as compost and its derivatives which not only provide plant nutrients but also microorganisms, are a convenient alternative.

Methods: The main objective of this work was to select protocols for the preparation of compost-based aqueous extracts having biofertilizing activity. For that, four extraction protocols differing on temperature and time incubation were applied to compost samples obtained from agri-food waste. Subsequently, a physicochemical and biological characterization of these extracts was carried out by analyzing pH, conductivity, Total Organic Carbon (TOC) and Biological Oxygen Demand (BOD). In addition, the phytostimulant capacity of each of them was determined by calculating the Germination Index (GI) of *Lepidium sativum* seeds and microbial functional diversity was evaluated using *Biolog EcoPlates*.

Results: In general terms, the TOC content showed noticeable differences between protocols. Extracts obtained at 70 °C had a highly phytotoxic character and the highest BOD values, while those obtained applying mild thermal conditions showed phytostimulant character and had high functional biodiversity.

Conclusions: Based on the results obtained, applying an optimal compost extraction protocol would lead to biofertilizers, which will enrich microbial soil functionality and provide beneficial effects to plant. Work funded by the project FEDER-UAL2020-BIO-B1964.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

EXPLOITING REGULATORY CIRCUITS TO UNLOCK THE BIOSYNTHETIC POTENTIAL OF ACTINOMYCETES

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Background and Aims: Actinomycetes, particularly the genus *Streptomyces*, are the most versatile producers of natural compounds. They synthesize about two-thirds of all antibiotics known to date. The antibiotic biosynthesis in these organisms is highly coordinated and subject to diverse environmental and physiological (pre)conditions. Important nodes of regulation are represented by pleiotropic, as well as pathway-specific regulators. A better understanding of the regulatory circuits controlling antibiotic biosynthesis and the targeted use of regulators can help to exploit the full biosynthetic potential of actinomycetes. In my talk, I will give an example how pathway-specific SARP-type regulators can be used to activate (silent) gene cluster expression in actinomycetes.

Methods: SARP regulators occur in many different actinomycetes (e.g. *Streptomyces, Salinispora, Micromonospora, Amycolatopsis*, etc.), where they act as transcriptional activators of different types of secondary metabolites. By using the SARP regulatory binding sequence as a search motif on actinobacterial genomes it is possible to predict the BGCs that will be activated upon SARP expression in the respective host strain.

Results: As a proof of principle, we have activated the silent undecylprodigiosin gene cluster in *Streptomyces lividans* by overexpressing the SARP-type regulator PapR2 from *Streptomyces pristinaespiralis*. Furthermore, we were able to activate an amicetin/plicacetin gene cluster in the novel Indonesian strain isolate *Streptomyces* sp. SHP22-7.

Conclusions: The advantage of the newly developed SARP-based activation strategy is that it allows to activate antibiotic gene clusters in diverse actinomycetes in a relatively easy but targeted manner.

EP162 / #392

E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

POTENTIAL FOR BIOTECHNOLOGICAL APPLICATIONS AND BIOLOGICAL CONTROL ACTIVITY OF AGRICULTURAL SOIL BACTERIAL ISOLATES

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Background and Aims: Excessive use of agrochemicals as pesticides and fertilisers to increase agricultural production deteriorates soil quality and has an environmental impact. A more ecologically balanced agriculture is possible by using strategies based on the beneficial effects of soil microorganisms. In the present study, a collection of soil bacteria was isolated and characterised to determine their potential for improving agricultural production.

Methods: Bacterial isolates were identified by partial 16S rRNA gene sequencing, and were tested for their ability to biofertilization concerning phosphorus, nitrogen and iron, as well as bioproduction of exoenzymes concerning organic compound recycling. Their antagonism activity against phytopathogenic fungi as *Verticillium dahliae* and *Fusarium pseudograminearum* was also considered.

Results: Isolates were mostly *Bacillus* spp., and almost 100% of them positive in at least one of the tested activities. Phosphates were solubilized, atmospheric nitrogen was fixed, siderophores were produced and hydrolytic enzymes were synthesized in 15%, 66%, 49% and 94% of the bacterial isolates, respectively. Effective inhibitory activity on *V. dahliae* growth was observed in 26 of the isolates by diffusible compounds, with two of them also producing VOCs (*volatile organic compounds*) against this pathogen. The same two isolates produced VOCs against *F. pseudograminearum*, which was additionally inhibited by VOCs from two other of the tested isolates.

Conclusions: The high activity observed in these soil bacterial isolates points out to their great potential to contribute efficiently to the sustainability of the agrosystems. BM is a grantee of a Training Fellowship by IMIDRA. Authors thank funding from IMIDRA and BACPLANT Group from Universitat de València.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

FORENSIC APPLICATION OF ORAL BACTERIAL DNA DETECTION FOR THE IDENTIFICATION OF HUMAN SALIVA

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Background and Aims: Forensic microbiology aims to resolve legal issues through the use of the unique microorganisms found in the environment and human body. Saliva is one of the forensically relevant body fluids, and its identification using biological markers, from casework samples, contributes to proving criminal acts. This study evaluated the applicability of a novel oral bacterial DNA marker to identify human saliva.

Methods: To detect Neisseria mucosa DNA from forensic saliva samples, an asd gene-targeted PCR primer set (amplicon size: 79 bp) was newly designed using the Primer-BLAST software. The optimization of the PCR condition and verification of the amplification products were conducted by touchdown endpoint PCR using genomic DNA of N. mucosa JCM12992. Subsequently, the animal species specificity of the N. mucosa DNA marker was evaluated by quantitative PCR using human, dog, and cat saliva stains.

Results: Single PCR amplification products with expected sizes were obtained from N. mucosa DNA. Non-specific amplicons were not observed from negative control. N. mucosa DNA was detected from all human saliva stains, and the relative DNA quantity in the human saliva was significantly higher than that of others.

Conclusions: Our results indicate that N. mucosa DNA could be detected from saliva stain samples, and its relative quantity could distinguish the saliva of humans from that of other animals. We consider the newly designed N. mucosa DNA marker to be a useful saliva marker in forensic casework.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

PROCESS OPTIMIZATION FOR EFFECTIVE HYDROLYSIS OF MUSHROOMS USING LOW-COST CRUDE MULTI-ENZYME EXTRACTS

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Background and Aims: Pre-treatment of mushrooms with acids, alkali, or enzymes is one method for improving the recovery of bioactive compounds. The aim of this study is to determine the best reaction conditions for mushroom hydrolysis using low-cost crude enzymatic extracts.

Methods: Initial screening of single parameters for mushroom hydrolysis was conducted using one variable at a time method and the results were then optimized by employing response surface methodology based on central composite design (CCD) values to achieve maximum response. A five-level, four-factor CCD was used to fit a second-order response model. Glucose yield from mushroom hydrolysis was analyzed with a second-order polynomial equation and data were fitted to the equation by a multiple regression procedure. Three-dimensional surface plots were drawn to show the effect of the independent variables on the response, and a quadratic polynomial equation was proposed to describe the mathematical relationship between the variables and the response. Experiments were performed to validate the predicted optimum response using the maximum conditions predicted by the model.

Results: The model developed by this study predicted 1.49 mg mL⁻¹ of glucose from mushrooms when hydrolyzed at optimal conditions of pH 6.5, temperature 50°C, and enzyme loading of 5% (v/v) for 12h. Experiments performed under these conditions yielded glucose concentration 1.1 fold higher than the value predicted by the model.

Conclusions: This study demonstrated that crude enzymatic extract is effective in hydrolyzing mushrooms under optimal conditions. Furthermore, the study found that pineapple peel is the best feedstock for producing enzymatic extracts under solid-state conditions for mushroom bioprocessing.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

A NOVEL A-1,3-GLUCAN PROBE CONSISTING OF A-1,3-GLUCAN-BINDING DOMAINS AND A TETRAMERIC RED FLUORESCENT PROTEIN

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Background and Aims: The cell wall of filamentous fungi is composed of multiple polysaccharides, which are responsible for maintaining cell morphology and protection against external stresses. Since the interest in fungal cell walls has turned to their function and application in fungal control, the visualization of the fungal cell walls is considered important. In our previous study, α -1,3-glucan (α -glucan) binding domains DS1, CB6 and DS2 (DCD) from Agl-KA (α -1,3-glucanase from *Bacillus circulans* KA-304) were fused with AcGFP1 (monoGFP) to detect α -glucan in *Aspergillus oryzae* and successfully detected. In this study, we aimed to improve binding activity of fluorescent probe for α -glucan by using a tetrameric red fluorescent protein DsRed Express2 (tetraRFP). Whereas DCD-monoGFP has three binding domains, DCD-tetraRFP is expected to have twelve binding domains per protein, which should increase binding strength.

Methods: DCD-tetraRFP and DCD-monoGFP expression vectors were constructed and expressed in *E. coli*. The weight and size of these proteins were compared by gel filtration chromatography and dynamic light scattering, respectively. Substrate binding activity was measured by pull-down assay and quartz crystal microbalance (QCM) method.

Results: As expected, DCD-tetraRFP was observed to be heavier and have a larger particle size than DCD-monoGFP. Furthermore, DCD-tetraRFP had higher substrate binding activity than DCD-monoGFP in the pull-down assay and the QCM measurement.

Conclusions: The steric arrangement technique for binding domains with a multimeric fluorescent protein core developed in this study can enhance the binding ability of α -glucan fluorescent probes. This technology is expected to be applied to other cell wall polysaccharide probes such as β -glucan and chitin.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

SUSTAINABLE BIOLOGICAL AMMONIA PRODUCTION USING YEAST

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Background and Aims: Ammonia, with a worldwide production of 235 million tonnes is one of the main chemical commodities produced, extensively used as fertilisers with growing interest as an energy storage vector. The conventional Haber-Bosch synthesis requires about 2-3% of total world's energy commonly derived from fossil fuels, generating disastrous effects for the environment. To achieve a carbon-free society, among the green approaches, we propose a new biological ammonia production exploiting yeasts potential and its ability to use different nitrogen-rich sources.

Methods: So far, few research reports the use of successful biological technologies for the sustainable secretion of Ammonia. Some bacteria and yeasts have already been engineered in order to produce ammonia from biomass, respectively, by metabolic engineering and by displaying an amino-acid-catabolizing enzyme on the cell surface. Intracellular production of ammonia in yeasts is difficult because strongly assimilate this compound and knockout of genes involved in nitrogen metabolism are lethal or cause poor growth.

Results: In our laboratory, we discovered different *Saccharomyces cerevisiae* strains with a natural ability to release ammonia opening new process possibilities. To further increase this feature, yeast background and medium composition were evaluated, and adaptive laboratory evolution strategy were successful adopted to promote an enhanced and natural nitrogen release metabolism. Furthermore, the preliminary results showed very promising extracellular ammonia accumulation using careful fermentative strategy allowed reaching the highest published titre.

Conclusions: The final goal of this project will be to create a cell factory able to valorise different waste biomasses for a renewable and sustainable process.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

SHELTERIN PROTEINS AND TELOMERIC DNA INTERACTION RESEARCH WITH DEDICATED BIOSENSORS USING BLITZ TECHNOLOGY

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Background and Aims: Shelterin complex is essential for a telomere function. It ensures the stability of the chromosome by remodelling the telomeric DNA into a specific t-loop structure and protects the telomeric DNA against undesirable activity of the DNA repair pathway (DDR). To control this process, a number of accessory proteins are involved, including six telomere-associated proteins: hTRF1 (human telomeric repeat-binding factor 1), hTRF2 (human telomeric repeat-binding factor 2), POT1, RAP1, TIN2 and TPP1. Only hTRF1 and hTRF2 exhibit high affinity for a double-stranded telomeric DNA and bind to DNA as homodimers. Evaluation of the recombinant TRF1/2 usefulness in study of the, protein-DNA interaction *in vitro.* The developed, simplified model of the shelterin complex will be used for testing of several chemical compounds, which may be potential anti-cancer drug candidates.

Methods: We expressed synthetic genes encoding the selected proteins from the shelterin complex. Recombinant proteins were purified by affinity chromatography methods. Protein-DNA interactions were studied by SDS-PAGE and BLItz analysis.

Results: Synthetic genes, coding for recombinant, full-length hTRF1/2 proteins as well as their isolated DNA binding domains (Myb1/Myb2) were overexpressed in *Escherichia coli* system and the proteins were purified. Its role in forming of the specific DNA-TRF1/2 complex was evaluated.

Conclusions: The ability of the obtained, recombinant protein variants to bind selectively to a telomeric DNA was confirmed.
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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

SCREENING AND SELECTION OF MICROBIAL CONSORTIA FOR AGRI-FOOD PLASTIC WASTES DEGRADATION

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Background and Aims: Plastics accumulate on environment due to their resistance to degradation resulting in contamination. Recently, treatments involving the use of microorganisms are being developed as an alternative to chemical and physicochemical recycling methods. In nature, microorganisms act in community to degrade recalcitrant compounds, so it is expected that microbial consortia are more effective in plastic biodegradation than pure cultures. The aim of this work was the screening and selection of microbial consortia to degrade the plastics most frequently used in agri-food industry.

Methods: Microorganisms were tested for the expression of plastic-degrading related enzymes: lipases, cutinases and ligninases. Plastic biodegradation was determined by their capability to grow in low linear density polyethylene, polyethylene terephthalate or polystyrene as the sole carbon source. Forty-three strains isolated from different environments were analyzed, of which twenty-seven expressed plastic degrading activities.

Results: Six of these strains, including bacteria and fungi, were used to build up eight consortia, which cover the whole enzymatic spectrum to break down plastic polymers. Four of these consortia grew from two or more plastics whereas one was able to grow from all plastics. In addition, this consortium resulted in a plastic weight loss between 2–14%.

Conclusions: The microbial consortia selected in this work are very promising tools for the treatment of heterogeneous mixtures of plastic wastes. **Acknowledgements**: This project was received funding from the Bio based Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 887648.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

ISOLATION, BIOACTIVITIES AND DEREPLICATION OF MARINE ACTINOMYCETOTA FROM PORTUGAL

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Background and Aims: Oceans are home to a stunning number of unique microorganisms that hold particular interest for the biotechnological and pharmaceutical fields. Actinomycetota are great reservoirs of bioactive molecules and responsible for more than 6000 different described compounds.

Methods: In this work, we detail the diversity of isolates present in various sampling places from the Portuguese coast. Isolates were identified by 16S rRNA gene analysis, searched for the putative presence of secondary metabolism genes associated with polyketide synthase I (PKS-I) and non-ribosomal peptide synthetases (NRPS), screened for bioactivity against a panel of antibacterial, antifungal and cytotoxic targets. Bioactive extracts were dereplicated by LC/HRMS.

Results: A total of 114 Actinomycetota were searched for PKS-I and NRPS associated genes, of which 63 possessed at least one copy of these genes. Ten out of the 63 strains proved to be bioactive against Escherichia coli ATCC 25922, 1 against Staphylococcus aureus ATCC 29213, 1 against both pathogens and 3 against Aspergillus fumigatus ATCC46645. Strains cytotoxic bioactivity was also observed against human hepatocellular carcinoma. Further bioactivities were explored using an "one strain many compounds" approach. Extract dereplication revealed the presence of several known bioactive molecules and potentially novel natural products in the bioactive extracts.

Conclusions: These results point to a potentially relevant use of the isolated bacteria, as source of novel natural products.

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PAOX1 EXPRESSION IN MIXED-SUBSTRATE CONTINUOUS CULTURES OF KOMAGATAELLA PHAFFII (PICHIA PASTORIS) IS COMPLETELY DETERMINED BY METHANOL CONSUMPTION REGARDLESS OF THE SECONDARY CARBON SOURCE

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Background and Aims: The expression of recombinant proteins by the AOX1 promoter of *Komagataella phaffii* is typically induced by adding methanol to the cultivation medium. Since growth on methanol imposes a high oxygen demand, the medium is often supplemented with an additional "secondary" carbon source which serves to reduce the consumption of methanol, and hence, oxygen. Early research recommended the use of glycerol as the secondary carbon source, but more recent studies recommend the use of sorbitol because it leads to higher P_{AOX1} expression. Our goal was to quantify the extent to which P_{AOX1} expression is affected by addition of a secondary carbon source to the medium.

Methods: We measured the steady state concentrations of biomass, residual methanol, and AOX1 over a wide range of dilution rates (0.02–0.20 h⁻¹) in continuous cultures of the Mut⁺ strain fed with methanol, methanol+glycerol, and methanol+sorbitol.

Results: We find that under those conditions, the specific AOX1 expression rate is completely determined by the specific methanol consumption rate regardless of the existence (present/absent) and type (repressing/non-repressing) of the secondary carbon source. Analogous results have been reported in *Escherichia coli* where it has been shown that the specific expression rate of the *lac* operon is also completely determined by the specific lactose consumption rate regardless of the nature of secondary carbon source.

Conclusions: By combining the simple unstructured model developed by Egli and co-workers with our data, we derive a simple 2-parameter formula that predicts the protein expression rates and levels of single- and mixed-substrate cultures over a wide range of conditions.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

METAL BIOSORPTION PROCESS UNDER STRONGLY ACIDIC CONDITIONS: A PART OF SUSTAINABLE METAL RECYCLING SYSTEM

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Background and Aims: The demand for metal resources as components of electronic devices has been increasing steadily owing to the widespread use of the Internet of Things and smart society technology. Recycling end-of-life products is important for ensuring a sustainable metal supply and for environmental safety. Conventional pyrometallurgical and hydrometallurgical methods require high energy consumption and large amounts of neutralizers. We propose a novel biotechnological method using acid-tolerant bacteria, growing under neutral conditions, and surviving under acidic conditions (Figure 1). In this study, we successfully designed a process for metal recovery under strongly acidic conditions in a laboratory setting.



Figure 1: Concept of the novel metal recycling system

Methods: We developed a unique screening method called a 'pH shift culture' and isolated acid-tolerant metal recovery bacteria from neutral environments. The metal recovery process from the simulated metal

leachate (pH 1.5, containing 1 g/L Co, Cu, Li, Mn, and Ni, respectively) was developed using *Micrococcus luteus* JCM1464 and four isolates.

Results: With this method, five metal species were successfully recovered under pH 1.5 conditions. The maximum recovery abilities were as follows: Co: 13.9, Cu: 20.2, Li: 22.6, Mn: 20.2, and Ni: 14.1 mg/g-dry cell, respectively.

Conclusions: The results indicate that our technique for metal biosorption under strongly acidic conditions was successful. This is the first step in the development of a process for a sustainable metal recycling system using acid-tolerant bacteria. Further studies are required to improve the metal recovery efficiency and to develop the metal desorption process.

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COMPARISON OF THE FDA BAM AND ISO METHODS FOR THE DETECTION OF B. CEREUS 3A SPORE SUSPENSIONS FROM ARTIFICIALLY PRESERVED AND CONTAMINATED WIPES

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Background and Aims: Despite the presence of preservatives, cosmetic wipes can become contaminated during processing steps and usage, which may lead to skin infections and other health issues for consumers.No validated method exists for the microbiological testing of cosmetic wipes. FDA BAM Chapter 23 uses a 1-g sample size and adds 1ml of Tween 80 (T80) for sample analysis. Conversely, ISO 21322:2020 recommends that the entire wipe be tested but does not require T80. FDA developed a protocol to prepare artificially contaminated and preserved wipes for method comparison.

Methods: Dry wipes were placed individually in sterile containers and wetted with benzalkonium chloride (BAC) solution inoculated with B. cereus 3A spore suspensions at three concentration levels. Wipes wetted with uninoculated BAC solution were the negative controls. After 14 days, B. cereus was enumerated from all wipes using BACARA and MLA plates, and the TEMPO® instrument, to compare the efficiencies of BAM, ISO and ISO with T80 (i.e., modified ISO) methods.

Results: The results showed no difference (P>0.05) of cell recovery among the three enumeration methods. Similarly, B. cereus recovery rates were comparable from the BAM method and the modified ISO method (P=0.822). However, B. cereus recovery rates were significantly higher from the BAM method compared to the ISO method (p=0.004). Similarly, counts from the entire wipe with T80 were significantly higher (p=0.047) than those without T80.

Conclusions: In conclusion, BAM and modified ISO methods were comparable for B. cereus recovery from wipes artificially preserved with BAC. T80 improves recovery of B. cereus cells.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

INHIBITION OF MULTIDRUG-RESISTANT PATHOGENIC BACTERIA BY THE EXTRACELLULAR BROTH CULTURE OF A PARACONIOTHYRIUM SP. STRAIN

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Background and Aims: The resistance to antibiotics by pathogenic bacteria has become a worldwide public health problem, generating a continuous search for new metabolites with antibacterial activity from natural sources such as fungi. The species within *Paraconiothyrium* ascomycete genus synthetize compounds with antimicrobial activity, but its extracellular metabolite production research is scarce. In this work, the antibacterial activity of the extracellular metabolites of basal/induced broth cultures of the CMU-196 *Paraconiothyrium* sp. strain against bacteria multi-resistant to antibiotics was determined.

Methods: The CMU-196 strain was cultured in peptone (P-B) and malt extract (ME-B) broths in basal condition and induced using autoclaved *Salmonella enterica* cells as inductor (P-IN, ME-IN, respectively). The extracellular filtrates in the stationary growth phase were lyophilized, and the production of metabolites was analyzed by thin layer chromatography (TLC). The antibacterial activity was assessed by a 96-well microplate assay, against multi-drug resistant strains of *S. enterica, Escherichia coli,* and *Staphylococcus aureus*.

Results: The CMU-196 strain cultivated in P broths showed a pigmented mycelium, aggregated in pellets, which is a characteristic related to high extracellular secondary metabolites production. TLC analysis of the extracellular lyophilizates showed four bands in both ME broths, five bands in P-B broth and seven in P-IN. Four of the bands recovered from the TLC plates showed significant inhibitory activity against the Gram-negative (10%-25%) and *S. aureus* (10%-35%) tested bacterial strains.

Conclusions: These results shows that CMU-196 strain synthetize extracellular metabolites active against multi-drug resistant bacteria that could be phenolic acids or flavonoids, as suggested by the visualization of bands under UV_{254} light.

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E-Poster Viewing Topic: AS19 Microbial biotechnology and applied microbiology

TOWARDS ENZYMATIC PLASTIC DEGRADATION, THE PROMISE OF SOIL BACTERIA

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Background and Aims: Enzymatic degradation of plastics is a promising approach that can be performed using mild reaction conditions and leads to well-defined intermediates. In search of novel plastic degrading enzymes, we screened a collection of Actinobacteria for their bis(2-Hydroxyethyl) terephthalate (BHET) and Poly(ethylene terephthalate) (PET) degrading capacities.

Methods: 94 Actinobacteria strains were screened for their PET degrading abilities by monitoring precursor BHET degradation on minimal medium with and without inducer substrate. Promising strains were screened individually and the most active strains were chosen for further research. Strains were grown in liquid NMMP medium containing BHET and inducer substrate, BHET degradation was monitored using LC-MS. Strains were also grown in liquid medium in the presence of PET films, degradation of plastic was monitored after 10 days using scanning electron microscopy.

Results: 37% of all strains showed BHET degrading activity when screened in arrays of multiple strains on plates. The active strains were screened individually; of those, two strains were selected for further investigation. Using LC-MS degradation of BHET was clearly visualized indicating that both strains were able to degrade all solubilized material within 24h. Incubation of plastic films with the strains resulted in a clearly damaged surface as visualized using SEM.

Conclusions: We focused on screening of a collection of the well-known soil bacteria species Actinobacteria, for its BHET and PET degrading capabilities. Two promising strains were selected for further investigation showing they can enzymatically degrade BHET and actively damage PET films. Further research will be required to characterize the specific enzymes involved.

EP175 / #779

E-Poster Viewing Topic: AS20 Extremophiles and archaea

DNA POLYMERASE B1 BINDING PROTEIN 1 IS IMPORTANT FOR DNA REPAIR BY HOLOENZYME POLB1 IN THE EXTREMELY THERMOPHILIC CRENARCHAEON SULFOLOBUS ACIDOCALDARIUS

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Background and Aims: DNA polymerase B1 (PolB1) is a member of the B-family DNA polymerase and is a replicative DNA polymerase in crenarchaea. PolB1 is responsible for the DNA replication of both the leading and lagging strands in the thermophilic crenarchaeon *Sulfolobus acidocaldarius*. Recently, two subunits, PolB1-binding protein (PBP)1 and PBP2, were identified in *Saccharolobus solfataricus*. Previous *in vitro* studies suggested that PBP1 and PBP2 influence the core activity of apoenzyme PolB1 (apo-PolB1). However, it has not been determined whether these subunits are essential for the activity of apo-PolB1.

Methods: We attempted to construct *S. acidocaldarius* strains completely lacking the *pbp1* and *pbp2* genes and characterized their mutant phenotypes, examining sensitivity to numerous types of DNA damage (i.e., UV irradiation, DNA-damaging agents, heat shock, and DNA replication inhibitors) and mutation rates.

Results: A *pbp2* deletion strain was not obtained, indicating that PBP2 is essential for replication by holoenzyme PolB1. A *pbp1* deletion strain was sensitive to various types of DNA damage and exhibited an increased mutation rate, suggesting that PBP1 contribute to the repair or tolerance of DNA damage by holoenzyme PolB1.

Conclusions: The results suggested that holoenzyme PolB1 contributes to both replication and repair. PBP1 is involved in the repair or tolerance of various types of DNA damage, although it is not essential for the activity of apo-PolB1. On the other hand, PBP2 is essential for replication by apo-PolB1. Thus, holoenzyme PolB1 of *S. acidocaldarius* is versatile. These results provide new genetic evidence of the biological function of holoenzyme PolB1.

EP176 / #782

E-Poster Viewing Topic: AS20 Extremophiles and archaea

GENETIC STUDY OF FOUR CANDIDATE HOLLIDAY JUNCTION PROCESSING PROTEINS IN THE THERMOPHILIC CRENARCHAEON SULFOLOBUS ACIDOCALDARIUS

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Background and Aims: Homologous recombination (HR) is thought to be important for the repair of stalled replication forks in hyperthermophilic archaea. Previous biochemical studies identified two branch migration helicases (Hjm and PINA) and two Holliday junction (HJ) resolvases (Hjc and Hje) as HJ-processing proteins; however, due to the lack of genetic evidence, it is still unclear whether these proteins are actually involved in HR *in vivo* and how their functional relation is associated with the process. In this study, we constructed *hjm*-, *pina*-, *hjc*-, and *hje*-knockout strains and double-knockout strains in *S. acidocaldarius* to investigate the functional role of Hjm and PINA *in vivo*, and the relationship between two helicases and two nucleases in archaeal HR.

Methods: A multiple-gene-knockout system with one-step PCR (MONSTER) was used to prepare the *hjc* (Saci_1558), *hje* (Saci_1741), *hjm* (Saci_0263), and *pina* (Saci_1557) MONSTER cassettes (MONSTER-*hjc*, MONSTER-*hje*, MONSTER-*hjm*, and MONSTER-*pina*, respectively) and to construct the *hjc*-, *hje*-, *hjm*-, and *pina*-disrupted strains and double-knockout strains.

Results: We succeeded in isolating the *hjm*- and/or *pina*-deleted strains. Growth retardation in $\Delta pina$ was observed at low temperatures. When deletion of the HJ resolvase genes was combined, $\Delta pina \Delta hjc$ and $\Delta pina \Delta hje$ exhibited severe cold sensitivity. Δhjm exhibited severe sensitivity to interstrand crosslinkers.

Conclusions: The results suggested that a function of Hjm and PINA is not essential for cellular growth in this archaeon. PINA is important for cellular growth at lower temperatures, and PINA and HJ endonucleases are functionally linked at lower temperatures. Hjm is important for the DNA repair of interstrand crosslinks, as previously demonstrated in euryarchaea. This study provides new insights into HR processes in thermophilic crenarchaeon.

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E-Poster Viewing Topic: AS20 Extremophiles and archaea

POLYPHASIC TAXONOMIC ANALYSIS OF STYGIOLOBUS SP. KN-1, FACULTATIVELY ANAEROBIC AND HYPERTHERMOPHILIC ARCHAEON, ISOLATED FROM THE UNZEN HOT SPRING IN JAPAN

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Background and Aims: *Stygiolobus* was established in 1991 as a novel genus of anaerobic and extremely thermoacidophilic Archaea in the order *Sulfolobales*. Since its original description, *Stygiolobus* has been considered the only genus comprising obligately anaerobic Archaea in the order *Sulfolobales*. Recently, we isolated a novel facultatively anaerobic archaeon, strain KN-1, which phylogenetically belongs to the genus *Stygiolobus*. In this study, we conducted a polyphasic taxonomic analysis of strain KN-1.

Methods: The following characteristics of strain KN-1 were examined: 1) cell morphology; 2) growth pH/temperature ranges; 3) utilization of complex-substrates/sugars; 4) chemolithotrophic growth; 5) aerobic/microaerobic/anaerobic growth; 6) core lipids; 7) phylogenetic placement (16S rRNA genebased); 8) complete genome sequence.

Results: Cells of KN-1 were irregular cocci that grew at 55–87.5 °C and pH 1.0–5.5. The chemolithoautotrophic growth occurred in the presence of S^o or H₂ under oxic conditions. Under anoxic conditions, KN-1 grew on S^o, Fe(C₆H₅O₇), and FeCl₃. The closest species to KN-1 is *S. azoricus*, with 98.9% 16S rRNA gene sequence identity, indicating that strain KN-1 belongs to the genus *Stygiolobus*. This genus has been known to consist of obligate anaerobes; in contrast, KN-1 grew under oxic, microoxic, and anoxic conditions. Moreover, KN-1 utilized various complex substrates and some sugars as a carbon or an energy source, which is also different from *S. azoricus*. The average nucleotide identity between KN-1 and *S. azoricus* was 79.4%, indicating that KN-1 represents a novel species.

Conclusions: Based on the polyphasic taxonomic analysis, we propose a novel species, *Stygiolobus caldivivus* sp. nov. to accommodate strain KN-1.

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E-Poster Viewing Topic: AS20 Extremophiles and archaea

CULTUROMICS OF HYPERSALINE SOILS: CHARACTERIZATION OF NEW AQUIBACILLUS SPECIES.

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Background and Aims: Previous metagenomic studies of hypersaline soils located in the Marismas del Odiel Natural Park, Spain permitted us to determine the prokaryotic diversity of these soils. In order to isolate and characterize bacterial and haloarchaeal taxa not previously described but that were determined to be present on these soils, we carried out extensive culture-dependent studies. Among these new isolates, a collection of 130 strains were characterized as members of the family *Bacillaceae*. We determined that a group of 30 isolates were members of the genus *Aquibacillus*. This study is focused on the taxonomic characterization of these new isolates, not previously determined by metagenomic analysis.

Methods: We characterized the new isolates according to the polyphasic approach, as well as by genomic methods (ANI, digital DDH, AAI, comparative genomics and phylogenomic analysis based on the comparison of the core-genomes).

Results: The phenotypic and genomic characterization of some representative strains showed that they represented new taxa of this genus. The comparative genomic analysis and the determination of several OGRI indexes, such as the orthoANI, digital DDH (GGDC) and the AAI as well as the phylogenomic analyses supported that they represented a new species of the genus *Aquibacillus*, for which we propose the new name *Aquibacillus haloterrestris* sp. nov.

Conclusions: Althought metagenomic analysis of the hypersaline soils studied did not determine that the members of Aquibacillus were abundant on these habitats, the use of a culturomic approach permitted us to isolate new strains and to characterize a new species of this genus.

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

INVESTIGATING THE PHYSIOLOGICAL ROLE OF TYPE III PKS GENES OF MYCOBACTERIUM MARINUM THROUGH THE GENERATION OF CRISPRI BASED GENETIC KNOCKDOWNS

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Background and Aims: *Mycobacterium marinum* (Mmar) is responsible for tuberculosis-like disease in aquatic animals and skin and soft tissue infections in immune-compromised humans. It has 80% genome-wide protein sequence homology with Mycobacterium tuberculosis. Polyketide synthases (*pks*s) genes, which belong to the class of secondary metabolism genes that encode enzymes involved in lipid metabolization and cell wall remodeling, are found in a variety of Mycobacterium species. In mycobacteria, these gene families play a role in the biosynthesis of specific lipids and virulence factors.

Methods: Mmar contains four type III pks genes, which are organized into three gene clusters along with other modifying genes. Two of these clusters are conserved in MTb, therefore we used a combination of *in silico*, gene editing, and phenotypic studies, to study their significance.

Results: Gene ontology analysis of *mmar_2190* gene cluster resulted in enrichment of the pathways related to the biosynthesis and metabolism of sulfolipids and chalcones. The CRISPRi based genetic knockdown of *mmar_2190* revealed interesting insights regarding the *in vivo* relevance of this gene. The gene expression analysis showed ~80% downregulation of the *mmar_2190* transcript in the pGrna-*mmar_2190* k/d strain. The mutant strain also displayed a retarded growth profile compared to pGrna in the planktonic and solid cultures and an altered morphological growth pattern on the nutrient-rich agar plates. Additionally, the mutant strain showed a defect in sliding motility behavior together with increased sensitivity to cell wall stresses. In comparison to the pGrna strain, the mutant strain showed impaired biofilm growth.

Conclusions: mmar_2190 have significance in adaptation and growth in the stationary phase.

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

THE CHARACTERIZATION OF TWO PROTEINS RELATED TO THE BIOFILM FORMATION OF CANDIDA GLABRATA

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Background and Aims: Candida glabrata is capable of developing biofilm on the surface of host cells and medical materials. Until now, the mechanism of biofilm formation of C. glabrata has not been studied well. Then the aim of our study was to isolate and characterize some genes related with the biofilm formation of C. glabrata.

Methods: The in vitro model of biofilm formation was constructed in multi-well plates and the metabolic activity were detected by XTT assay.

Results: Upon the result of a genetic comprehensive screening by using C. glabrata mutant library, two gene mutants decreased metabolic activity of biofilms drastically and their transcriptional expressions in biofilm formation showed more than two times higher than those of planktonic cell growth. One gene was named as CgSYN8 which encodes a SNARE protein. The syn8Δ mutant was defective in the adhesion ability during the biofilm formation, which may link to its abnormal vacuolar function. Another gene was named as CgQDR2 which encodes MFS transporter. This mutant exhibited reduction in fluconazole resistance during biofilm formation. QDR2 deletion caused an impaired ability to maintain pH homeostasis, then might lead to the reduction of cell growth at neutral-basic pH conditions, eventually affect biofilm susceptibility.

Conclusions: CgSYN8 and CgQDR2 were suggested to be involved in biofilm formation of C. glabrata. These findings provide more understanding on the biofilm formation of this fungus and more information for the development of clinical treatment in future.

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

GENETIC RECOMBINATION BETWEEN A MAJOR HUMAN SKIN COMMENSAL FUNGUS (MALASSEZIA RESTRICTA) AND M. GLOBOSA IS MEDIATED BY AN AGROBACTERIUM TUMEFACIENS GENE TRANSFER SYSTEM

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Background and Aims: *Malassezia restricta* is the main fungus of the skin microbiome, followed by *M. globosa*. These fungi trigger or exacerbate *Malassezia*-associated skin, seborrheic, and atopic dermatitis, as well as pityriasis versicolor. The virulence factors of *M. restricta* and *M. globosa* remain unknown because fungal genetic recombination has hitherto been impossible. We thus used an *Agrobacterium tumefaciens*-mediated gene transfer (ATMT) system to generate mutants of the two major *Malassezia* species.

Methods: The binary vector pAg1- Δ fkb1::NAT1 was introduced into *M. restricta* CBS 7877 and *M. globosa* CBS 7966 using the ATMT system; the *FKB1* gene was replaced and then re-introduced into *FKB1*-deficient mutants (genetic complementation).

Results: A mutant in *FKB1* that encodes the FKBP12 protein (which binds to the calcineurin inhibitor tacrolimus) was generated using the ATMT system. Wild-type *M. restricta* and *M. globosa* were sensitive to tacrolimus, while the *FKB1* mutants were resistant. Drug susceptibility was restored by reintroducing *FKB1*. The *FKB1* mutants were not resistant to cyclosporine A. Phenotypic analysis revealed the crucial role played by FKBP12 when tacrolimus inhibited *M. restricta* and *M. globosa*.

Conclusions: Our gene recombination system will aid elucidation of the molecular mechanisms of *Malassezia*-associated dermatitis.

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

Y12F MUTATION IN PSEUDOMONAS PLECOGLOSSICIDA S7 LIPASE ENHANCES ITS THERMAL AND PH STABILITY

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Background and Aims: Appropriate amino acid substitutions are critical to protein engineering to redesign catalytic properties of industrially important enzymes like lipases. The present study aimed for improving the environmental stability of lipase from *Pseudomonas plecoglossicida* S7 through site-directed mutagenesis driven by computational studies.

Methods: Lipase gene was amplified and sequenced. Three *in silico* point mutations were carried out in the lipase protein and docking of the resultant 3D models with palmitic acid was carried out with UCSF Chimera. The mutant (MT) with the highest binding affinity was further selected for molecular dynamic simulations (MDS) to check the stability along with the wild type (WT). Both WT and MT lipase genes were expressed into the pET SUMO system. The expressed proteins were purified and characterized for pH and thermostability.

Results: The lipase gene belonged to subfamily I.1 lipase. The Y12F-palmitic acid complex had a greater binding affinity (-6.3 Kcal/mol) than WT (-6.0 Kcal/mol) complex. Interestingly, MDS showed that the binding affinity of WT-complex (-130.314 +/- 15.11 KJ/mol) was more than mutant complex (-108.405 +/- 69.376 KJ/mol) with a marked increase in the electrostatic energy of mutant (-26.969 +/- 12.646 KJ/mol) as compared to WT (-15.082 +/- 13.802 KJ/mol). Y12F mutant yielded 1.12 folds and 1.27 folds increase in lipase activity at 40°C and 55°C respectively as compared to purified WT protein. Also, Y12F mutant showed increased activity (1.2 folds each) at both pH 6 and 10.

Conclusions: *Pseudomonas plecoglossicida* S7 Y12F mutant lipase with better pH and thermal stability can be used in nonaqueous biocatalysis.

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

FCRX, A NEW GLOBAL REGULATOR OF CELL CYCLE IN FREE LIVING CONDITIONS AND DURING SYMBIOSIS IN SINORHIZOBIUM MELILOTI

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Background and Aims: *Sinorhizobium meliloti* is a soil bacterium that establishes a symbiosis with *Medicago sativa*, where it fixes the atmospheric nitrogen into ammonia and in return the plant shares carbon sources with bacteria. In this symbiosis, *S. meliloti* undergoes a drastic cellular change leading to an intracellular terminal differentiation (bacteroid) characterized by genome endoreduplication, cell enlargement and high membrane permeability. The bacterial cell cycle regulation is closely implicated in this differentiation. Indeed, in free cells, the bacterial regulator, CtrA, among other functions, activates cell division (controlled by ring forming FtsZ), and inhibits DNA replication, while during symbiosis CtrA and FtsZ downregulations are essential for bacteroid differentiation. Little is known about regulators of CtrA and FtsZ in *S. meliloti* that control bacteroid development.

Methods: Here we describe results of cell biology, biochemistry and genetics approaches deployed to understand the function(s) of FcrX in free and symbiotic life.

Results: Depletion of the essential gene *fcrX* lead to minicells formation in which levels of FtsZ and CtrA are abnormally high. Using several techniques we showed that FcrX (an alpha-helix-rich protein) is able to interact with FtsZ and CtrA. Its transcription is controlled by CtrA itself. Further we showed that, despite a weak homology with FliJ-like proteins, only closely-related species FcrXs are able to complement *S. meliloti fcrX* deletion. Finally mutants of FcrX showed abnormal symbiotic behaviors in plants suggesting a putative role of this factor during bacteroid differentiation.

Conclusions: In conclusion FcrX is the first known cell cycle regulator that acts directly on CtrA and FtsZ.

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

SYNONYMOUS MUTATIONS IN THE GENE ENCODING THE RNA CHAPERONE HFQ AFFECT THE FITNESS, YIELD AND DUPLICATION RATE OF ESCHERICHIA COLI

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Background and Aims: The genetic code is redundant, which means that more than one codon can decode the same amino acid (synonymous codons). The choice of synonymous codons in each gene is not random, since it can affect the efficiency of translation, folding and levels of proteins. In bacteria, translation is regulated partly by the sRNA-mRNA interaction in which the RNA chaperone Hfq plays a crucial role specially in stress response. In *E. coli, while* coding region of *hfq* mRNA is biased to preferred codons, regions of mRNAs that interact with sRNAs have a bias for non-preferred codons. Evaluate the effect of codon bias in the function of Hfq carrying out replacements of optimal to non-optimal codons in two regions in *E.coli* strain BW25113.

Methods: Based on ribosome profile of Hfq mRNA, two regions were randomly mutated and the resultant mutant genes were replaced in the genome of *E. coli* giving rise to a collection of mutants.

Results: Analyses of mutant cells revealed that some replacement of optimal for non-optimal codons affected the fitness, growth rate and the yield of the cells. Thus, natural codon bias for optimal codons in *hfq* not necessarily implies that function of the protein is optimized. Probably other effects of the synonymous codons in gene expression are taking place. Also levels of the mRNA was affected in some mutants without effecting protein levels. Transcriptomic analysis of mutant cells as well as functional and structural properties of mutant Hfq are in progress.

Conclusions: Synonymous changes in Hfq generate diverse phenotypic alterations

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

DOMAIN ANALYSES AND PHOSPHOREGULATION OF A TRANSCRIPTION FACTOR MXR1 RESPONSIBLE FOR METHANOL-INDUCED GENE EXPRESSION IN KOMAGATAELLA PHAFFII

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Background and Aims: Methylotrophic yeasts can utilize methanol as the sole source of carbon and energy. They have been used as hosts for heterologous protein production, which is achieved with their strongly and tightly regulated methanol-induced promoters. When methylotrophic yeasts grow on methanol, methanol-induced genes (e.g., *AOX1, DAS1*) in *Komagataella phaffii* (*Pichia pastoris*) are expressed depending on the methanol concentration in the medium. A transcription factor Mxr1 is necessary for methanol-induced gene expression and its function is known to be regulated by phosphorylation. In this study, we investigated phosphorylation status of Mxr1 under various methanol concentrations and determined functional domains for methanol concentration-dependent gene expression.

Methods: Phosphorylation level of Mxr1 was analyzed by phos-tag SDS-PAGE or western-blot with antiphosphorylated peptide antibodies. We constructed the strain expressing C-terminal truncated mutants of Mxr1 and investigated the expression level of methanol-induced genes by qRT-PCR.

Results: The phosphorylation status of Mxr1 changed corresponding to the methanol concentration. Mutation of putative phosphorylated amino acid residues in Mxr1 resulted in a partial deficiency of methanol concentration-dependent gene expression. Moreover, we identified functional domains of Mxr1, which were involved in methanol-dependent gene expression, and several of them were found to be conserved among methylotrophic yeast strains.

Conclusions: We concluded that phosphoregulation of Mxr1 is responsible for methanol-induced gene expression corresponding to methanol concentration.

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

IDENTIFICATION AND PRELIMINARY CHARACTERIZATION OF RYHB-1 AND RYHB-2 SRNA IN YERSINIA ENTEROCOLITICA

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Background and Aims: Small regulatory RNAs modulate gene expression on a post-transcriptional level and play an essential role in rapid response to environmental changes. RyhB of *Escherichia coli* is an sRNA involved in maintaining iron homeostasis. Recent studies demonstrate that this sRNA modulates the virulence of some pathogenic bacteria. This study aimed to characterize the RyhB sRNAs of *Yersinia enterocolitica*.

Methods: The sRNA sequences were predicted *in silico*. Primer extension assays were used to determine the transcriptional start sites of identified sRNAs. Northern Blots established the influence of iron and Fur protein on sRNA expression. Mutant strains were obtained by homologous recombination using suicide vector pDS132. Vectors pBR322 with cloned sRNAs were used for overexpression. Growth assays were assessed under stress conditions.

Results: Bioinformatic analyses revealed the presence of *ryhB*-1 and *ryhB*-2 genes, located separately within the genome of *Y. enterocolitica*, similar to *Y. pestis*. The transcription start sites of sRNAs were estimated and the corresponding promoter regions were predicted using BPROM. The expression of RyhB-1 and RyhB-2 is inhibited by iron and Fur protein, the master regulator of iron homeostasis in bacteria. The growth of *Y. enterocolitica* is slightly inhibited by investigated RNAs under tested conditions.

Conclusions: *Y. enterocolitica* poses two RyhB homologs, RyhB-1 and RyhB-2, which are negatively regulated by iron and Fur. Their potential role in *Y. enterocolitica* pathophysiology will be examined. **Acknowledgments** This work was supported by the National Science Center, Poland (Preludium-17 grant UMO-2019/33/N/NZ1/00484).

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E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

KRAFT LIGNIN/AROMATIC COMPOUND-INDUCED PROMOTERS IN WHITE ROT FUNGI CERIPORIOPSIS SUBVERMISPORA

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Background and Aims: White rot fungi are the only known organisms that can completely degrade lignin in plant cell walls. However, it is obscure whether lignin and its degradants regulate the lignin-modifying enzyme (LME) genes expression. In this study, the expression of LME genes in white rot fungi *Ceriporiopsis subvermispora*, as well as *cis*-elements response to lignin and its putative degradants were investigated.

Methods: The expression of *C. subvermispora* LME genes when grown with Kraft lignin or 10 different lignocellulose-related aromatic monomers was measured by qRT-PCR. Promoter analyses for *lcc1* and *lcc4* were done with the luminous shrimp luciferase gene (Nano*Luc*) as a reporter introduced into *C. subvermispora* pyrG locus.

Results: Significant differences in the gene expression of LMEs were observed between the cultivations supplemented with the different aromatic compounds and Kraft lignin. Specifically, the expression *lcc1* and *lcc4* were totally induced by Kraft lignin, ferulic acid, and coniferyl alcohol. Some *mnps* also show the similar phenomenon. To confirm whether *lcc1*, *lcc4* promoter contains a ciselement(s) responsible for Kraft lignin/coniferyl alcohol induction, 1774-bp (*lcc1*) and 2000-bp (*lcc4*) promoters were fused with Nano*Luc*, respectively. The luciferase activities of the transformants were significantly higher upon cultivation when supplemented with Kraft lignin/coniferyl alcohol compared to the control.

Conclusions: Coniferyl alcohol and ferulic acid were reported to possibly originate from lignin degradation/fragmentation, these results suggesting the specific transcriptional regulation response to lignin and its degradation products in *C. subvermispora*.

EP188 / #100

E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

TRANSCRIPTOME ANALYSIS OF ALCALIGENES FAECALIS DURING HETEROTROPHIC NITRIFICATION

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Background and Aims: Nitrification is the microbial function that is essential to the nitrogen cycle in the environment. Not only autotrophic nitrifiers but also some heterotrophic microorganisms have been known to possess ammonia-oxidizing activity, however, the biochemistry and genetics of heterotrophic nitrification are still poorly understood. The aim of this study is to provide the genetic framework of heterotrophic nitrification.

Methods: Alcaligenes faecalis was cultivated in a synthetic medium containing organic acid and ammonium as sole carbon and nitrogen sources, respectively, to elucidate the cultivation condition that is appropriate for the induction of nitrifying activity. Comparative transcriptome analysis was performed using the nitrifying and non-nitrifying bacterial cells to evaluate the differentiation of the gene expression during induction of heterotrophic nitrification.

Results: Heterotrophic nitrification was induced in *A. faecalis* when cultivated in a nitrogen-rich medium (C/N ratio = 5). Transcriptome analysis demonstrated that four gene clusters, *pod* cluster containing gene encoding pyruvic oxime dioxygenase (POD), a key enzyme in heterotrophic nitrification, *podh* cluster containing gene encoding POD homolog, *suf* cluster for the biosynthesis of iron-sulfur centers, and *dnf* cluster encoding enzymes of a novel ammonia oxidation pathway, were activated in the nitrifying cell.

Conclusions: This study suggested the involvement of four gene clusters in the heterotrophic nitrification process and provide valuable insight into the molecular mechanism of heterotrophic nitrification. To confirm the function of these genes, phenotype analysis of the gene knockout strains is now in progress.

EP189 / #104

E-Poster Viewing Topic: AS21 Gene expression, gene regulation, and development

IN SILICO IDENTIFICATION AND CHARACTERIZATION OF CIRCRNAS AS POTENTIAL MIRNA SPONGES FROM VIRULENT AND NONVIRULENT STRAINS OF ENTAMOEBA HISTOLYTICA AND ENTAMOEBA INVADENS.

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Background and Aims: Ubiquitous eukaryotic non-coding circular RNAs regulate transcription and translation. We have reported full-length intronic circular RNAs (flicRNAs) in Entamoeba histolytica with esterified 3'ss and 5'ss. Their 5'ss GU-rich elements are essential for their biogenesis and their suggested role in transcription regulation. Here, we explored whether exonic, exonic-intronic, and intergenic circular RNAs are also part of the E. histolytica and E. invadens ncRNA repertoire, as well as their possible functions.

Methods: To search for differentially expressed circular exonic RNAs (between the virulent HM-1:IMSS and nonviruent Rhaman E. histolytica strains) we mined the available RNA-seq libraries using the CIRI-full sofware. The search was extended to the reptile parasite E. invadens. The robustness of the analyses was validated using synthetic decoy sequences with bona fide back splice junctions. In addition, the miRNA sponging potential of the circular RNAs was analyzed using the intaRNA software.

Results: 188 and 605 reverse overlapped circRNAs from the E. invadens and E. histolytica libraries were identified, respectively. The sequence composition of the circRNAs was mostly exonic. 416 circRNAs from E. histolytica were virulent-specific and 267 were avirulent-specific. In addition, out of the common circRNAs, 32 were differentially expressed between strains, showing no correlation with the expression of their linear primary transcripts and mRNA counterparts. Finally, we predicted that 12 of the differentially expressed circRNAs could function as sponges of the reported miRNAs in E. histolytica, whose functions are still unknown.

Conclusions: Our results extend the RNAome of E. histolytica and allow us to devise working hypothesis to test circRNAs/miRNAs interactions in determining the virulent/nonvirulent phenotypes.

EP190 / #285

E-Poster Viewing Topic: AS22 Food microbiology

GROWTH OF SALMONELLA SPP. ON READY-TO-EAT FRESH-CUT LETTUCE AT 7.1°C AND 9.5°C: COMPARISON WITH COMBASE PREDICTOR

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Background and Aims: Ready-to-eat (RTE) salads are minimally processed foods. In spite of their healthy and convenient aspects, microbiological safety should be a concern as the growth of pathogens may occur during refrigerated storage, particularly at abusive temperatures often observed at the household level. In this way, it is important to monitor the microbiological quality of fresh-cut packaged salads. The aim of this study was to evaluate the behavior of *Salmonella* spp. in organic and conventional salads in abusive temperatures (7.1 and 9.5 °C) and comparing the data obtained with experimental data with predictions from the ComBase Predictor model.

Methods: Individual packages of pre-washed lettuce salad were inoculated with a cocktail of five *Salmonella* spp. strains. Pathogens were enumerated during storage (8 days) at different abusive temperatures (7.1 and 9.5°C) simulating what is observed in domestic settings. Growth under the same conditions was predicted using the ComBase Predictor software.

Results: Growth of *Salmonella* spp. was observed on salads stored at temperatures 7.1 °C and 9.5 °C with an increase between 2 and 3 log at end of the experience (8 days) and higher growth rates were determined when ComBase Predictor was used.

Conclusions: Salmonella spp. can grow in fresh-cut lettuce even at short abuse of refrigeration temperature during storage.

EP191 / #69

E-Poster Viewing Topic: AS22 Food microbiology

OCCURRENCE OF LISTERIA SPP. AND LISTERIA MONOCYTOGENES IN HORSE MEAT AT RETAILS STORE IN SPAIN

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Background and Aims: Horse meat is not usually consumed in many countries and therefore, studies about the microbiological safety of this matrix are scarce. However, the increasing of consume around the world associated with the presence of pathogens in living animals, makes necessary investigations concerning this issue. This study evaluated the presence of Listeria spp. and Listeria monocytogenes in retail horse meat.

Methods: A total of 19 meat samples were collected from different retailers in 2020-2021, in Spain. For Listeria spp. enumeration, meat samples were homogenized and inoculated in Chromogenic Listeria Agar (OXOID) plates. For detection, homogenates were incubated for 24 h at 37 °C, and subsequently cultured in Fraser broth. A loopful of each culture-enriched sample was streaked on Chromogenic Listeria Agar plates. From each positive sample five colonies were isolated and later identified by MALDI-TOF/MS (Bruker Daltonics, Bremen, Germany).

Results: Counts of Listeria spp. were below 1 log cfu/g in all samples. However, the presence of Listeria spp. was detected in 6 samples (31.57%) while L. monocytogenes was identified in 4 samples (21.05%). Besides L. monocytogenes, it was also found L. welshimeri and L. innocua isolates in one sample each

Conclusions: Despite the low counts of Listeria spp. observed, the number of samples contaminated with L. monocytogenes suggests attention of food industry against possible food-borne pathogens contamination. This work has received funding from the European Union's H2020 research and innovation program under Marie Sklodowska-Curie grant agreement No 801586, Pre-doctoral UR-CAR fellowship and POCTEFA Project (INTERREG Program) TESTACOS EFA 152/16.

EP192 / #163

E-Poster Viewing Topic: AS22 Food microbiology

PANGENOME AND PHYLOGENETIC ANALYSIS OF SALMONELLA TYPHIMURIUM GENOTYPE ST19 IN MEXICO

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Background and Aims: Salmonella enterica is one of the main etiological agents related to foodborne diseases, a global public health problem. *S. enterica* subsp. *enterica* causes 90% of cases of salmonellosis in humans and animals. The Typhimurium serotype is the most relevant globally, and its sequence type ST19 is the most widely distributed genotype worldwide, being considered the founding genotype. Most epidemiological studies have been carried out on bacterial populations at the serotype level, but subtle genomic differences at genotype level are currently considered relevant to outbreak and strain dispersion analysis. The objectives of this work were to obtain the pangenome and to determine the phylogenetic relationships of *Salmonella enterica* subsp. *enterica* serotype Typhimurium ST19 in Mexico.

Methods: The quality of analyzed genomes and *de novo* assembly and annotation were performed with Tormes and Prokka packages, the pangenome was constructed with Roary. 45 genomes of ST19 strains from different sample types that were retrieved from public databases, and one genome of a strain from food sample was here sequenced using Illumina platform.

Results: The pangenome of Mexican ST19 strains is made up of 6092 genes, of which 4023 genes constitutes the coregenome and 2879 genes belongs to accessory genome. The phylogeny obtained using the Maximum-likelihood criterium cluster genomes of strains according to the sample type (e. g. bovine origin) and year of isolation, but not by geographical origin.

Conclusions: These results showed that reconstruction of pangenome from Mexican ST19 strains allows to a subtle sublineages differentiation and to know its patterns of dispersion.

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E-Poster Viewing Topic: AS22 Food microbiology

POSTBIOTIC METABOLITES PRODUCED BY LACTIPLANTIBACILLUS PLANTARUM LRCC5195 AMELIORATES ATOPIC DERMATITIS BY REGULATION OF THE GUT-SKIN AXIS IN OVALBUMIN (OVA)-SENSITIZED BALB/C MICE

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Background and Aims: According to the continuous occurrence of atopic dermatitis (AD), research on effects of probiotics has been reported. However, definitive recommendation is limited as the effects of probiotics in the treatment of AD are still controversial. Postbiotics is attract attention as candidate for treatment of diseases as it exerts a beneficial effect directly or indirectly and the risks associated with their intake are minimized. In this study, we investigated the postbiotic effect from *Lactiplantibacillus plantarum* LRCC5195 regarding gut microbiota regulation and immune responses in ovalbumin (OVA)-sensitized Balb/c mice.

Methods: Mice were OVA-sensitized for 8 weeks, followed by oral administration of postbiotic *Lactiplantibacillus plantarum* LRCC5195 for 8 weeks. Mice in negative group was treated only with 200 μ L of PBS. After 16 weeks, the mice were sacrificed, and tissue and blood samples were collected for blood chemistry and cytokine analysis. Faecal samples from individual mice were collected after 8, 12, and 16 weeks for gut microbiome and SCFA analysis.

Results: Postbiotic *Lactiplantibacillus plantarum* LRCC5195 increased the production of butyric acid, acetic acid, and propionic acid in feces. This increase was associated with increased abundance of *Butyricicoccus*, *Ruminococcus*. Furthermore, Postbiotic *Lactiplantibacillus plantarum* LRCC5195 significantly suppressed T cell-mediated T helper cell type 2, IgE levels, eosinophils and mast cells.

Conclusions: Postbiotic *Lactiplantibacillus plantarum* LRCC5195 has the potential to be applied as a supplement to alleviate AD symptoms by modulating the immune response and gut microbiota.

EP194 / #68

E-Poster Viewing Topic: AS22 Food microbiology

PREVALENCE OF LISTERIA SPP. AND LISTERIA MONOCYTOGENES IN RETAIL PORK MEAT IN LA RIOJA, SPAIN

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Background and Aims: Pork meat is one of the most consumed in the world and can be a source of foodborne pathogens. For this reason, it is necessary investigations about safety of this food. Listeria monocytogenes is a bacterium that causes listeriosis. The aim of the present was to analyze the prevalence of Listeria spp. in pork marketed in La Rioja, Spain.

Methods: Twenty-nine meat samples were analyzed for enumeration and presence of Listeria spp. and L. monocytogenes. Regarding presence of Listeria spp., 25 grams of meat were weighed and enrichment was carried out in half Fraser and Fraser, followed by plating in Chromo Listeria Agar. For enumeration of Listeria spp. samples were homogenized and inoculated in Chromo Listeria Agar. The suspected strains isolated were identified by MALDI-TOF/MS.

Results: The presence of Listeria spp. was found in 10 of the 29 samples analyzed (34.48%). L. monocytogenes was isolated in 4 samples (13.79%) at levels below 2 log cfu/g. Among the other Listeria spp. non-L. monocytogenes, the following species were detected: L. welshimeri (75% of the samples), L. innocua (12.5%), L. grayi (6.25%) and L. ivanovii (6.25%).

Conclusions: The present work shows that L. monocytogenes can be present in pork meat at retail level. In order to control the presence of this pathogen, it is advisable to implement disinfection systems at all levels of meat production. This work has received funding from POCTEFA Project (INTERREG Program) TESTACOS EFA 152/16, Pre-doctoral UR-CAR fellowship and European Union's H2020 research and innovation program under Marie Sklodowska-Curie grant agreement No.801586.

EP195 / #145

E-Poster Viewing Topic: AS22 Food microbiology

POTENTIAL OF MIXED STARTER CULTURE FOR FERMENTED COFFEE PROCESSING AS THE FUNCTIONAL BEVERAGES WITH ANTI-COLON CANCER PROPERTY

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Background and Aims: Coffee is the world's most popular non-alcoholic beverage which has a high economic value due to its taste, flavor, and health benefits such as antioxidant activity and antibacterial activity. Furthermore, its biological active compounds are commonly reported for health promoting. In this study, microbial fermented coffee was formulated and investigated for the biological activity on colon cancer inhibition.

Methods: By enzymatic production selection for the specific Lactobacillus sp. formulated with one of individually yeast including Pichia kluyveri, Saccharomyces cerevisiae, Yarrowia lipolytica, and Torulaspora delbrueckii as the mixed starter culture, five fermented coffees were generated and examined. The antibacterial activity was investigated with foodborne bacterial pathogens including B. cereus, L. monocytogenes, E. coli, Ps. aeruginosa, S. enteritidis, and S. aureus.

Results: Interestingly, the fermented coffee extracts expressed the inhibitory effect to all gram positive of tested strains including Ps. aeruginosa, at 500 mg/ml. In addition, antioxidant activity and total phenolic compound of the extracts were determined by ABTS⁺ and Folin-Ciocalteu assay, respectively. The activity to scavenge ABTS⁺ of fermented coffee revealed the better activity than control. To determine biological active compounds in fermented coffee, LC-MS was performed and evaluated the major potential compounds. Moreover, the coffee extracts were determined for anti-colon cancer property in colorectal adenocarcinoma cell line, Caco-2 and showed the potential to inhibit the growth and proliferation in cancer cell by stimulating apoptotic activity.

Conclusions: The results in this study reveal the advantages of using selected mixed starter culture as the potential for further development of some functional food or beverages.

EP196 / #789

E-Poster Viewing Topic: AS22 Food microbiology

ASSESSMENT OF THE RELATIONSHIP BETWEEN MLST GENETIC DIVERSITY OF L. MONOCYTOGENES AND THEIR GROWTH UNDER SELECTIVE AND NON-SELECTIVE CONDITIONS

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Background and Aims: Listeriosis remains a severe foodborne disease with a mortality rate up to 20%. *L. monocytogenes* can grow under stressing conditions and contaminate various food categories. Its MLST genetic diversity is reflected by different prevalence of the "clonal complex" (CC) in food or infections. Understanding *L. monocytogenes* growth potential variability is essential for quantitative risk assessment, and to ensure efficiency detection of each CC.

Methods: Using optical density (OD) measurements overtime with Bioscreen C spectrophotomer, the growth rates of 36 Lm strains from 12 different CCs and various origins were determined. Three broth medium conditions mimicking stressing food conditions (8°C, aw 0.95 and pH5) and two ISO Standard enrichment broths (Half Fraser and Fraser) were tested. The OD Time to Detection (TD) method using successive serial dilutions was chosen to determine the maximum growth rates (µmax) of the 36 isolates under the fifth growth conditions.

Results: No significant differences were identified between μ max of frequent CC versus rare CC, and between "infections associated" and "food associated" strains. Similarly, no differences were found according to strain origin (dairy, meat, fish and vegetables products). However CC6 and CC8, and CC6 and CC321 had lower μ max than some other CC respectively at pH 5 or in Fraser broth (p < 0.05).

Conclusions: This is important as growth rate could influence risk through pathogen multiplication in food, and lack of detection in case of enrichment problems. Despite this limited difference highlighting natural intraspecific variability, it is interesting to note that µmax can't explain higher CC "virulence" or prevalence.

EP197 / #185

E-Poster Viewing Topic: AS22 Food microbiology

IMPROVEMENT OF LOW-CALORIE INDIAN GOOSEBERRY WATER KEFIR WITH INULIN ADDITION AND ITS ANTI-LIPOGENESIS CAPABILITY

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Background and Aims: The consumption of health promoting foods/beverages has been favorable all over the global population. Water kefir (sugary kefir) is an outstanding alternative beverage for those who do not consume dairy kefir/products since it contains several beneficial bioactivities. The consumption demand of water kefir is increased annually even it contain relatively high calorie level since it traditionally made from sugary substrates e.g., fruit juice or sucrose solution. This study, an alternative low-calorie Indian gooseberry water kefir with anti-lipogenesis capability in 3T3-L1 adipocyte was introduced by replacement of sucrose with inulin addition.

Methods: Supplementation of 10% (w/v) of inulin to Indian gooseberry kefir helped facilitate the growth of microbial communities inside the kefir grain starter during the optimum fermentation condition at 25 °C for 48 h. Cells viability of adipocytes was performed by MTT assay, lipid content was measured by oil red O staining, and glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity was evaluated by GPDH activity assay kit sufficient.

Results: The kefir was capable to reduce 15.21% of lipogenesis without toxicity to adipocytes compared with unfermented Indian gooseberry (18.71%). The GPDH enzyme activity was also reduced with positively correlation to lipogenesis.

Conclusions: This current discovery revealed that the novel kefir beverage had capability to promote health and potentially combat obesity by regulating lipid synthesis of adipocytes.

EP198 / #171

E-Poster Viewing Topic: AS22 Food microbiology

SEASONALITY AND GEOGRAPHY HAVE A GREATER INFLUENCE THAN THE USE OF CHLORINE-BASED CLEANING AGENTS ON THE MICROBIOTA OF BULK TANK RAW MILK

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Background and Aims: Cleaning of the production environment is vital to ensure the safety and quality of dairy products. Although cleaning with chlorine-based agents is widely adopted, it has been associated with detrimental effects on milk quality and safety, which has garnered increasing interest in chlorine-free cleaning. However, the influence of these methods on the milk microbiota is not well documented. This study investigated the factors that influence the raw milk microbiota, with a focus on the differences when chlorine-based and chlorine-free cleaning of milking equipment are used.

Methods: Bulk tank raw milk was sampled at three sampling months (Apr, Aug and Nov), from farms across Ireland selected to capture the use of different cleaning methods, i.e., exclusively chlorine-based (n = 51) and chlorine-free cleaning (n = 92), and farms that used chlorine-free agents for the bulk tank and chlorine-based cleaning agents for the rest of the equipment (n = 28).

Results: Shotgun metagenomic analysis revealed the significant influence of seasonal and geographic factors on the bulk tank milk microbiota, indicated by differences in diversity, taxonomic composition, and functional characteristics. Taxonomic and functional profiles of samples collected in November clustered separately from other months. In contrast, cleaning methods only accounted for 1% of the variation in the bulk tank milk bacterial community, and samples collected from farms using chlorine relative to chlorine-free cleaning did not differ significantly, suggesting that chlorine-free approaches used did not negatively impact microbiological quality.

Conclusions: This study shows the value of shotgun metagenomics in advancing our knowledge of the raw milk microbiota.

EP199 / #371

E-Poster Viewing Topic: AS24 Food Mycology/Taxonomy Penicillium and Aspergillus

BEADLE AND TATUM 2.0: ONE GENE, ONE PROTEIN, MULTIPLE VIRULENCE PATHWAYS IN THE BLUE MOLD FUNGUS.

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Background and Aims: Apples are stored for extended periods of time in controlled atmosphere. In storage, fruit are susceptible to blue mold caused *Penicillium expansum*. The disease manifests in multimillion-dollar losses and is a significant source of patulin. The main aim of our study was to identify genes, processes and pathways that control fungal virulence by integrating multiple modalities to develop such controls.

Methods: Random T-DNA mutagenesis was used to generate *Penicillium expansum* transformants. TAIL-PCR and qRT-PCR was used to assess the T-DNA location and its impact on gene expression. Electron Microscopy and standard microbiological assays were conducted. Mass spectrometry was used to monitor proteins secreted into liquid media during growth and quantitative mass spectrometry was used to explore changes in mycelial proteins.

Results: One transformant, T625, had reduced decay in apples, showed blistered mycelial hyphae, and had undetectable expression of the blistering1 locus. The Blistering1 gene encodes a single protein with a DnaJ domain. Secretome analysis of the T625 mutant showed mis regulated secretion of degradative enzymes, along with ones involved in patulin biosynthesis. The mutant had reduced capacity to degrade apple tissue and contained 30 times less patulin. Quantitative analysis of mycelial proteins revealed altered cellular networks controlling protein processing.

Conclusions: This is the first study to demonstrate that a single copy gene, Blistering1, encodes a polypeptide that regulates multiple processes crucial for fungal virulence in apple fruit. We hypothesize that Blistering1 interacts with other cellular proteins and machineries to aid in protein folding/secretion/sorting and is the subject of active investigation in our laboratory.

EP200 / #554

E-Poster Viewing Topic: AS30 Mycobiomes

AMELIORATION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY ACTIVATED MYELOID-DERIVED SUPPRESSOR CELLS-BASED THERAPY ASSOCIATES WITH SPECIFIC CHANGES IN GUT MICROBIOTA COMPOSITION

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Background and Aims: The therapeutic potential of myeloid-derived suppressor cells (MDSCs) for the treatment of autoimmune diseases has not been sufficiently studied. The aim was to investigate the potential of MDSC to ameliorate experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), and to assess how the efficacy of MDSC therapies associated with gut microbiota changes.

Methods: EAE was induced with MOG₃₅₋₅₅/CFA/pertussis toxin in C57BL/6 mice. Bone marrow-derived MDSC were differentiated with GM-CSF/IL-6 (MDSC), or GM-CSF/IL-6/PGE2 (MDSC-PGE2), MACS-purified, and then given as a cell therapy in 5 doses (from day 3 to day 11) post-immunization. The microbiota composition was analyzed by shotgun metagenomics analysis from feces collected before EAE induction, and at the disease peak.

Results: We demonstrated that MDSC-PGE2, but not control MDSC, inhibited the symptoms of EAE accompanied by a reduction of inflammatory infiltrates, and an increase in regulatory lymphocyte populations in the spinal cord. Both MDSC or MDSC-PGE2 therapies prevented the reduction of gut microbiota diversity induced by EAE. Although the samples of all groups were marked with genus *Romboutsia*, previously associated with EAE and MS, only the fecal samples from MDSC-PGE2-treated mice were enriched with short-chain fatty acids producing genera *Odoribacter* and *Butyricimonas*, and with *Turicibacter* and *Rikenella*, previously reported as an anti-inflammatory.

Conclusions: These results indicate that MDSCs obtained *in vitro* have a great potential in reducing the symptoms of EAE, which correlated with the enrichment of immunoregulatory members of gut microbiota. These results can be used to further develop and improve the therapy for acute phases of MS.

EP201 / #202

Spain

E-Poster Viewing Topic: AS31 Climate change and microbial life

EFFECT OF TEMPERATURE AND SALINITY ON VIBRIO-MUSSELS INTERACTIONS

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Background and Aims: Vibrios are ubiquitous inhabitants of the aquatic ecosystems, where they live in intimate contact with autochthonous fauna. In fact, Vibrio spp. can be entrapped by filter feeding invertebrates, i.e. mussels, which can serve as reservoirs for vibrios and other pathogens. To compare the direct impact of temperature and salinity on the capacity of mussels to accumulate vibrios.

Methods: Mytilus galloprovincialis was used as host of a V. harveyi strain modified to express the GFP protein to monitor the interaction between these organisms. Mussels were collected in the estuary of Plentzia (Basque Country, Spain) and, after acclimation, were transferred to tanks containing sterile natural seawater (NSW) or simulated estuarine water (SEW; mix of freshwater and seawater, 1:2) inoculated with V. harveyi gfp (10⁷cells/mL), and incubated at 12°C or 20°C. Periodically, mussels were collected and dissected. Grills, gonads and digestive glands were sampled separately for V. harveyi quantification. Additionally, the planktonic cells of V. harveyi remaining in NSW or ESW were enumerated.

Results: Our results showed that mussels actively removed V. harveyi from both types of water at 12°C and 20°C. In few minutes Vibrio reached its maximum density in the organs, where was retained for some hours or days. Subsequently, the bacteria content decreased progressively.

Conclusions: Vibrio was rapidly accumulated in the mussels' organs, mainly in the digestive gland. No great differences were detected due to changes in temperature or salinity conditions. Projects PIBA_2021_1_0047 (Basque Government) and GIU20/074 (University of the Basque Country).

EP202 / #283

E-Poster Viewing Topic: AS31 Climate change and microbial life

PHOSPHATE SOLUBILIZING BACTERIA ABILITY TO FORM BIOFILM UNDER ABIOTIC STRESS

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Background and Aims: Phosphate solubilizing bacteria (PSB) are responsible for most P acquisition by plants in cultivated soils. This is mainly achieved by effective PBS colonization of the plant rhizosphere. Biofilm development by PBS is considered as a survival strategy for growth under stress and natural conditions. Bacteria with efficient colonization ability and exhibiting P solubilization are expected to stimulate better the plant growth. Meanwhile, many biological processes which occur in soils (including P solubilization) can be affected by abiotic factors like pH, temperature and salinity due to the current climate changes. The main goal of our study was to identity bacterial strains able to solubilize tricalcium phosphate (TCP) and to form biofilm, under abiotic stress.

Methods: Therefore, seven bacterial strains were tested for their ability to solubilize TCP using phosphomolybdate ammonium method and for biofilm formation using crystal violet assay.

Results: Our findings showed that the tested strains have different solubilization potential, the amounts of solubilized P ranging between 11.35 and 17.06 µg/ml. Regarding biofilm formation, four strains are strongly adherent, and three strains are moderately, weakly, respectively non-adherent. One of the strongly adherent strains (P3.3S) was tested for biofilm formation under abiotic stress (pH variations and different concentrations of NaCl). This strain was able to form biofilm even at 30 g/L NaCl and at pH of 5 and 9.

Conclusions: Our results support the hypothesis of using P3.3S strain as biofertilizer in soils affected by abiotic stress.
EP203 / #807

E-Poster Viewing Topic: AS31 Climate change and microbial life

COLD-ADAPTED YEASTS LURKING IN SPRING TREE FLUXES IN TEMPERATE CLIMATES

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Background and Aims: Global warming has already significant effects on many Earth's habitats. Climate change and dramatic cryosphere reduction in space and time can negatively affect psychrophilic and psychrotolerant species, including prominent cold-dwelling yeasts. Their habitats outside threatened due to climate change polar and non-polar cold environments are little known.

Methods: Tree fluxes caused by tree injuries were sampled in early spring (2013–2022) in the Northern Germany. Yeasts were isolated by dilution plating and enrichment techniques, and identified using common ribosomal DNA-barcodes.

Results: Yeast communities developing in tree fluxes were strongly dominated by basidiomycetous yeasts. Occasionally isolated ascomycetous yeasts *Hanseniaspora osmophila*, *Kazachstania servazzii*, *Komagataella pastoris*, *Pichia fermentans*, and *Zygotorulaspora florentina* were associated with insects (e.g., *Drosophila*) feeding on tree fluxes. Basidiomycetous yeasts were represented by rarely observed pigmented phylloplane species and strongly prevailing cold-adapted yeasts of genera Cystofilobasidium, *Goffeauzyma*, *Holtermanniella*, *Leucosporidium*, *Mrakia*, and *Tausonia*.

Conclusions: Large temperature contrasts in spring favour development of cold-adapted yeasts in tree fluxes. Whether or not cold-adapted yeasts survive warm temperatures in a dormant state or remain active throughout the year is yet unknown. Spring tree fluxes are an ephemeral but rather widespread substrate that could potentially provide a refuge for cold-adapted species, even outside polar and non-polar cold environments.

EP204 / #281

E-Poster Viewing Topic: AS32 Complex systems, disturbances and disruption

DUAL "OMICS": INTEGRATED BIOMARKER DISCOVERY AT RIVERINE SEDIMENT-WATER INTERFACE ALONG A GRADIENT OF AGRICULTURE NUTRIENT CONTAMINATION.

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Background and Aims: There is significant interest in incorporating next generation "omics" techniques into monitoring frameworks for aquatic environments. However, conventional taxonomic indicators, or "eDNA", can lack sensitivity in predicting ecosystem dynamics, especially with respect to microbial communities at ecosystem-interfaces. Thus, there is need for the development of tools that provide for increased responsiveness to environmental perturbations, such as increased nutrient input into aquatic systems from agricultural or industrial runoff. Such contamination can have significant impact at the level of microorganism community function, given their role as primary drivers of sediment nutrient and energy cycling, and often leads to algal development and downstream eutrophication.

Methods: The complexity of agricultural contaminants (nutrients, herbicides, etc.) and phenotypic and functional plasticity of microbial communities necessitates the use of advanced omics to understand ecosystem disruption. Herein, we jointly use metatranscriptomic (active gene expression) and metabolomics (pool of metabolites; GC-MS) to profile microbial community activity at the sediment-water interface of an agriculturally impacted river system in Ontario, Canada. Data from bench-scale annular flumes and biofilm bioreactors modelling the impacts of acute *vs* chronic nutrient-contamination on sediment microbial function is presented.

Results: reveal metabolic markers associated with particular land-use (e.g., recreational beachuse *vs* agriculture activity), and the ratio of lipid:plant tissue related compounds can potentially indicating agriculture-contamination. Specific markers also show potential to distinguish between industrial agriculture and naturally vegetated regions. Microbial stress response signatures are potentially more closely associated with compounds known to be antimicrobial.

Conclusions: The collective "omics" datasets can be used in assessment and monitoring of sediment nutrient contamination.

EP205 / #101

E-Poster Viewing Topic: AS34 Innate immunity as a first-line against viruses, bacteria and fungi

PRETREATMENT WITH ZYMOSAN MODULATES DOUBLE STRANDED RNA OR TOLL-LIKE RECEPTOR 7 LIGAND-INDUCED PRODUCTION OF PROINFLAMMATORY CYTOKINES AND INTERFERON-BETA

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Background and Aims: Heat-killed Candida albicans augments Toll-like receptor (TLR) 2 or 4 ligandinduced proinflammatory cytokine production by mouse macrophage-like J774.1 cells. In this study, we investigated whether zymosan regulated cytokine production by the cells incubated with polyinosinicpolycytidylic acid (poly(I:C)), a ligand for TLR3 and retinoic acid inducible-gene I (RIG-I), or imiquimod, a TLR7 ligand.

Methods: J774.1 cells were pretreated with or without zymosan, a TLR2 and dectin-1 ligand, curdlan, a dectin-1 ligand, or Pam₃CSK₄, a TLR2 ligand in 96-well flat-bottomed plates. Cells were then washed three times and incubated with poly(I:C) or imiquimod. Culture supernatants were analyzed by ELISA for secreted mouse interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF)- α , and interferon (IFN)- β . Caspase-11, nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), caspase-1 and RIG-I were analyzed by western blotting.

Results: Pretreatment with zymosan or curdlan augmented poly(I:C)-induced production of IL-6, MCP-1, TNF- α , and IFN- β by J774.1 cells. Zymosan or curdlan also upregulated the expression of caspase-11, NLRP3 and RIG-I in the cells. In addition, pretreatment with zymosan augmented imiquimod-induced production of proinflammatory cytokines by J774.1 cells, but not curdlan. However, IFN- β production induced by imiquimod was reduced when cells were pretreated with zymosan or curdlan. Similar results were shown in pretreatment of cells with Pam₃CSK₄.

Conclusions: These results suggest that pretreatment with zymosan augments poly(I:C)-induced cytokine production by J774.1 cells through upregulation of RIG-I expression. In addition, zymosan might down-regulate TLR7 ligand-induced IFN-β production by activating inflammasome via TLR2 and dectin-1.

EP206 / #702

E-Poster Viewing Topic: AS34 Innate immunity as a first-line against viruses, bacteria and fungi

CAN WE MODULATE THE MACROPHAGE POLARIZATION BY INDIRECT ACTIVITY OF PLANT EXTRACTS?

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Background and Aims: The increasing occurrence of chronic infections and inflammatory diseases necessitates the search for therapeutic solutions based on immunomodulatory effects. The aim of this study was to determine whether the supernatants of staphylococcal cultures exposed to *Viburnum opulus* L. extracts could influence the polarization of macrophages to M1 or M2 phenotype *in vitro*.

Methods: Planktonic and biofilm cultures of *Staphylococcus aureus* ATCC 43300 were exposed to *V. opulus* extracts (100 µg/ml) and vancomycin for 24h at 37°C. Human macrophages derived from THP-1 cell line were exposed to staphylococcal culture supernatants and cell wall components. The expression of surface CD molecules characteristic for the macrophages of M1 (CD11c) and M2 (CD206) phenotype was assessed by flow cytometry. The profile of secreted cytokines was tested by ELISA.

Results: There was no statistically significant effect of the staphylococcal culture supernatants on CD molecules expression. Nevertheless, exposition of the macrophages to biofilm supernatants or staphylococcal cell components caused slight decrease of CD11c expression. Similar effect was observed for CD206 expression after cell exposition to both planktonic and biofilm supernatants. While, increased production of TNF-alpha and IL-10 was noticed for majority of stimulated cells.

Conclusions: The indirect immunomodulatory activity of natural plant products against phagocytic cells consists more in modifying cytokine production than in changing the expression of surface CD molecules. However, further detailed research is required to confirm this thesis. The study was supported by the funds of University of Lodz – Doctoral Research Grant 11/DGB/IDUB/2022.

EP207 / #154

E-Poster Viewing Topic: AS37 Emerging diseases in 'One Health' context

SYMPTOMATIC PROFILING OF DENGUE-CHIKUNGUNYA CO-INFECTION BY LATENT CLASS CLUSTER ANALYSIS (LCCA). A CROSS-SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL IN NORTHERN INDIA

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Background and Aims: Cases of dengue and chikungunya fever are escalating all over India. Both the virus shares a common vector, the Aedes mosquitoes. Due to similar clinical symptoms, both the dengue (DENV) and chikungunya (CHIKV) virus can circulate as co-infection. In this study, Using Latent Class Cluster analysis (LCCA), we thoroughly examined clinical data from DENV-CHIKV suspected people to determine the presence of a group of individuals with similar and distinct characteristics.

Methods: A cross-section study was carried out in a tertiary care hospital in Northern India from January 2018 to December 2019. Based on clinical symptoms, a clinical profile of 11 symptoms was derived using Latent Class Cluster Analysis (LCCA) by Latent GOLD 6.0. All samples were tested for dengue NS1 antigen, dengue IgM antibody, and chikungunya IgM antibody by ELISA.

Results: Of 279 suspected individuals of DENV-CHIKV infection, five mutually exclusive latent clusters of individuals were classified with divergent and statistically significant characteristics, implying the existence of discrete DENV-CHIKV suspected subpopulations. Fever, rash, arthralgia, and vomiting were present commonly in all 5 clusters. The majority of LCCA-derived clusters are made up of males, demonstrating a distinct phenotypic response pattern when compared to females (χ 2= 44.7, df=1, p= <0.0001) ELISA result shows that 222 (79.57%) samples came positive for dengue, and 16 (5.73%) samples for CHIKV. 4 (1.43%) samples were positive for both DENV and CHIKV.

Conclusions: Repeated episodes of DENV, CHIK, and co-infection in this region and LCCA analysis indicate that any symptomatic case should be tested for both DENV and CHIKV infection.

EP208 / #596

E-Poster Viewing Topic: AS37 Emerging diseases in 'One Health' context

POTENTIALLY PATHOGENIC ENVIRONMENTAL STRAINS OF NON-TUBERCULOUS MYCOBACTERIA FROM WASTEWATER SAMPLES FROM THE MUNICIPALITIES OF YOPOUGON AND KOUMASSIMARCORY IN ABIDJAN

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Background and Aims: *Mycobacteria* represent a large group of bacteria commonly found in the environment. They are involved in several infections ranging from lung infections to skin infections. In Côte d'Ivoire, very little information is available on these species apart from the best known, namely *M. ulcerans* and *M. tuberculosis*, responsible for Buruli ulcer and tuberculosis respectively. The cultivation of these species is a real challenge, especially in developing countries such as Côte d'Ivoire. However, there are reports in the literature of infections caused by these mycobacteria and few species have been described in cases of human or animal infections. Mycobacteriosis due to these mycobacteria is difficult to estimate because these diseases are not reportable illness. These pathologies are difficult to treat because of their resistance to most anti-tuberculosis antibiotics. The aim of our study was to identify the strains of potentially pathogenic non-ulcer and non tuberculosis environmental mycobacteria circulating in the wastewater in the city of Abidjan

Methods: Mycobaterium Colonies were looking for in waters samples by usual microbiology methods. The amplification products corresponding to IS6110 gene identified by classical PCR were sequenced by the CRCHU sequencing and genotyping platform of Quebec-CHUL

Results: The strains isolated in this study were fast-growing mycobacteria and slow-growing mycobacteria. Thanks to the sequencing of the amplification product, 5 species of mycobacteria were identified, namely *mycolicibacterium fortuitum*; *mycolicibacterium mageritense*; *mycolicibacterium europaeum*; *mycolicibacterium neworleansense* and *mycolicibacterium Brumae*.

Conclusions: This study would be the first to identify these fast-growing and slow-growing species in wastewaters in Côte d'Ivoire.

EP209 / #751

E-Poster Viewing Topic: AS37 Emerging diseases in 'One Health' context

PAN-GENOMIC APPROACH OF MDR SALMONELLA INFANTIS STRAINS ISOLATED FROM A POULTRY FARM IN CHILE.

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Background and Aims: The emergence of new *Salmonella* serotypes is a great concern in the food industry; in particular, the serotype Infantis is associated with critical profiles of Multiple Drug Resistance (MDR) that are challenging for epidemiology control. We aimed to characterize *Salmonella* Infantis strains from a poultry farm in Chile and determine possible associations between the strains and the unique genes contributing to adaptation and persistence within the production lines despite rigorous disinfection protocols.

Methods: The analysis was carried out following the anvi'o pangenomic workflow, eliminating all contigs with less than 200 nucleotides, and hierarchically clustering the gene (based on their distribution) and the genomes (based on the gene clusters they share); generating a comprehensive anvi'o Pan-DataBase for downstream analyses.

Results: We found that 53.9% of the identified gene clusters belong to the core-genome most of which are single-copy core genes, and only 3.7% were unique genes (the remaining 42.3% belong to the disposable genome). We also detected antimicrobial resistance genes and virulence elements using ABRicate and the ARG-ANNOT, VFDM, and PlasmidFinder databases. The analysis of all genes and their variations among the sampled strains shows no associations between a particular strain and its isolation site, suggesting widespread contamination throughout the facility. Furthermore, the 181 unique genes identified are distributed between ten strains with frequencies from 1 to 140 unique genes per strain, some with functions associated with virulence and stress resistance.

Conclusions: We conclude that disinfection protocols are ineffective in eliminating *Salmonella* from the poultry meat and this work contributes with knowledge for improving food safety.

EP210 / #303

E-Poster Viewing Topic: AS37 Emerging diseases in 'One Health' context

COXIELLA BURNETII SHEDDING OCCURRENCE AND MOLECULAR DIVERSITY IN EWES FROM ST. KITTS

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Background and Aims: The aim of this study was to detect and characterize the genetic diversity of *C. burnetii* bacteria shed by ewes.

Methods: Vaginal secretions, fecal and milk samples were obtained from pregnant and post-parturient Barbados Blackbelly ewes (n=187) in Saint Kitts, West Indies. *Coxiella burnetii* was screened using IS1111 qPCR. For molecular characterization, six strong qPCR positive samples (5 vaginal secretions, 1 fecal) were interrogated with IS1111 conventional PCR. nBLAST, phylogenetic and haplotype analyses were then performed.

Results: Overall, 86% (161/187) of ewes were shedding *C. burnetii*. According to nBLAST analyses, 4/6 IS1111 sequences were related (identity 99.8%) to *C. burnetii* from humans in Kyrgyzstan (CP014563.1). The rest IS1111 sequences (2/6), were closely related (identity 100%) to *C. burnetii* isolated from in goats in Germany (CP018150.1). Phylogenetic analyses revealed that the IS1111 sequences from St. Kitts were closely aligned to *C. burnetii* detected in ruminants from Colombia. Haplotype analyses of sequences from this study, along with worldwide sequences from ruminants, and humans, revealed a total of 5 haplotypes. All sequences from this study belonged to haplotype #1, and seemed to be the most widespread. They were also seen in humans from Australia, and Kyrgyzstan, as well as ruminants from Germany, China, and India.

Conclusions: In nutshell, there is evidence of frequent shedding of *C. burnetii* by ewes in St. Kitts. Although the shed bacteria had low diversity, the observed haplotype has worldwide distribution and is shared with humans, highlighting the zoonotic potential of *C. burnetii* in the country.

EP211 / #34

E-Poster Viewing Topic: AS37 Emerging diseases in 'One Health' context

INVESTIGATION ON THE COVID-19 PANDEMIC: HEALTH, INFECTION, AND VACCINATION

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Background and Aims: COVID-19, a highly contagious and progressive infectious disease that is still posing a major global health challenge for our world, after the emergence of the seventh zoonotic pathogenic novel member of the human coronaviruses SARS-CoV-2.

Methods: we launched an "open to everyone" survey on social media, to estimate the use of preventive measures, or distinguish the rate of infection, and vaccine hesitation for members according to their gender, age, and place of residence; it contained questions on the novel human coronavirus, the applied preventive measures, and vaccination, plus the impact of the pandemic on their lives.

Results: We found out that many believed in the disease and knew how to prevent it, but mostly didn't understand the transmission process, also some of the partakers did confirm the infection by the different available tests, others didn't, and unfortunately, half of the participants were not vaccinated and the other half refused to get a vaccine.

Conclusions: Many sides of this pandemic are still unknown, and important data is needed, to understand the key to ending it.

EP212 / #73

E-Poster Viewing Topic: AS38 Other

RESEARCH OF THE EFFECTIVENESS OF SIMULTANEOUS USE OF ANESTHETICS AND ANTISEPTICS FOR LOCAL TREATMENT OF INFECTIOUS COMPLICATIONS AND PAIN AFTER COMBAT TRAUMA

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Background and Aims: Background. Infections after combat trauma are common and complex, requiring a multidisciplinary approach to treatment. The aim: To study in vitro antimicrobial activity of surfactant active antiseptics decamethoxin 0.1%, chlorhexidine 0.05%, local anesthetics (0.5% bupivacaine, 2% lidocaine) and their simultaneous use against strains of opportunistic pathogens A.baumannii as pathogens of infectious complications in patients with combat trauma.

Methods: In total, the study used 21 clinical strains of A. baumannii isolated from purulent-inflammatory wounds in patients injured in combat. Minimum bacteriostatic and bactericidal concentrations (MIC and MBC) of antiseptics and bacteriostatic effect (IPC) of local anesthetics with the addition of a subbacteriostatic dose of antiseptics were investigated by the methods of disco-diffusion and double serial dilutions.

Results: The antimicrobial properties of 0.5% bupivacaine and 2.0% lidocaine relative to A.baumannii, as evidenced by clear zones of growth retardation of microorganisms around the wells with analgesics (p <0,05). It has been proven that the bacteriostatic action of bupivacaine and lidocaine is within the effective concentrations of these drugs, providing an analgesic effect when adding a subbacteriostatic dose of antiseptics. A strong bactericidal effect of decamethoxine ($19.5 \pm 9.5 \mu g / ml$) was found, and chlorhexidine showed weak activity against this pathogen (MIC - 54.8 $\mu g / ml$, MBcK - 33.4 $\mu g / ml$).

Conclusions: Local antiseptics and anesthetics, along with the main pharmacological activity, have antimicrobial action on leading opportunistic pathogens, and can be used together in complex treatment for patients with combat trauma in need of immediate medical attention.

EP213 / #237

E-Poster Viewing Topic: AS38 Other

ASSOCIATION OF GUADELOUPE MOSQUITO VIRUS WITH MOSQUITO-BORNE FLAVIVIRUSES IN FEMALE ADULT AEDES AEGYPTI MOSQUITOES

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Background and Aims: Guadeloupe mosquito virus (GMV) is an insect-specific Luteo-sobemo-like virus that has firstly been identified in female adult Aedes aegypti collected from northeastern Thailand. Recent studies indicated the roles of insect-specific viruses (ISVs) in mosquito-borne flaviviral interference in such hosts. However, the relationship between identified GMV and mosquito-borne flaviviruses remains not clarified. In this study, we aimed to examine whether GMV could be negatively correlated with arboviruses in Aedes aegypti mosquitoes.

Methods: Total RNA was extracted from the pools of field-caught Aedes aegypti and then sequenced on the Illumina Miseq platform. Viral contigs were queried against NCBI viral database. The viral evolutionary relationship was reconstructed using Maximum-likelihood phylogenetic tree. The relations between the considered arboviruses (i.e., dengue virus and zika virus) and GMV presence were explored using Pearson's correlation. Based on the infection rate of GMV, these arboviruses are categorized by principal component analysis (PCA).

Results: GMV was found in the abdomens of 97.21% of female adult Aedes aegypti. Dengue virus and zika virus were detected in 1.32% and 0.06% of these samples, respectively. Interestingly, GMV was statistically significantly negatively associated with arboviral prevalence (OR = -6.28), whereas no association with other ISVs. Consistently, using Kaiser Index and PCA analysis, there are significant differences between the presence of GMV in the mosquito and these arboviruses.

Conclusions: Collectively, arbovirus prevalence is negatively associated with GMV infection, suggesting that GMV may act as an important anti-arboviral factor in female adult Aedes aegypti.

EP214 / #230

E-Poster Viewing Topic: AS38 Other

ANALYSIS AND CHARACTERIZATION OF NON-RETROVIRAL RNA VIRUSES ENDOGENOUS VIRAL ELEMENTS IN FIELD-CAUGHT AEDES AEGYPTI COLLECTED FROM NORTHEASTERN THAILAND

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Background and Aims: Aedes aegypti is one of the potential mosquito vectors for several pathogenic arboviruses, including dengue virus, zika virus, and chikungunya virus. With no specific vaccines and mosquito control approaches for recent arboviral epidemics prevention, understanding the mechanisms of immune response in mosquitoes could provide alternative strategies to limit arbovirus spread. Recent evidence indicates that the sequences of non-retroviral RNA viruses endogenous viral elements (nrEVEs) with high sequence identity to a contemporary RNA virus have highly been found in the Aedes aegypti genome which can play important role in the PIWI-interacting RNAs (piRNAs) biogenesis, serving as an immunological memory source in such Culicidae species. However, the roles of nrEVEs- derived piRNAs in antiviral infection in field-caught Aedes aegypti are poorly understood.

Methods: Here, we used genome assemblies from next-generation sequencing (NGS)-based metagenomic data to analyze and characterize the nrEVEs landscape and its function across 10 pools of field-caught Aedes aegypti genomes.

Results: We found that nrEVEs are prevalent in these genomes and predominantly link to unclassified RNA viruses, including Luteo-sobemo-like viruses and families belonging to the Flaviviridae, Rhabdoviridae, and Chuviviridae. Notably, these sequences were enriched in clusters of piRNA. Moreover, we steadily found nrEVEs-derived piRNA with targeted processing of persistently infecting virus genomes supporting the contribution of nrEVEs in the immune response of the mosquito.

Conclusions: Our work affords data that antiviral piRNAs are fabricated in the presence of naturally occurring nrEVEs and their endosymbiotic viruses, revealing a functional network between nrEVEs and antiviral immunity in natural mosquito holobiont.

EP215 / #767

E-Poster Viewing Topic: AS38 Other

FUNGAL BIOAUGMENTATION FOR BIOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL IN KITWE, ZAMBIA

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Background and Aims: Filamentous fungi present in heavy metals (HMs) contaminated environments have a great potential for bioaugmentation, although they are still poorly investigated in developing countries such as Zambia. Therefore, this research aimed at exploring the bioaugmentation potential of a consortium of filamentous fungi in heavy metal contaminated soil.

Methods: Three highly tolerant indigenous fungal species (Aspergillus oryzae, Cladosporium pseudocladosporioides, and Geotrichum candidum) were isolated from heavy metal-contaminated soil by conventional streak-plate method and identified using a combination of morphological and molecular techniques. Their tolerance to heavy metals was evaluated by the radial growth diameter technique. The three indigenous fungi were used as a consortium for the bioaugmentation experiment to bioremediate copper and cobalt contaminated soil; simultaneously, using uncontaminated as a control

Results: The maximum tolerance index of 1.0 was observed for all three species in the presence of high concentrations of Cu, Co, Fe, Mn, Pb, and Zn. The maximum Cu, Co, Fe, Mn, Pb, and Zn bioremoval capacities of a highly tolerant blended fungal consortium of treated soil were examined after 90 days and was recorded as Cu:75.09 %, Co:99.8 %, Fe:62.9 %, Mn:84.7 %, Pb:100 %, and Zn:100 %.

Conclusions: There was a significant difference between the treated and untreated contaminated soil. The present study has demonstrated the potential of highly resistant fungal isolates to remediate the heavy metal contamination.

EP216 / #587

E-Poster Viewing Topic: AS38 Other

CHARACTERIZATION OF GENES ENCODING SECRETORY ASPARTYL PROTEINASE (SAP1-6) AMONG CANDIDA ALBICANS ISOLATED FROM PATIENTS WITH SURGICAL SITE INFECTIONS

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Background and Aims: Surgical site infection (SSIs) is one of the major complications following surgery. Candida albicans is commensal of host epithelial tissues can cause life-threatening infections in immunocompromised patients. The aim of this study was to determine the prevalence of C. albicans in patients with SSIs and to analyze genetic versatility of six virulence genes (SAP1-SAP6) of secretory aspartyl proteinase to highlight the role of these virulent genes in SSIs.

Methods: Pus Swabs (n=450) were collected from patients having SSIs . Candida isolates were identified by standard microbiological methods. Prevalence of six virulence genes (SAP1-SAP6) of secretory aspartyl proteinase of C. albicans was determined by PCR.

Results: Culture of the wounds yielded Candida spp. 128 (20.94%). Species identification of Candida strains revealed most frequent presence of C. albicans 54 (42.18%). All isolates of C. albicans possessed genes SAP1-3. Gene SAP4 was detected in genome of 46 C. albicans strains, SAP5 in 23, and SAP6 in 28 strains. Among all 54 C. albicans tested, SAP5 and SAP6 genes were synchronously lacking in eight isolates while six isolates were found to be synchronous deficient for SAP4 and SAP5 genes. However, sixteen C. albicans showed synchronous presence of all tested genes except SAP5.

Conclusions: Presence of Candida spp. in surgical wounds demonstrated the role of fungi in surgical site infections. The high frequency and high rate of association patterns of SAP genes in C. albicans strains draw attention to comprehend the existence and function of these genes may involve to enhance the risk of infection in patients undergoing surgeries.

EP217 / #132

E-Poster Viewing Topic: AS38 Other

MYCOBACTERIUM TUBERCULOSIS PANGENOME ANALYSIS TO IDENTIFY GENES ASSOCIATED TO EXTRAPULMONARY TUBERCULOSIS

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Background and Aims: Tuberculosis (TB) is a global disease caused by *Mycobacterium tuberculosis* (MTB). MTB may spread by lymphatic drainage or through the bloodstream to any organ causing extrapulmonary TB (EPTB). Epidemiological and experimental studies have shown that MTB genetic diversity plays an important role in the clinical presentation, however the genetic determinants associated with EPTB remains unclarified. This study evaluates the composition of the MTB pangenome constructed from the genomes of pulmonary and extrapulmonary strains to identify putative genes associated with clinical presentation of the disease.

Methods: Different software was used to analyze 490 genomes of MTB strains retrieved from the SRA database, and the genomes of 10 Mexican strains here sequenced. A quality analysis was performed with Fastqc and TrimGalore, then the assemblies and annotations were conducted with SPADES and Prokka, the pangenome was constructed with Roary. The association between genes and EPTB was performed with query_pan_genome and SCOARY.

Results: It was found that the presence of the *aftc* gene is associated with the EPTB phenotype (p<0.0001, OR: 10.5) while the presence of a gene encoding a hypothetical protein is associated with pulmonary tuberculosis (PTB) (p<0.0001, OR: 10.2). The analysis of the distribution of genes in the accessory pan-genome determine the association of PTB with the deletion of *hspR*, *plcD* (virulence genes) and *pe_pgrs5, pe_pgrs25, Rv1759c, pe_pgrs57, Rv817c, Rv2818c, Rv2816c, Rv3740c* and *Rv2550c*. Deletion of virulence genes *aceA, plcA, esxR* and *Rv2275* is associated with EPTB.

Conclusions: These results show EPTB associated genetic markers and guide further experimental work to evaluate MTB virulence factors.

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DETECTION OF POLYMICROBIAL CANDIDAEMIA BY SINGLE STRAND CONFORMATION POLYMORPHISM

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Background and Aims: Candidaemia is a life threatening clinical infections. While most candidaemia is caused by infections by a single *Candida* species, mixed candida bloodstream infections had been reported. The timely detection of resistant organism such as *Candida glabrata* is important to guide appropriate treatment regimen. This study aimed to assess the usefulness of single strand conformation polymorphism (SSCP) in delineating different Candida species in mixed infections.

Methods: Type strains of *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, and *Candida tropicalis* were cultured and then serially diluted from 10⁷ to 10³ cfu/mL. DNA were extracted from these serially dilutions. A 280bp amplicon was targeted from the 28S rRNA for PCR-SSCP. Mixtures of different dilutions of *C. albicans*, *C. glabrata*, and *C. tropicalis* were simulated and subjected to PCR-SSCP. The SSCP patterns of mixed cultures were then compared with those from monocultures.

Results: The detection limit of monocultures were 10⁴ cfu/mL. In mixed cultures of *C. albicans* and *C. glabrata*, discernable bands were only observed when *C. albicans* was at 10⁵ cfu/mL and *C glabrata* at 10⁶ cfu/mL. In mixed cultures of *C. albicans* and *C. tropicalis*, the detection limit was 10⁵ cfu/mL for both organisms. In mixed cultures of *C. albicans*, *C. glabrata* and *C. tropicalis*, the detection limits were further increased to 10⁶, 10⁷, and 10⁶ cfu/mL respectively.

Conclusions: In mixed infections by candida organisms, the detection limit PCR-SSCP were raised. In clinical candidaemia where inoculum is likely low, PCR-SSCP had limited usefulness in speciation of mixed Candida infections.

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URINE CYSTOLOGICAL CHANGES IN PATIENTS WITH CHRONIC RECURRENT CYSTITIS OF BACTERIAL AND PAPILLOMAVIRUS ETIOLOGY

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Background and Aims: Over 10 years, the incidence of human papillomavirus (HPV) in the urogenital tract has increased significantly. Thestudy aimed to investigate the cytological urine characteristics in women with chronic recurrent cystitis (CRC) of bacterial and HPV etiology.

Methods: Study included 118 female patients with CRC aged between 20 and 50 years old. Depending on etiology, all patients were divided into two groups: group 1 (n=65) had HPV etiology and group 2 (n=53) bacterial etiology. All patients were subjected to a urine cytological study. For the study, we centrifuged 2.5 ml of urine for 10 minutes at a speed of 2000 rpm. Urine sediment was aspirated with a pipette. Staining was performed by the polychrome method used in cytological examination. Smear staining was performed according to the Romanovsky-Giemsa method

Results: Cytological study showed that both groups had a different pattern of cytological changes in urine. The total koilocytic transformation of urothelium in group 1 was detected only in 11 (16.9%) cases. Group 1 patients had many lymphocytes compared to a low count of neutrophils (23.1%) per FoV. At the same, the high count of neutrophils was found in all patients of group 2. Most patients in both groups had epithelial cells without signs of koilocytosis and atypia (Table).

Cytological characteristics of urine, n (%)	Group 1 (n = 65)	Group 2 (n = 53)
Presence of total koilocytic transformation of urothelium	11 (16.9)	0 (0.0)
Single koilocytes per FoV	12 (18.5)	5 (9.4)
Neutrophils	15 (23.1)	53 (100)
Lymphocytes	41 (63.0)	6 (11.3)

Conclusions: Cytological urine study in patients with chronic recurrent cystitis cannot determine HPV-related changes of the urothelium in all cases and requires a large amount of epithelial cells in urine sediment.

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MORPHOLOGICAL CHARACTERISTICS OF THE BLADDER UROTELIUM IN WOMEN WITH CHRONIC RECURRENT CYSTITIS OF BACTERIAL AND HUMAN PAPILLOMAVIRUS ETIOLOGY

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Background and Aims: Currently, the role of human papillomavirus infection (HPV) in the genesis of chronic recurrent cystitis (CRC) is controversial. The study aimed to investigate the morphological characteristics of CRC of bacterial and HPV etiology.

Methods: Study included 118 female patients with recurrent UTI aged between 20 and 50 years old. Depending on etiology identified previously, all patients were divided into two groups: group 1 (n=65) had HPV etiology and group 2 (n=53) bacterial etiology. All patients were subjected to a cystoscopy and biopsy. The samples were fixed in a 10% neutral PBS buffer (24 hours), formed into paraffin blocks, stained with hematoxylin and eosin, microscopied with magnification x100, x200, x400.

Results: Most patients of group 1 (93.8%) had a total koilocytic transformation of the bladder urothelium combined with local and diffuse metaplastic changes in 36.4% and 18.4% cases, respectively. Furthermore, all patients in group 1 had lymphocytic infiltration along with inclusion of plasmatic cells, whereas neutrophils were identified in 11 (16.9%) cases. At the same time, the morphological picture of all patients in group 2 presented neutrophilic infiltration with alow count of lymphocytes (16.9%) and local metaplastic changes (26.4%) (Table).

Morphological characteristics, n (%)	Group 1 (n = 65)	Group 2 (n = 53)
Absence of koilocytes	0 (0.0)	48 (90.8)
Presence of koilocytes	65 (100.0)	5 (9.2)
Total koilocytic transformation	61 (93.8)	0 (0.0)
Local metaplasia	24 (36.4)	14 (26.4)
Diffuse metaplasia	12 (18.4)	0 (0.0)

Conclusions: Total koilocytic transformation of the bladder urothelium is a pathognomonic morphological sign of CRC of HPV etiology.

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BACTERIAL MEMBRANE VESICLES MAINTAIN THE BIOPHYSICAL HOMEOSTASIS OF THE MEMBRANE OF PSEUDOMONAS AERUGINOSA

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Background and Aims: *P. aeruginosa* is an opportunistic pathogen that infects immunocompromised patients. The capability of this pathogen in maintaining cellular homeostasis in a variety of lifestyle-related and environmental stress is outstanding. In particular, membrane vesicle (MVs) production is among the coping strategies to resist the challenges. In this study, we investigated the role of MVs in maintaining the homeostasis of two important biophysical characteristics of the membrane of *P. aeruginosa*, i.e., i) membrane fluidity and ii) membrane potential.

Methods: We defined two dedicated models for our investigations; i) To explore the role of membrane vesicles in altering the bacterial membrane fluidity, we studied the effect of the MVs of *P. aeruginosa* in interplaying between the planktonic and biofilm bacteria. ii) To study the role of MVs in maintaining the homeostatic membrane potential, we perturbed the membrane potential by mildly acidifying the bacterial growth medium. Then, we studied the membrane potential in knocked-out mutants for the system responding to the low pH stress ($\Delta pmrB$), ($\Delta phoQ$) and induced membrane vesiculating phenotype (I-alg).

Results: showed; i) MVs produced by biofilm *P. aeruginosa* induce membrane rigidification in the planktonic bacteria (Figure 1). Hence, the bacteria possessing the rigidified membrane are more capable of resisting the hostile environment of the biofilm. Besides, ii) we observed that hyper-vesiculating strain is more capable of coping with the perturbation of the membrane potential (Figure 2).



• Figure 1 Fluorescence lifetime imaging (FLIM) and viscosity analysis. Top panels: Intensity images. Middle panels: Pseudo-colored FLIM images. planktonic control *P. aeruginosa* (A), planktonic *P. aeruginosa* incubated with MVs from the planktonic bacteria (B), planktonic *P. aeruginosa* incubated with MVs of the biofilm bacteria (C). control biofilm *P. aeruginosa* (D), the biofilm bacteria incubated with MVs of the planktonic bacteria (E), and biofilm *P. aeruginosa* (D), the biofilm bacteria incubated with MVs of the planktonic bacteria (E), and biofilm *P. aeruginosa* (D), the biofilm bacteria incubated with MVs of the planktonic bacteria (E), and biofilm *P. aeruginosa* (D). The data were obtained from two individual experiments, and in each experiment, at least 10 bacteria or three slides were considered to measure the lifetimes of the bacteria. The data were presented as mean ± SD from independent replicates in each condition. Statistical analysis was performed by one-way ANOVA with Tukey's multiple comparison test. ******: *p* <0.0001, ***: *p* <0.05, ns: not significant. (The image is in press in mSphere; DOI:10.1128/msphere.00187-22)



 Figure 2 Membranes and MVs zeta-potential. Zeta potential of the bacterial membranes (Panels A and C); wild type P. aeruginosa (WT), P. aeruginosa hyper vesiculation phenotype (LalgU). P. aeruginosa deleted mutant for pmrB (ΔpmrB), P. aeruginosa deleted mutant for phoQ (ΔphoQ). Zeta potential of the MVs (Panels B and D). The data were presented as mean ± SD from independent replicates in each condition. Statistical analysis was performed by multiple t-test. **: p <0.01, ,*: p <0.05, ns: not significant.

Conclusions: Conclusion: These results showed; the production of MVs is a resistant strategy benefiting the bystanders and the producing bacteria. **Acknowledgment:** This work is supported by FNRS.

EP222 / #801

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SPATIALLY SEGREGATED TRANSMISSION OF CO-OCCLUDED BACULOVIRUSES LIMITS CELLULAR COINFECTION AND VIRUS-VIRUS INTERACTIONS

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Background and Aims: Occlusion bodies (OBs) of certain baculovirus are polyhedrin-rich structures that mediate the collective transmission of tens of viral particles to the same insect host. In addition, in multiple nucleopolyhedroviruses, occlusion-derived viruses (ODVs) form nucleocapsid aggregates that are delivered to the same host cell. It has been suggested that, by favoring coinfection, this transmission mode promotes evolutionarily stable interactions between different baculovirus variants.

Methods: To investigate this, we obtained OBs from cells coinfected with two viral constructs, each encoding a different fluorescent reporter, and used them for inoculating Spodoptera exigua larvae.

Results: Microscopy analysis of midguts revealed that the two reporter genes were typically segregated into different infection foci, suggesting that ODVs do not promote the coinfection of cells with different baculovirus genetic variants. However, a polyhedrin-deficient mutant underwent inter-host transmission by exploiting the OBs of a fully-functional virus and re-acquired the lost gene through recombination, demonstrating cellular coinfection.

Conclusions: Our results suggest that viral spatial segregation during transmission and primary infection limits interactions between different baculovirus variants, but that these interactions still occur episodically.

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SYNERGISTIC ACTIVITY OF ANTIBODIES IN MULTIVALENT VACCINES

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Background and Aims: Vaccines based on multiple antigens often induce an immune response which is higher than that triggered by each single component. This effect may be due to antibodies targeting multiple antigens that act cooperatively and synergistically in tackling the infection. The multicomponent 4CMenB vaccine, currently licensed for the prevention of *Neisseria meningitidis* serogroup B (MenB) contains four antigenic components: Factor H binding protein (fHbp), Neisseria adhesin A (NadA), Neisserial Heparin Binding Antigen (NHBA) and Outer Membrane Vesicles (OMV).

Methods: Serum bactericidal assays (hSBA) : Bacteria were subcultured overnight on Chocolate Agar and resuspended to an OD_{600} of 0.25 and incubated with antibodies and human complement . SBA titers were calculated as the last dilution corresponding to at least a 50% reduction in CFU compared to time 0.

Results: Here we provide evidence that antibodies induced by the recombinant antigens and OMV components can act in concert and be functional against meningococcal strains not predicted to be covered by classical MenB prediction tools. Different antibodies can bind simultaneously the different antigens, reaching the threshold for triggering the complement mediated bacterial lysis and overcoming the limitations of a low surface expression and/or a high antigenic diversity.

Conclusions: These data support the hypothesis that mechanisms of antibody-mediated protection by multicomponent vaccines is mainly due to a complex interplay of antibodies acting in synergy. 3D structures and protein modeling studies may provide key insights on the structure of antigen-antibody complexes and on the specific epitopes engaged in simultaneous binding, allowing a complete understanding of the immune response induced

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EVALUATION OF THE GANODERMA CURTISII MYCELIUM CONSUMPTION EFFECTS ON MURINE MODEL OF OBESITY

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Background and Aims: Obesity is a relevant health problem worldwide, related with a fat-rich food consumption habits and a sedentary lifestyle, being associated with several metabolic disorders. Mushroom consumption, including species belonging to the *Ganoderma* genus, have shown to have a variety of beneficial effects to body weight control and its associated diseases. This work evaluates the effects of *Ganoderma curtisii* (GC) mycelium as a diet additive in a murine model of obesity.

Methods: C57BL/6J mice fed with normal diet (ND, 10.50 g/kg fat) or high-fat diet (HFD, 353.33 g/kg fat) were supplemented with 200 mg/kg/day GC mycelium extract or vehicle. For a 5-week period of treatment, a record of the animal's weight was kept daily, and insulin and glucose resistance tests were performed. A fasting glucose control was performed at the beginning and at the end of treatment, and blood biochemistry at the end.

Results: HDF feeding increased mice weight (39.61%) and serum cholesterol (107.75 mg/mL) compared with ND (84.51 mg/dL. Fasting blood glucose levels were significatively higher in HFD-fed mice (179.1 mg/dL) compared to ND-fed mice (142.8mg/dL). Glucose sensitivity was deteriorated in obese mice (35190 AUC) related to non-obese (25870 AUC). All these alterations didn't show significant changes at the indicated dose of GC consumption, as well as total cholesterol, triglycerides, glucose levels or ameliorate glucose resistance during 5-week period of treatment.

Conclusions: It is important to conduct further research with higher GC biomass used as food additive to see those favorable anti-obesity results reported in other *Ganoderma* spp.

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EVALUATION OF THE METABOLIC VERSATILITY AND ANTIFUNGAL ACTIVITY OF CMU-08 STRAIN OF TRICHODERMA ATROVIRIDE

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Background and Aims: Species within *Trichoderma* genus are efficient antagonists of phytopathogenic fungi. Both metabolic plasticity and the capability to synthetize a great diversity of extracellular secondary metabolites are linked to antagonism capability of *Trichoderma* spp. Intraspecific physiological diversity has been documented in *Trichoderma atroviride*, resulting in significant differences to antagonize phytopathogens. This makes necessary to characterize new geographical isolates with potential for biocontrol. The aim of this work was twofold: (i) to analyze the metabolic plasticity of the novel CMU-08 strain of *T. atroviride*, and (ii) to evaluate the antifungal activity of the broth in which CMU-08 was incubated.

Methods: The metabolic and conidiation capacity of the CMU-08 strain was evaluated using the phenotypic microarrays assay (PMs) with Biolog® FF plates. The extracellular broth (EB) at stationary growth phase of the studied strain was recovered from basal culture (BC) using Vogel's minimal medium (VM) and induced culture (IC) by supplementing VM with lyophilized mycelium of *Botrytis cinerea* (1% w/v). The recovered EB was concentrated twofold using a rotary evaporator.

Results: PMs showed that glycogen and D-raffinose substrates are the best for mycelium development of CMU-08 strain. The optimal substrate for conidiation is β -methyl-D-galactoside, followed by L-sorbose. Inhibition assays in 96-well plates show that the BC extract (15% v/v) inhibits the phytopathogen growth by 85.9%, while the IC extract inhibits it by 61.6%.

Conclusions: These results show that CMU-08 strain is capable of optimally assimilating different carbon sources for mycelial growth and conidiation. The incubation broth of CMU-08 strain contains extracellular metabolites with antifungal activity.