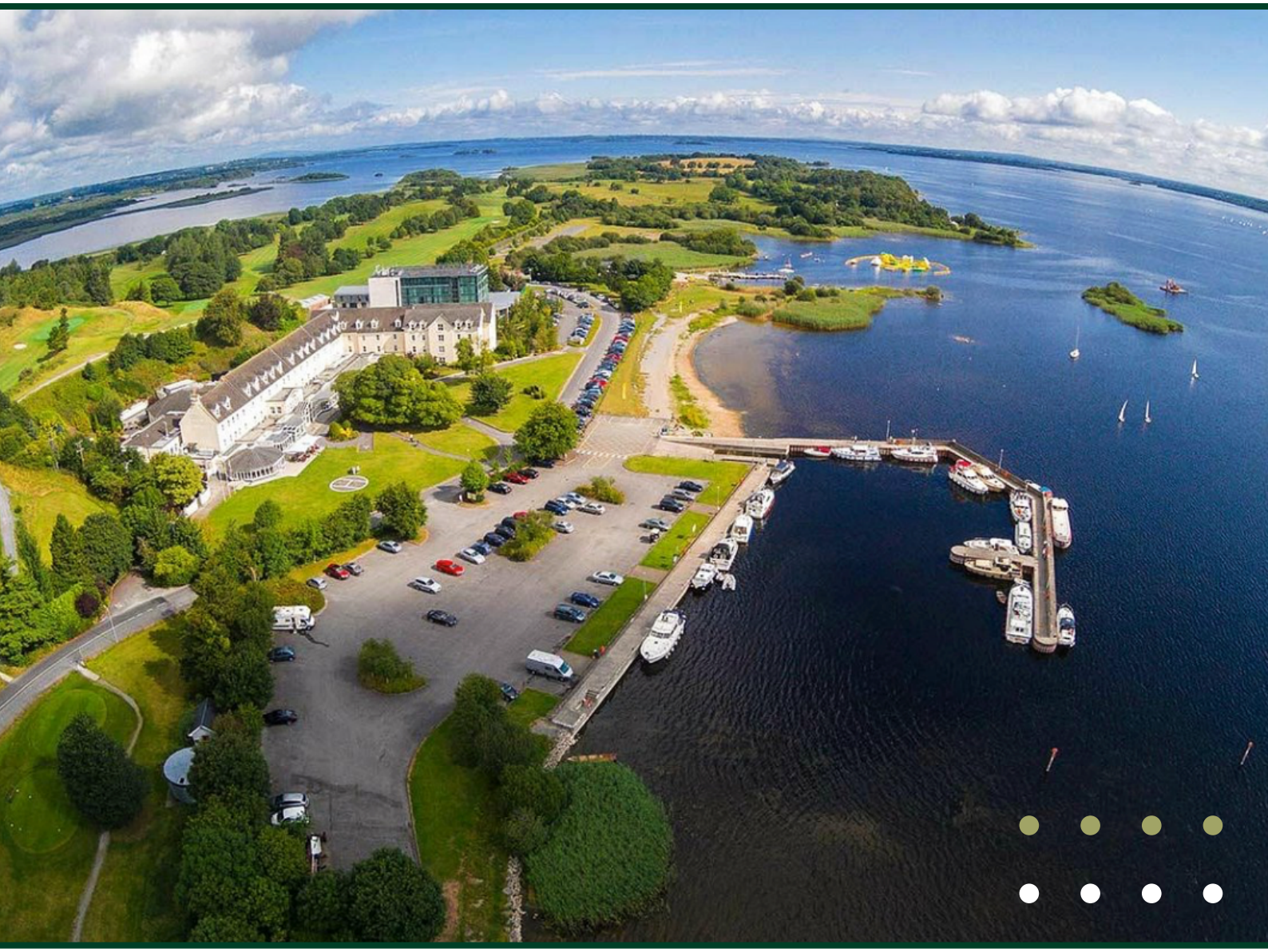
MICROBIOLOGY SOCIETY ANNUAL MEETING IN IRELAND

Hodson Bay Hotel, Ireland
4–5 November 2025

INVITED AND OFFERED TALKS



#MicrobioIrish25

MICROBIOLOGY SOCIETY

Invited talk : Transmission of human intestinal spore-forming bacteria

Hilary Browne

University College Cork, Cork, Ireland

Abstract

In order to ensure long-term survival and maintain human symbiosis, our intestinal microbiota must successfully transmit between and colonise new hosts. As most gut bacteria are strict anaerobes, maintaining viability in environmental conditions long enough until ingested by a new host poses challenges. Some members of the Bacillota (formerly Firmicutes) phylum produce dormant endospores that promote transmission as they are highly resistant to ambient temperatures and oxygen encountered during transmission. Recent culturing and genomic studies have revealed the taxonomic diversity of human-associated spore-formers and their prevalence in human populations highlighting a distinct ecology compared to non-spore-forming bacteria. In this talk, I will discuss the life-cycle of commensal intestinal spore-formers, their ecology and their therapeutic potential.

Offered talk (10 minutes)

18

Serum triggers increased biofilm formation and antibiotic tolerance in *Staphylococcus aureus* via two independent mechanisms

Elizabeth Ledger[1], Niamh Horgan[1], Ruth Massey[1,2]

[1]University College Cork, Cork, Ireland. [2]University of Bristol, Bristol, United Kingdom

Abstract

Staphylococcus aureus is the leading cause of fatal bloodstream infections, in large part due to its ability to disseminate around the body, causing complications such as endocarditis, osteomyelitis and deep tissue abscesses. These infections frequently have a biofilm component and are extremely challenging to treat as they protect the bacteria within from antibiotics and the immune system. The staphylococcal cell envelope is crucial for biofilm formation and exposure to human serum induces major structural changes in the cell wall. However, how these changes affect biofilm formation is unknown. Here, we show that incubation of *S. aureus* in human serum triggers increased biofilm formation in laboratory strains and in clinical isolates. This increased biofilm formation is due to specific activation of the GraRS and SaeRS two component signalling systems by serum, as well as the non-specific binding of human serum proteins to the staphylococcal surface. Activation of these stress responses results in changes in the cell wall including increased wall teichoic acids and altered surface charge, enhancing adhesion and biofilm formation. Additionally, exposure to serum significantly reduces the daptomycin susceptibility of the biofilms, compared to biofilms formed in the absence of serum, possibly contributing to the high rates of treatment failure observed with this last resort antibiotic. Finally, serum-induced biofilm formation can be inhibited with existing antibiotics, including the cell wall synthesis inhibitor fosfomycin, providing a viable therapeutic approach to prevent biofilm formation and improve treatment outcomes in invasive staphylococcal infections.

19

Biofilm-Resistant Dental Implants: Exploring Omega-3 Fatty Acids as a Functional Coating

Aoife Mulry

TUS, Athlone, Ireland

Abstract

Biofilms are central to development of oral diseases, particularly dental caries, which exhibits heightened resistance to conventional treatments. This study investigated antibiofilm properties of Omega-3 (n-3) fatty acids Eicosapentaenoic acid (EPA, C20:5) and Docosahexaenoic acid (DHA, C22:6) against *Streptococcus mutans*ATCC 25175, a primary cariogenic pathogen.

Using a modified McBain medium simulating salivary conditions, bacterial growth was assessed at concentrations of 250, 100, 50, 25, and 10 µg/ml. Minimum biofilm inhibitory concentration (MBIC) was determined on titanium discs preconditioned with artificial saliva and assessed using colony enumeration, crystal violet staining, and scanning electron microscopy (SEM). Cytotoxicity and proliferation effects on TR146 oral cells were assessed using lactate dehydrogenase (LDH) release and XTT assays. Additionally, n-3 incorporated medical-grade shellac coatings were developed and evaluated for biofilm prevention.

EPA and DHA significantly inhibited *S. mutans* growth and biofilm formation at 250-100 µg/ml. EPA was most effective in the presence of artificial saliva, with 25 µg/ml effecting growth and biofilm formation when exposed to salivary components. DHA was less effective at the same concentration, suggesting that salivary components may buffer or diminish its antibiofilm activity. Lowest concentration 10 µg/ml displayed no significant antibiofilm activity however, exhibited limited cytotoxicity compared to chlorhexidine. Shellac coatings containing Omega-3's effectively reduced surface biofilms in a dose-dependent manner, with SEM confirming biofilm inhibition at higher concentrations.

These findings highlight the potential of Omega-3 fatty acids, particularly EPA, as alternative antibiofilm agents against *S. mutans*, offering a promising approach for caries prevention with reduced cytotoxicity.

22

Spaceflight alters host-gut microbiome interactions: lessons from multiomics in space

nicholas brereton

UCD, Dublin, Ireland

Abstract

In spaceflight biology and aerospace medicine, understanding microbial responses to spaceflight is essential for supporting astronaut health and enabling sustainable life beyond Earth. The rodent habitat on the International Space Station has provided crucial insights into how spaceflight affects mammals, including symptoms characteristic of liver disease, insulin resistance, osteopenia and myopathy. While these responses involve the microbiome on Earth, the specific effects of spaceflight on host-gut microbiome interactions remain unclear.

Here, NASA GeneLab multiomic data from the Rodent Research-6 mission were used to determine changes in gut microbiota and host colon and liver gene expression after 29 and 56 days of spaceflight. Using amplicon and whole metagenome sequencing analysis, significant spaceflight-associated alterations in 44 microbiome species were identified. These included relative reductions of bacteria associated with bile acid and butyrate metabolism, such as *Extibacter muris* and *Dysosmobacter welbionis*. Functional prediction suggested over-representation of fatty acid and bile acid metabolism, extracellular matrix interactions, and antibiotic resistance genes within the gut microbiome, while host intestinal and hepatic gene expression reflected corresponding changes in bile acid and energy metabolism, and immune suppression from spaceflight.

Taken together, these changes imply that interactions at the host-gut microbiome interface contribute to spaceflight pathology and highlight how these interactions might critically influence human health and the feasibility of long-duration spaceflight. Ireland's expertise in microbiome science, multiomics and translational research offers a valuable contribution to this growing field. Spaceflight microbiology provides a platform to apply and extend these capabilities, with benefits for both exploration and life on Earth.

62

Genome-Wide Analysis of Methylation Dynamics in Salmonella enterica Using Oxford Nanopore Technology

Anna Ershova, Carsten Kröger

Trinity College Dublin, Dublin, Ireland

Abstract

Epigenetic regulation in bacteria is a growing area of interest, but most studies focus on individual DNA methyltransferases or a single bacterial state, which is typically the stationary phase. In this study, we compared genome-wide DNA methylation patterns associated with all eleven methyltransferase genes encoded by *Salmonella enterica* strain 4/74 at two distinct growth stages: mid-exponential and late stationary. Oxford Nanopore sequencing was used to generate methylation profiles, which were integrated with transcriptomic and genomic data to explore links between methylation and DNA methyltransferase gene expression.

Genomic DNA was extracted from cells harvested at OD_{600} 0.3 (mid-exponential phase, MEP) and after 16 hours of growth (late stationary phase, LSP), with two biological replicates per condition. Libraries were prepared using the Genomic DNA Ligation Sequencing Kit V14 and sequenced on MinION flow cells (R10.4). Methylation calling was performed using Dorado v0.8.3 and Modkit v0.4.4, with downstream analysis and visualisation using custom R scripts.

We defined the minimum sequencing depth required for robust methylation analysis and validated all six methylation motifs previously detected by PacBio sequencing. While genome-wide methylation patterns shifted between growth phases, these changes did not directly correlate with expression levels of the corresponding methyltransferases. Notably, regulatory methyltransferases and those associated with Restriction–Modification systems displayed distinct methylation patterns.

Our findings demonstrate that DNA methylation in *S. enterica* is growth-phase dependent, highlighting the potential for complex epigenetic regulation in bacterial adaptation.

79

ChiVariARIBA: a modular, editable workflow and database for characterising chitin gene variation in Vibrio spp. and related bacteria

Evan P. Naughton[1,2], Matthew J. Dorman[1,2]

[1]University of Galway, Galway, Ireland. [2]Trinity College Dublin, Dublin, Ireland

Abstract

Chitin is a highly abundant biopolymer of bioeconomic, biochemical and commercial importance. This carbohydrate is a source of nutrients for chitinolytic bacteria and can influence natural competence, surface adsorption and other fundamental aspects of prokaryote physiology. Bacterial enzymatic degradation of chitin is mediated by a well-studied set of hydrolytic enzymes, transcriptional regulators and carbohydrate transport proteins. Many of these gene products have been functionally characterized in vitro or in vivo, but there is a reliance on in silico genomic approaches to study the variation of these metabolic components amongst diverse bacteria. Computational surveys of bacterial genomes to date have tended to focus on determining the presence and absence of chitin metabolism genes in diverse genomes, but not on the diversity of sequences amongst these gene families. To enable future research into chitin metabolism variation in vibrios and other bacteria, we present ChiVariARIBA, a workflow for extracting chitin metabolism genes from published genome sequences of chitinolytic Vibrio species and their relatives, compatible with the rapid gene-finding and variant-characterizing tool ARIBA, with which to describe the presence of chitin-metabolising genes in genomes of interest and to characterize the sequence variation of these genes across diverse bacteria.

Published in Microbial Genomics: DOI 10.1099/mgen.0.001439

96

Unmasking the microbial interactions which underpin successful ruminant methane mitigation via novel oxygen-releasing feed additives

Alison Graham[1], Camilla Thorn[1], Anna Trego[1], Umer Ijaz[2], Sinead Waters[1], Vincent O'Flaherty[1]

[1]University of Galway, Galway, Ireland. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Ruminant livestock contributes significantly to global methane (CH_4) production and its mitigation is of great importance. Feed additives represent a cost-effective and realistic means of achieving this. Previous research demonstrated that slight elevations of the rumen oxidation reduction potential (ORP) using oxygen-releasing feed additives serves to hinder enteric methanogenesis. This is due to the niche specialisation of ruminant methanogens, which are typically only active at ORPs below -300 millivolts. *In-vitro* assessment of these compounds, including both calcium and magnesium peroxide (CaO_2, MgO_2), and encapsulated liquid H_2O_2 for controlled, slow release has demonstrated effective CH_4 mitigation potential, with consistent CH_4 reductions of >50% observed. Encapsulated formats of CaO_2 and MgO_2 offer potential feasibility as a CH_4 mitigation feed additive solution in both intensive and pasture-based production systems. However, the impact these oxygen-releasing compounds have on the rumen microbiome, specifically what might be occurring when hydrogen (H_2) or carbon dioxide (CO_2) are diverted away from ruminant CH_4 production requires further investigation. This study focuses on the influence of ORP modulating compounds on *in-vitro* rumen microbial communities. Nucleic acids were co-extracted from rumen fluid. Amplicon sequencing of the 16S and 18S genes was performed on all samples (n=64) comprising inoculum (time zero), CaO_2, MgO_2, liquid H_2O_2, encapsulated liquid H_2O_2, and controls, over the course of a 21-day RUSITEC trial. Microbial community dynamics were integrated with process data and revealed the extent to which ORP can alter the rumen microbial community, thus elucidating the microbial mechanisms which underpin the CH_4 reductions observed.

100

Cold Atmospheric Plasma Reprograms Biofilm Susceptibility and Host Responses in Chronic Infection Models

Thomas Thompson[1], Carly Smith[2], Amanda Watkins[3], Bryan Sim[1], Paula Bourke[4], Thomas Schaer[3], Noreen Hickok[2], Theresa Freeman[2], Brendan Gilmore[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Thomas Jefferson University, Philadelphia, USA. [3]University of Pennsylvania, Philadelphia, USA. [4]University College Dublin, Dublin, Ireland

Abstract

Cold atmospheric plasma (CAP), a non-thermal ionised gas producing reactive oxygen and nitrogen species (RONS), has emerged as a promising adjuvant to antibiotics for disrupting biofilms and modulating tissue responses. In a series of transnational studies across Ireland, the UK, and the US, we investigated CAP's role in reprogramming infection microenvironments.

Short exposures (90–120 s) to CAP significantly enhanced the susceptibility of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms to antibiotics, achieving up to 512-fold reductions in MBEC. These synergistic effects were not attributable to direct killing alone. Real-time structural and lipidomic profiling (REIMS, fluorescence imaging) confirmed biofilm matrix collapse and phospholipid depletion. Transcriptomic analyses revealed oxidative stress responses and membrane-associated gene upregulation, consistent with transient outer membrane disruption and increased antimicrobial penetration.

Building on these in vitro findings, we assessed CAP's effects in vivo. In a sterile muscle injury model, CAP induced rapid neutrophil and mast cell recruitment, antioxidant gene expression, and sustained activation of pro-regenerative programs, promoting myogenesis over adipogenesis. In contrast, surgical revision of *S. aureus* implant infections alone exacerbated virulence factor expression and triggered a multi-tissue inflammatory response without reducing bacterial burden.

These findings demonstrate that CAP not only sensitises biofilms to antimicrobials but also modulates host inflammatory and repair pathways. This dual antimicrobial–immunomodulatory activity supports its development as a non-antibiotic, stewardship-aligned intervention for recalcitrant infections.

103

Strain-specific activation of the SOS pathway limits carbapenemase gene dissemination in *Vibrio cholerae* in response to selective antibiotic stress environment.

Shashi Kumari

THSTI, Faridabad, India

Abstract

Background: The bacterial SOS response is a highly conserved global regulatory system that is activated in response to DNA damage or cellular stress. In this study, we explored the role of the SOS response in the transmission dynamics of the *bla*$_{NDM}$ -*sh-ble* gene cassette, a clinically significant determinant of carbapenem resistance. We specifically investigated the expression of SOS-related genes during horizontal gene transfer events involving *ISAba125*-linked *bla*$_{NDM}$-*sh-ble* gene cassette in *V. cholerae* strains N16961 and C6709 under selective antibiotic stress environments.

Methods: We applied **comparative genomics** and **transcriptomic analysis** in model pathogen *Vibrio cholerae.*

Results: Our findings reveal a distinct correlation between SOS gene expression and the frequency of *bla*$_{NDM}$-*sh-ble* gene cassette transmission in strain N16961. This suggests that the activation of the SOS response enhances the mobilization of resistance genes, likely through upregulation of recombination and transposition enzymes, as well as increased competence genes for natural transformation.

In contrast, strain C6709 exhibited significantly reduced transformation frequency, and this reduction appeared to be independent of SOS gene expression. Interestingly, when exposed to sublethal concentrations of antibiotics, strain C6709 showed a further decline in transformation frequency. This is a particularly notable observation, as sub-inhibitory antibiotic levels have been previously reported to induce the SOS response and enhance AMR genes spread in various bacterial species.

The lack of such an effect in C6709 implies the existence of strain-specific regulatory mechanisms or a differential stress response that suppresses or fails to activate the SOS pathway in the same manner as observed in N16961.

113

Fungal Biodegradation and Defluorination of PFOS, 6:2 FTSA, and 6:2 FTAB by *Cunninghamella elegans*: Linking Metabolite Formation to Reduced Ecotoxicity.

GAURAV CHUGH[1], MOHD FAHEEM KHAN[1], EVA NAUGHTON[2], DANIEL MOLLOY[2], CAMILLA FONTANA[3], PATRIZIA COLUCCI[3], BRENDAN N KENNEDY[3], JAMES A SULLIVAN[2], CORMAC D MURPHY[1]

[1]UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland. [2]UCD School of Chemistry, University College Dublin, Dublin, Ireland. [3]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

Abstract

Per- and polyfluoroalkyl substances (PFAS), such as perfluorooctanesulfonic acid (PFOS), 6:2 fluorotelomer sulfonic acid (6:2 FTSA), and 6:2 fluorotelomer amido betaine (6:2 FTAB), are environmentally persistent contaminants with significant ecological and health concerns due to their bioaccumulation and toxicity. This study investigates the fungal degradation and defluorination of PFOS, 6:2 FTSA, and 6:2 FTAB by the fungus *Cunninghamella elegans*. Resulting degradative metabolites were analyzed using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Detected transformation products included perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), and 5:3 fluorotelomer carboxylic acid (5:3 FTCA), indicating active transformation of long- and short-chain PFAS. Fluoride ion release, measured by a fluoride ion-selective electrode and fluorine nuclear magnetic resonance (^{19}F NMR), confirmed partial defluorination. To evaluate the ecological relevance of fungal treatment, toxicity assays were performed using *Danio rerio* (zebrafish) embryos, *Daphnia magna*, and *Aliivibrio fischeri*. Results demonstrated significantly reduced toxicity in PFAS-treated samples post-fungal degradation across all models, suggesting a detoxification effect linked to metabolite formation. This multi-tiered approach highlights the potential of *C. elegans* as a promising agent for PFAS bioremediation and provides new insights into microbial degradation pathways and their implications for environmental safety.

123

Aspergillus-Pseudomonas Interactions Alter *P. aeruginosa* Lipopolysaccharide, reshaping membrane properties and bacterial behaviour.

Natasha Sarwar, Emma Reece, Naoise Mc Garry, Manuel Ruether, Julie Renwick

Trinity College Dublin, The University of Dublin., Dublin, Ireland

Abstract

Pseudomonas aeruginosa and *Aspergillus fumigatus* frequently co-occur in cystic fibrosis (CF) lungs, where their interactions can alter virulence. Lipopolysaccharide (LPS), a complex glycolipid of the *P. aeruginosa* (PAO1) outer membrane, protects against host defences, mediates host-pathogen interactions, and acts as a potent endotoxin. This study examines how *A. fumigatus* secreted products impact LPS expression and surface phenotypes in *P. aeruginosa.*

PAO1 cultures were treated with sterile-filtered 72-hour *A. fumigatus* supernatant (AFsn). RNA sequencing used Illumina NextSeq 2000. LPS profiles were analysed by SDS-PAGE with Pro-Q Emerald 300 staining and quantified via ImageJ. Total LPS was measured by Purpald assay, and lipid A by ELISA. Structural changes were assessed by ^{1}H NMR spectroscopy. Cell surface charge was measured by cytochrome c binding. Motility phenotypes were assessed on agar media.

RNA-seq revealed significant upregulation of LPS biosynthesis genes (*wzz, wzy, wzx, wbpB, wbpG, wbpH, hisH2, hisF2*). SDS-PAGE demonstrated significant increases across O-antigen regions: short-chain (9.29%, $p = 0.0157$), long-chain (8.6%, $p = 0.049$), very long-chain (12.59%, $p = 0.0172$), and core regions: uncapped (16.36%, $p = 0.029$), capped (20.61%, $p = 0.0475$), core+1 (16.5%, $p = 0.038$). Total LPS content increased 69.9% ($p < 0.0001$) and lipid A by 12.4% ($p = 0.0007$). NMR detected subtle structural differences. Cell surface charge decreased 1.3% ($p = 0.014$). Motility assays revealed decreased twitching and swimming with increased swarming.

A. fumigatus supernatant induces structural and compositional changes in *P. aeruginosa* LPS alongside altered surface properties and motility, potentially contributing to bacterial virulence in CF lungs.

128

Assessing the presence of foodborne pathogens in Irish horticultural production settings

Elena Anedda[1], Elena- Alexandra Alexa[2], Michael Arthur[1,2], Michael Gaffney[1], Catherine Burgess[1]

[1]Teagasc Food Research Centre, Dublin, Ireland. [2]Technological University Dublin, Dublin, Ireland

Abstract

Due to the nature of their production horticultural crops are at risk of microbiological contamination from a range of sources. It is therefore imperative that growers are aware of where risks may arise on farm so that targeted interventions can be put in place to minimise transference of pathogens onto crops.

The objective of this study was to identify the areas most likely to harbour foodborne pathogens in commercial horticultural settings in Ireland. Produce, water and environmental swab samples were collected from commercial production units (n=12) for four crops, namely strawberries, carrots, lettuce and spinach, on four different occasions. Each sample was tested for the presence of *Listeria monocytogenes, Salmonella* spp. and Shiga toxin producing *E. coli* by standard methodologies.

In total 765 samples were collected, including 585 environmental swabs, 61 produce samples and 119 water samples. *L. monocytogenes* (n = 20 isolates) was detected in 13 samples (2.1%). Samples from seven out of the 12 sites harboured *L. monocytogenes* on at least one occasion. No *Salmonella* was detected in any sample .Eight enrichments from the 765 samples tested positive for *stx* genes but no STEC were recovered. Whole genome sequencing was utilised to compare the *L. monocytogenes* isolates, as well as to provide information on their serotype and virulence gene properties.

This study indicated that the horticultural production and processing environment can harbour *L. monocytogenes,* particularly on surfaces, and therefore particular emphasis should be placed on rigorous cleaning and disinfection and minimising cross contamination of the crop.

143

Beyond phosphorus availability: Exploring the intricate regulatory mechanisms of organophosphonate metabolism

Leanne Murray[1], John McGrath[1], Katharina Pallitsch[2], Jason Chin[1]

[1]Queens University, Belfast, Ireland. [2]Institute of Organic Chemistry, Vienna, Austria

Abstract

Recently, organophosphonates, a chemically distinct class of organic-phosphorus compounds characterised by a C–P bond, have become increasingly recognised as vital constituents of marine biogeochemistry, especially in phosphorus-depleted oceans. Previously thought to be recalcitrant, they are known to be metabolised by diverse marine microbes as PHO-regulated alternative phosphorus sources, and sometimes nitrogen and carbon under non-PHO control. Future predictions indicate phosphonate degradation will intensify under increasingly stratified oceans. However, phosphonate metabolism genes, their distribution, and regulation remain poorly resolved, particularly in-vivo.

Here, we report the first in-vivo characterisation and regulatory mechanisms of a novel phosphonate catabolic pathway in the marine isolate *Roseovarius nubinhibens*. Previous work described PbfA function in-vitro, but our findings confirm its physiological role in-vivo. Using bioinformatics and cell-free extracts, we demonstrate direct co-utilisation of (R)-1-hydroxy-2-aminoethylphosphonate (HAEP) and 2-aminoethylphosphonate (2AEP) via a single catabolic pathway. While 2AEP degradation follows the traditional PhnWYA route, HAEP is degraded through a novel PbfA–PhnYA pathway the first in-vivo demonstration of this system in any organism. The accessory enzyme PbfA expands the pathway's substrate range by converging on the shared intermediate phosphonoacetaldehyde, produced by both PbfA and PhnW, then funnelled to PhnYA.

Degradation of both substrates is substrate-inducible and regulated by a LysR-type transcriptional regulator, rather than the classical PHO-regulon. Bioinformatic analysis revealed that 43% of bacterial type-strains carrying PhnWYA also possess PbfA, underscoring the ecological importance of this pathway across diverse lineages.

This study provides the first evidence of exogenous HAEP utilisation and LysR-regulated degradation via the PbfA–PhnYA pathway.

154

After a century of nisin research - where are we now, and where are we going?

Des Field

University College Cork, Cork, Ireland. Biosciences Research Institute, Cork, Ireland

Abstract

Nearly a century ago, nisin was discovered in fermented milk cultures, coinciding with the first description of penicillin. Since then, this small, post-translationally modified polycyclic peptide has achieved notable success in the food industry as a preservative that prevents spoilage and foodborne illness, while also serving as a model for understanding the genetic organization, expression, and regulation of lantibiotic biosynthetic gene clusters. Recent advances in elucidating nisin's complex biosynthesis have revealed details about the cellular localization of its modification and transport machinery, as well as the precisely coordinated spatio-temporal processes that generate and secrete active nisin. Furthermore, the ongoing discovery of natural variants in the gastrointestinal tracts of humans and animals has renewed interest in the potential of nisin to modulate the microbiome, especially given the expanding awareness of microbial influences on health and disease. In addition, bioengineering strategies offer promising solutions to overcome the inherent limitations of nisin, including its potency, target range, and protease sensitivity, thereby broadening its prospects as a precision therapeutic.

Offered short talk (3 minutes)

P014

Investigating the therapeutic potential of scorpion venom

Dayle Leonard, Aoife Boyd, Michel Dugon

University of Galway, Galway, Ireland

Abstract

Every year 1.2 million people are stung by scorpions resulting in over 3,250 deaths. The long term effects of scorpion envenoming have not been systematically studied but reports include severe infections. These infections can result in further disfigurement through amputations or mortalities. Our understanding of venom system associated microbiomes is still in its infancy. During envenomings, vectoring is often not considered until the onset of infection. Part of our research seeks to shed light on the microbial association with venom systems in the Moroccan Brown scorpion *Scorpio mogadorensis* to advance knowledge that could lead to therapeutic and health benefits.

To achieve this, we utilised *S. mogadorensis* and associated soils collected from three habitats in the Tiout Oasis of Morocco. Through a mix of culture-dependent and culture-independent methods, microbial communities were identified. Comparative bioinformatic analysis was conducted to investigate unique microbial communities in the scorpion venom system, potential pathogens, and correlations between scorpion and soil microbiomes.

A number of cultured microbes were capable of haemolysis, suggesting scorpions possess molecules in their venom to counter potential infections. We assessed the venom of 17 scorpion species for antimicrobial and anti-biofilm activity. The most active venoms of *Androctonus crassicauda* and *Androctonus mauritanicus* inhibited biofilm formation by methicillin resistant *Staphylococcus aureus* and methicillin sensitive *Staphylococcus aureus* by 80-90% and *Escherichia coli* by 60-70%.

Our work establishes the bacteriome of an African scorpion with insights into patterns of composition and assesses the defence mechanism of the venom system of scorpions as a source of novel therapeutics.

P022

Shifts of nutrient stoichiometry as a new form for a sustainable management of soils

Jianqing Tian[1], Patrick Forrestal[2], Gary Bending[3], Michael Schloter[4], Achim Schmalenberger[1]

[1]University of Limerick, Department of Biological Sciences, Limerick, Ireland. [2]Teagasc, Environment, Soil and Land Use, Wexford, Ireland. [3]School of Life Sciences, University of Warwick, Coventry, United Kingdom. [4]Technical University of Munich, School of Life Sciences, Munich, Germany

Abstract

The extensive use of synthetic fertilizers alters soil physiochemical properties and disrupts microbial diversity, contributing to long-term declines in soil fertility. Bio-based fertilizers offer a promising alternative by reducing dependency on chemical inputs. Optimizing the stoichiometric balance of soil carbon (C), nitrogen (N), and phosphorus (P) is crucial for regulating nutrient turnover and improving nutrient use efficiency of plants (NUE). However, the mechanisms by which bio-based fertilizers influence soil and microbial nutrient stoichiometry, as well as the structure and function of the soil microbiome, remain poorly understood. This study aims to: (1) assess the effects of various bio-based fertilizers (including straw, insect frass, and dairy-processing residues) on soil physicochemical properties, microbial nutrient stoichiometry and the structure – function relation of the soil microbiome, through both long-term field-scale trials in Ireland, the UK, and Germany, as well as greenhouse experiments; and (2) elucidate microbial-mediated C, N, and P turnover in response to these amendments, and their subsequent impact on NUE, crop yields, and plant–microbe interactions. Preliminary results using quantitative PCR and enzyme assays indicate that different types of bio-based fertilizers distinctly affect the abundance of microbes driving key processes in N- and P-turnover. Both soil enzyme activities and abundance of certain functional groups of microbes are more responsive to fertilization in grasslands than in croplands. Furthermore, fertilization significantly shifts soil microbial nutrient limitations. These findings contribute to a deeper understanding of how bio-based fertilizers can support sustainable nutrient management, reduce reliance on synthetic fertilizers, and promote long-term soil health.

P029

Identification and Multi-omics Validation of Minimal Microbial Communities for the Production of Novel Water Kefir

Anik Khan[1,2,3], A. Kate O'Mahony[1,2], Wendy Izedonmwen[1], Orla O'Sullivan[1,4,5], Paul D. Cotter[1,2,5], Sinead McCarthy[6], Jennifer Mahony[2,3], John Kenny[1,2,5]

[1]Teagasc Food Research Centre, Moorepark, Cork, Ireland. [2]APC Microbiome Ireland, Cork, Ireland. [3]School of Microbiology, University College Cork, Cork, Ireland. [4]APC Microbiome, Cork, Ireland. [5]VistaMilk Research Ireland, Cork, Ireland. [6]Teagasc Food Research Centre, Ashtown, Dublin, Ireland

Abstract

Background

Water kefir (WK) is a traditional plant-based fermented beverage. This study used genome-scale modelling (GSM) to design minimal microbial communities (MinComs) for novel WK fermentations. These MinComs were then validated through multi-omics analyses following fermentation with two sustainable substrates from Ireland. The aim was to assess the performance of GSM-designed MinComs in terms of fermentation efficiency (e.g., acidification and microbial growth), and to evaluate the safety and health-promoting potential of the resulting beverages.

Methods

Six MinComs were used for the fermentation of two sustainable substrates for 48 hours. The pH and viable colony-forming units (CFUs) were monitored every 24 hours. The ethanol and residual sugar contents were quantified using HPLC. Shotgun metagenome sequencing was performed to confirm the community composition after fermentation. Safety assessments, including mycotoxin screening and cytotoxicity evaluations using a gut epithelial cell model, were also performed. Gut microbiota modulatory effects of the fermented foods were evaluated using an in vivo model of the distal colon. A comprehensive metabolomic analysis was performed to quantify non-volatiles, volatiles, and organic acids.

Results

All MinCom successfully fermented the novel WK, lowering pH to ~3.5. Ethanol content remained within the non-alcoholic range (<0.1% ABV), and the sucrose content was below 0.5%, resulting in non-alcoholic, low-sugar beverages. Shotgun metagenomics and microbial culturing confirmed MinCom composition. Metabolomic analysis revealed the presence of favourable health-promoting compounds and volatiles, resulting in promising novel WK.

Conclusion

This study demonstrates the successful application of MinComs for the fermentation of novel and healthy WK.

P031

The S. pneumoniae toxin pneumolysin is released via an interaction with the cell surface associated beta-galactosidase, BgaA.

Jenna Callaway[1], Dora Bonini[1,2], Nathan Palk[1], Ruth Massey[1,3]

[1]University of Bristol, Bristol, United Kingdom. [2]University of Sheffield, Sheffield, United Kingdom. [3]University College Cork, Cork, Ireland

Abstract

Streptococcus pneumoniae (*Sp*.) is an opportunistic pathogen that produces a single toxin, pneumolysin (Ply). Ply causes host cell lysis, induces inflammation and is key to *Sp*. evasion of the complement cascade. However, Ply does not have a secretion signal sequence and its mechanism of release from the cell remains unknown. In a recent GWAS to identify genes that affect Ply activity, the *bgaA* gene was identified. This encodes a beta-galactosidase enzyme, BgaA, which is cell-wall associated and has an interesting structure: the initial 1000 amino acids are homologous to beta-galactosidases in other species, however the second ~1000 amino acids bear no resemblance to proteins found elsewhere. To confirm the association between Ply activity and *bgaA,* a knockout strain was constructed. In this strain, Ply was not released as efficiently however there was no difference in *ply* production, growth or autolysis between the wild type and *bgaA* mutant. Computational modelling of the protein-protein interaction suggest that BgaA may directly interact with Ply, specifically between residues within the uncharacterised region, and this was experimentally confirmed using a bacterial-2-hybrid assay. Complementation of the full *bgaA* gene and the uncharacterised section in isolation confirms the phenotype of Ply release being dependent upon the presence of the C-terminus section of BgaA. This strongly indicates that Ply uses BgaA to gain access to the cell envelope, subsequently allowing for its extracellular release. This work gives a new understanding of the previously unknown release mechanism of this toxin, which could have significant implications for tackling *Sp*. infections.

P039

Silencing the Competition: Concentration-Dependent Bacteriostasis of Vibrionaceae by the Pseudomonas aeruginosa Quorum Sensing Signal HHQ

Conor Hill[1], Sarah Lombard[1], Molly O'Callaghan[1], Stephanie McGimpsey[2], Avril Coghlan[2], Nicholas Thomson[2], Ruth Massey[1], Nicky O'Boyle[3], F. Jerry Reen[1]

[1]UCC, Cork, Ireland. [2]Wellcome Sanger Institute, Cambridge, United Kingdom. [3]Trinity, Dublin, Ireland

Abstract

Microbial communities are shaped by interspecies chemical signalling and competitive interference through quorum sensing (QS) molecules. Remarkably, although the concept of QS first originated from work done on the marine symbiont *Aliivibrio fischeri*, very little is known about cell-cell communication in the marine ecosystem. Marine sponges (Porifera) represent a rich chemical reservoir of natural bioactive compounds and understanding how QS operates in this niche would be an important ecological and translational advance. While more often viewed in the context of human disease and infection, *Pseudomonas aeruginosa* and *Vibrionaceae* species have been isolated from marine sponges sourced off the coast of Ireland. Here, we investigate the antibacterial effects of the *P. aeruginosa*QS signal 2-heptyl-4-quinolone (HHQ) against members of the *Vibrionaceae* family. Initial assays revealed that HHQ activity against *Vibrio* species is not binary but operates along a susceptibility gradient. While *Aliivibrio fischeri* was completely inhibited at low HHQ concentrations, species such as *V. alginolyticus* and *V. diabolicus* tolerated levels up to 100 µM. Comparative genomics revealed a second *cyd*AB operon in *V. parahaemolyticus*, also present in *P. aeruginosa*. However, deletion of the *cyd*AB genes did not sensitize the strain to 10 µM HHQ. Supernatant cross-feeding assays suggest an inducible, cell-associated mechanism of sequestration and/or detoxification, while prolonged exposure of *V. scophthalmi* to HHQ led to emergence of spontaneous resistant mutants, indicating the potential for adaptive resistance under sustained selective pressure. Our findings reveal that HHQ functions as a species-specific antimicrobial in marine environments, with species-level tolerance evident in *Vibrionaceae*.

P059

Potential of Large Language Models(LLMs) for the reassembly of Proteins from Peptide Data

Shrutik Kharkar, Florence Abram, Enda Howley

University of Galway, Galway, Ireland

Abstract

Proteomics and metaproteomics are key for the advancement of biomedical, environmental, and industrial research in the post-genomic landscape. Proteomics provides a closer connection to functional activity and phenotypes compared to genomics and transcriptomics, which show an organism's genetic potential. Protein identification depends on reliable reference databases, while standard proteomic analyses usually use tandem mass spectrometry (MS) and peptide digestion. However, for many environments such as soils, these databases are incomplete or unavailable. Peptide sequences can be directly retrieved from MS data using de novo peptide sequencing, but reassembling entire protein sequences remains a challenge.

As a first step to this goal, we evaluated the ability of LLMs to assign unknown peptides to their proteins. We fine-tuned BERT, RoBERTa, ALBERT, and ProteinBERT architectures on curated peptide datasets, testing how artificial data masking strategies to generate augmented data influence predictive performance. We were only able to achieve around 3% accuracy without applying data augmentation strategies, but after using the data augmentation strategies we were able to achieve a much higher prediction accuracy. Among the models, RoBERTa achieved the highest prediction accuracy of 83% when trained with augmented data, whereas ProteinBERT, designed primarily for dealing with protein sequences, reached around 63% accuracy when peptide sequences were used for training and prediction.

Our findings demonstrate the potential of LLMs for de novo protein reassembly, especially when coupled with strategic data augmentation methods. This approach could improve proteomic analyses in diverse or poorly characterised samples where conventional reference-based workflows fall short.

P061

PAD enzymes inhibit biofilm formation and quorum sensing in a multidrug resistant cystic fibrosis isolate of *Pseudomonas aeruginosa*

RORY BAIRD[1], Debananda Gogoi[1], Luke Forde[1], Sara Waqas Ahmed[1], Mengxin Niu[1], Brenton Cavanagh[2], Emer P. Reeves[1]

[1]Pulmonary Clinical Science, Department of Anaesthetics and Critical Care Medicine, RCSI University of Medicine and Health Sciences, Beaumont Hospital, Dublin, Ireland. [2]Cellular and Molecular Imaging Core, RCSI University of Medicine and Health Sciences, Dublin, Ireland

Abstract

Background: *Pseudomonas aeruginosa* is a major opportunistic pathogen implicated in chronic infections, especially in immunocompromised individuals. Its ability to form biofilms, combined with antibiotic resistance, plays a critical role in the persistence of these infections. Biofilms, composed of extracellular polymeric substances, shield bacteria from immune responses and antimicrobial agents, necessitating the development of novel therapeutic strategies. We recently demonstrated that peptidyl arginine deiminases, PAD2 and PAD4 isoforms, are localized in neutrophil granules and contribute to the phagosomal killing of *P. aeruginosa*. This study aimed to investigate the effects of recombinant (r)PAD2 and rPAD4 on biofilm formation and quorum sensing (QS)-regulated virulence in multidrug resistant *P. aeruginosa* strain PGO2330, isolated from a patient with cystic fibrosis.

Methods: Crystal violet biofilm assays and confocal laser scanning microscopy were used to assess biofilm development. Cell motility assays, qPCR for QS gene expression, and virulence factor analyses were also conducted.

Results: Treatment with 20 nM rPAD2 and rPAD4 reduced biofilm formation to 67.9±5.6% ($p=0.0002$) and 68.2±4.2% ($p=0.0002$), respectively. This reduction correlated with decreased surface attachment ($p<0.0001$ for both) and twitching motility (rPAD2 $p<0.0001$; rPAD4 $p=0.0190$). rPAD treatment significantly downregulated QS genes; *lasR* (rPAD2 $p=0.0004$; rPAD4 $p=0.0002$), *lasI* ($p<0.0001$), *rhlR* ($p<0.0001$), *rhlI* ($p<0.0001$), and *mvrf* ($p=0.0004$). Corresponding decreases in extracellular DNA (PAD2 $p=0.0343$; PAD4 $p=0.0055$), rhamnolipids ($p=0.0055$; $p=0.0021$), pyocyanin ($p=0.0004$; $p=0.00133$) and protease activity ($p=0.0037$; $p=0.0303$) were observed.

Conclusions: These findings highlight rPAD2 and rPAD4 as potential antimicrobial agents that disrupt biofilm formation and attenuate QS-regulated virulence in *P. aeruginosa*.

P079

Nisin-like biosynthetic gene clusters are widely distributed across microbiomes

David Hourigan[1,2], Des Field[1,2], Ellen Murray[1,2], Ivan Sugrue[1,2], Paula O'Connor[1,3], Colin Hill[1,2], Paul Ross[1,3]

[1]APC Microbiome Ireland, Cork, Ireland. [2]University College Cork, Cork, Ireland. [3]Teagasc, Cork, Ireland

Abstract

Bacteriocins are antimicrobial peptides/proteins that can have narrow and broad spectrums of inhibition and remarkable potency against clinically-relevant pathogens. One such bacteriocin that is extensively used in the food industry and with potential for biotherapeutic application is the post-translationally modified peptide, nisin. Recent studies have shown the impact of nisin on the gastrointestinal microbiome, but relatively little is known of how abundant nisin production is in nature, the breadth of variants that exist and their antimicrobial potency. Whether or not nisin production and immunity is widespread in gut microbiomes could be a deciding factor in determining the suitability of nisin as a prospective therapeutic for human and/or animal infections. Here, we used publicly available datasets to determine the presence of widespread and diverse nisin biosynthetic gene clusters (nisin-BGCs) across the biosphere. We show that 30% of these nisin-BGCs are predicted to be located on mobile genetic elements, with some found in pathogenic bacteria. Furthermore, we highlight evidence of horizontal gene transfer of nisin-BGCs between genera, including *Streptococcus suis*, *Enterococcus hirae* and *Staphylococcus aureus*. In all, we describe 107 novel nisin-like peptides. Five representatives were heterologously expressed and all exhibited antimicrobial activity. We further characterised nisin VP, a novel natural nisin variant produced by *Velocimicrobium porci* isolated from the porcine gut. The peptide has a completely novel hinge region "AIQ" not detected in other nisin variants to date. While nisin VP could be induced by nisin A, the latter could not be induced by nisin VP.

P092

The effect of ATP release by colorectal cancer cells on motility and invasion in *Streptococcus gallolyticus* subspecies *gallolyticus*.

Adam Ralph, Jennifer Mitchell

University College Dublin, Dublin, Ireland

Abstract

Streptococcus gallolyticus subspecies *gallolyticus* (*Sgg*) has been long associated with colorectal cancer (CRC). An opportunistic pathogen, *Sgg* colonises the gut and passes into the bloodstream of CRC patients through cancerous lesions causing bacteraemia and infective endocarditis.

Our lab identified that *Sgg* displays twitching motility via a Type IV Pilus and is capable of invading CRC cells. Both behaviours are altered in the presence of excess glucose and cAMP. Recently, it has been shown that mechanosensitive cell membrane channel, PIEZO1, signals the release of ATP as an immune response to invading bacteria.

To investigate whether extracellular ATP (eATP) alters motility and invasion in *Sgg*, CRC cell line, HT-29, was treated with PIEZO1 agonist, Yoda1, and Yoda1 competitive inhibitor, Dooku1, followed by incubation with *Sgg* to facilitate invasion and gentamicin treatment. Supernatants were harvested following incubation and used in motility assays.

Interestingly, invasion was significantly reduced following treatment by Yoda1 compared to DMSO control, while an increase was observed in the presence of Dooku1. However, motility was unaffected in both Yoda1 and Dooku1 groups compared to control.

As the release of eATP is promoted via PIEZO1 agonist Yoda1, it is likely that eATP plays a role in the decreased invasive capability of *Sgg*. The release of eATP by HT-29 cells induced by *Sgg* invasion will be evaluated. These results further the understanding of the mechanism utilised by *Sgg* to enter the bloodstream of CRC patients. This is the first time the release of DAMPs has been seen to directly inhibit bacterial pathogenesis.

Offered Flash talk

P002

Dissecting bacterial hosts and inferring evolution of class 1 integrons in environmental microbiomes with a single-cell fusion-PCR strategy

Qin Qi[1,2], Timothy Ghaly[2], Vaheesan Rajabal[2], Michael Gillings[2], Sasha Tetu[2]

[1]The University of Manchester, Manchester, United Kingdom. [2]Macquarie University, Sydney, Australia

Abstract

Class 1 integrons are versatile bacterial genetic elements that correlate with anthropogenic pollution and contribute to the dissemination of antimicrobial resistance (AMR). Over time, class 1 integrons have spread extensively from clinical bacterial pathogens to environmental bacterial hosts, and *vice versa*, via horizontal gene transfer. Understanding their dissemination patterns in environmental microbiomes offers the potential to better predict the evolutionary trajectories of class 1 integrons and their cargo genes. Our work applied a single-cell genomic strategy, epicPCR (emulsion, paired isolation and concatenation PCR), to link class 1 integrons to the 16S taxonomic markers of their respective hosts using single bacterial cells from vegetable phylloplane and environmental water microbiome samples as amplification targets. Through Nanopore sequencing of fusion-PCR products, this approach allowed us to assign class 1 integrons to diverse bacterial hosts, including novel hosts of class 1 integrons in environmental microbiomes. We identified and experimentally characterised altered integron gene cassettes with novel promoters, which increased the expression strengths of AMR cargo genes and conferred higher antimicrobial resistance phenotypes. Our work further characterised a gene cassette that confers resistance to chlorite, which is a by-product of many chlorinated disinfection agents. This chlorite dismutase-encoding gene cassette has the potential to further contribute to the global spread of AMR due to its physical linkage with AMR-encoding gene cassettes, which has been observed in an increasing number of countries in recent years. This necessitates a better understanding of the transmission dynamics and evolutionary trajectory of this globally emerging integron gene cassette.

P003

Temperature and pH universally govern protein diversity in hydrothermal spring communities, but they do so differently

Juan Rivas-Santisteban

Centro Nacional de Biotecnología (CNB-CSIC), Deparment of Systems Biology, Madrid, Spain

Abstract

It has been suggested that temperature and pH are strong explanatory causes of protein evolutionary constraint. However, a general understanding of how changes in temperature and pH may universally impact protein evolution is lacking. While pH is locally regulated in microbial compartments, temperature is not. These variables may act at different rates in a given genome, since there are genes encoding for proteins allocated in different compartments. To test whether this is true, we can use a proxy for the number of evolutionary events. One unregarded way is to calculate the diversity of unique sequences assigned to an ortholog across species. The expectation for any two KEGG orthology identifiers with a similar metagenomic representation is to be represented by a similar number of unique sequence variants. We examine sequence diversity among 17 metagenomes from El Tatio geothermal field (Chile), spanning temperatures of 45-62ºC, and pH values of 7.2-9.3. When controlling for metagenomic abundance, higher temperature increases, while basic pH decreases the expected by-function diversity. We thus provide evidence against temperature-induced random gene loss hypotheses. The inclusion of these variables improved the prediction of sequence diversity from metagenomic abundance. Finally, between-compartment sequence diversity was more affected by pH than by temperature, partially supporting our initial hypothesis.

P007

Anti-cancer compounds can alter the treatment efficacy of bacterial infections

Aaron Nolan, Merve Zeden, James P. O'Gara

University of Galway, Galway, Ireland

Abstract

The clinical burden of infections caused by antimicrobial resistant (AMR) pathogens is rising with future predictions suggesting that 10 million deaths will be caused by AMR infections by 2050 followed closely by cancer with a predicted 8.2 million deaths. Maintaining the effectiveness of antibiotics is pertinent to the ability to slow down the rising burden of AMR. β-lactam antibiotics are regarded as the primary choice for treatment of infections caused by *Staphylococcus aureus* but are limited by the prevalence of methicillin-resistant *S. aureus* (MRSA). Our data revealed the anti-MRSA activity of several compounds used during cancer chemotherapy, when used alone and in combinations with standard of care antibiotics. RNA-Seq, whole genome sequencing, checkerboard assays and microscopy was used to elucidate the anti-MRSA mechanism(s) of action of the chemotherapeutic drugs. These experiments led to the identification of MRSA pathways targeted by the anti-cancer drugs and novel insights into their synergistic or antagonistic interactions with beta-lactam antibiotics. These findings are important to improve our understanding of the most effective use of antibiotics for the treatment of MRSA infections in patients undergoing chemotherapy.

P008

Biochar-Enhanced Enrichment of Type I Methanotrophs with Relative Abundance Insights from a Custom Database

Brianna Casey, Gustavo Sambrano, Alma Siggins

University of Galway, Galway, Ireland

Abstract

Type I methanotrophs play an important role in methane cycling and have potential in biotechnological and environmental applications. Enriching these bacteria from environments like activated sludge is a key strategy for optimising applied research. As well as their environmental importance, some species can produce high-value products like ectoine, methanol, and bioplastics with significant commercial potential. These by-products offer a revenue stream that aligns with circular economy principles, turning waste methane into profitable, sustainable products.

This study provides a practical protocol for enrichment of Type I methanotrophs from activated sludge, with emphasis on accessible, low-cost techniques suited for small-scale testing. Activated sludge samples were taken from a municipal wastewater treatment plant and incubated at temperatures representative of their environment, under selective conditions using NMS medium and methane-limiting conditions.

The enrichment process was monitored through methane consumption levels, qPCR for relative abundance targeting the *16S rRNA* and *pmoA* genes, and shotgun metagenomic analysis to evaluate community composition. The raw data was compared to a custom curated methanotroph database. To address methane's low solubility in water, biochar, a carbon-rich, pyrolyzed waste product, was used for its adsorption potential. The aim was for biochar to adsorb methane onto its surface, making it more accessible to methanotrophs, thereby enhancing methane oxidation, and microbial growth, with the potential to increase by-product production. This process was successful in increasing type 1 methanotrophs.

The described method proposes an economical approach to enriching Type I methanotrophs without complicated tools and serves as a practical resource for further study.

P010

Defining the role of TcaA in cell envelope biogenesis of *Staphylococcus aureus*

Samuel Fenn[1], Nathan Palk[2], Marcia Boura[2], Ruth Massey[1,2]

[1]University College Cork, Cork, Ireland. [2]University of Bristol, Bristol, United Kingdom

Abstract

Staphylococcal cell envelope biogenesis is a highly coordinated process with synthesis and ligation of wall teichoic acid (WTA) essential for resistance to beta-lactams and glycopeptides. Ligation of WTA to peptidoglycan is performed by members of the LCP family of enzymes in a magnesium-dependent reaction, with LcpABC performing this function in *S. aureus*. Enzymes of this family posses pyrophosphatase activity to cleave WTA from a lipid carrier, whilst the phosphotransferase function is used attach cleaved WTA to peptidoglycan

We recently identified a novel protein implicated in WTA ligation, with mutation of *tcaA* reducing ligation of WTA in the cell envelope. Co-immunoprecipitation using TcaA as the bait protein revealed multiple interactions with components of peptidoglycan and WTA synthesis, whilst use of purified peptidoglycan and WTA demonstrated that TcaA binds to both polymers. This led us to hypothesize that *tcaA* encodes a novel WTA ligase or supports function of the LCP enzymes. Modelling of TcaA and biochemical assays revealed the presence of a conserved magnesium binding site, with site-directed mutagenesis (SDM) confirming the importance of the predicted residues in binding. Screening of TcaA and SDMs revealed that TcaA possesses pyrophosphatase activity, with SDMs with reduced magnesium binding demonstrating reduced catalysis. Overexpression of TcaA in a Δ*lcpA* mutant restores cell envelope WTA levels, with a double Δ*lcpA tcaA*::tn mutant leading to further loss of WTA from the cell envelope. Together with the pyrophosphatase activity, we conclude that TcaA is a novel WTA ligase which contributes to *S. aureus* cell envelope biogenesis during stress.

P012

A genome wide association analysis to understand the factors involved in serum adaptation of Streptococcus pneumoniae

Charlotte Richards[1,2], Cristian Voiculescu[1], Mario Recker[3], Ruth Massey[1,4]

[1]University of Bristol, Bristol, United Kingdom. [2]Cardiff University, Cardiff, United Kingdom. [3]University of Exeter, Exeter, United Kingdom. [4]University College Cork, Cork, Ireland

Abstract

Streptococcus pneumoniae (SP), a Gram-positive WHO priority pathogen, causes over one million deaths annually. To establish bacteraemia, SP must withstand the antimicrobial-rich environment of human blood, including antimicrobial peptides and fatty acids. While its evasion of phagocytes is well studied, less is known about its resistance to these soluble antibacterial factors. In this study, 165 sequenced clinical SP strains were exposed to 10% human serum for 90 minutes, and survival rates were quantified. A genome-wide association study (GWAS) using single nucleotide polymorphism data identified genetic determinants of serum adaptation. Survival ranged from 36% to 1055% relative to controls. GWAS revealed 84 genes and 34 intergenic regions significantly associated with serum resistance. Notably, the BceAB ATP-binding cassette transporter, known for antimicrobial peptide resistance via interaction with a two-component system (TCS), was highly represented. Additional hits included genes involved in carbohydrate metabolism (11), glycan production (2), ABC transporters (6), TCS components (5 across 3 systems), and metal metabolism (3). Compared to pathogens like *Staphylococcus aureus*, SP has fewer described mechanisms for serum adaptation. This top-down GWAS approach, paired with functional validation, offers a powerful strategy to uncover novel resistance mechanisms and define gene function.

P035

The CytePulse: Amping up antimicrobial susceptibility testing

Tudor Onose[1], Magdalena Karlikowska[2], Munehiro Asally[1]

[1]School of Life Sciences, University of Warwick, Coventry, United Kingdom. [2]Cytecom Ltd., Coventry, United Kingdom

Abstract

The forthcoming antimicrobial resistance (AMR) crisis highlights the urgent need for rapid and reliable diagnostic tools enabling effective, evidence-based screening and therapy. Current gold-standard susceptibility testing methods rely on measuring bacterial growth, resulting in time-to-result delays that limit their clinical responsiveness. Here, we present a novel bioelectrical susceptibility testing method, which can determine a culture's antimicrobial susceptibility using a 1-minute test, following a short incubation of bacteria with antibiotics, via an optical electrophysiology platform, the CytePulse. This method builds on our prior observation that antimicrobial-inhibited and non-inhibited bacteria exhibit distinct membrane potential dynamics following exogenous electrical stimulation. Using Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) as model organisms, we assessed whether these electrophysiological responses could reliably reflect antibiotic efficacy. We tested antibiotics from multiple classes, including cell wall synthesis inhibitors (such as meropenem, imipenem, and ceftriaxone), protein synthesis inhibitors (erythromycin, gentamicin), and DNA replication inhibitors (ciprofloxacin). Cultures were briefly incubated with antibiotics before being assessed using the CytePulse in conjunction with fluorescence microscopy. We observed that non-inhibited (unexposed) cells displayed hyperpolarisation, while antibiotic-exposed cells displayed depolarisation in response to electrical stimulation. This dynamic shift can serve as a rapid phenotypic marker of antibiotic efficacy. Importantly, the ability of our proposed method to detect antimicrobial effects across multiple bacterial species and antibiotic classes highlights its potential for broad-spectrum applicability -hence potential broad utility in diverse infection contexts.

P045

HUMID: Honing Our Understanding of Microbial Diversity in Tropical Peatlands

Leanne O'Donoghue, Jerry Reen, Barbara Doyle-Prestwich, Michelle McKeown

University College Cork, Cork, Ireland

Abstract

Tropical peatlands are among the world's most carbon-rich ecosystems, yet their microbial diversity and functional ecology remain poorly characterised, particularly in the South Pacific. The HUMID project investigates microbial community composition and environmental controls across two contrasting peatlands: high-elevation Lake Tagimaucia (Fiji) and low-elevation Lac Lanutuli (Uvea). We integrate prokaryotic DNA profiling, functional metabolic assays, and testate amoebae analysis to examine how microbial diversity changes across ecohydrological conditions and vegetation types. Preliminary results from RAPD-PCR reveal clear site-specific differences in community structure, with samples from Uvea exhibiting a greater level of prokaryotic diversity when compared to those from Fiji. Results from the Biolog EcoPlate assays reveal active carbon substrate utilisation across both sites, indicating the presence of functional microbial diversity despite distinct differences in microbial community composition. Testate amoebae assemblages exhibited significant site-specific variation, with vegetation type and water-table depth emerging as primary environmental drivers. Uvea was characterised by a dominance of smaller testate amoebae, likely bacterivorous in nature, whereas larger taxa predominated in Fiji—potentially contributing to the comparatively lower prokaryotic diversity observed at that site. Furthermore, 28 morphotypes were found to be unique to Fiji, in contrast to only a single morphotype exclusive to Uvea. By connecting contemporary testate amoebae with both present-day prokaryotic diversity and down-core amoebae assemblages, we aim to infer past microbial dynamics and their role in long-term carbon cycling. While further research is needed to fully characterise the microbial diversity of tropical peatlands, our preliminary findings underscore the microbial complexity of these important ecosystems

P046

Trans-kingdom conservation of antimicrobial peptide structure and function

Ivan Sugrue[1,2], Carolin Ade[3], Paula M. O'Connor[4], Jan-Martin Daniel[3,5], Paolo Innocenti[6], Nico Kirsch[7], Nathaniel I. Martin[6], Günther Weindl[7], Colin Hill[1,2], Tanja Schneider[3,5], R. Paul Ross[1,2]

[1]APC Microbiome Ireland, University College Cork, Cork, Ireland. [2]School of Microbiology, University College Cork, Cork, Ireland. [3]Institute for Pharmaceutical Microbiology, University of Bonn, University Hospital Bonn, Bonn, Germany. [4]Teagasc Food Research Centre, Cork, Ireland. [5]German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Bonn, Germany. [6]Biological Chemistry Group, Institute of Biology, Leiden University, Leiden, Netherlands. [7]Department of Pharmacology and Toxicology, Pharmaceutical Institute, University of Bonn, Bonn, Germany

Abstract

Antimicrobial peptides are widespread defence molecules found across all domains of life, and they hold promise for the development of new antimicrobial therapies. One such peptide, the bacteriocin actifensin from *Actinomyces ruminicola* DPC7226, exhibits potent activity against gram-positive bacteria, including vancomycin-resistant *Enterococcus* and *Clostridioides difficile*. Structurally, actifensin resembles cysteine-stabilized αβ-defensins found in fungi, insects, and molluscs. This study explored the therapeutic potential and mechanism of action of actifensin through detailed biochemical characterization, in comparison with eukaryotic defensins. Mechanistic assays demonstrated that actifensin acts by binding to the peptidoglycan precursor molecule, lipid II, thereby inhibiting cell wall biosynthesis. Notably, its binding does not require UDP-GlcNAc, indicated by its ability to bind lipid I equally, and it does not depolarize bacterial membranes, unlike related defensins. Actifensin halts peptidoglycan biosynthesis, causing indirect cell death via cell wall weakening, visualised by membrane blebs observed with phase-contrast microscopy. The peptide shows minimal cytotoxicity and haemolysis toward human cells and is not immunogenic, demonstrating no induction of LDH release in PBMCs or any effect on TLR-mediated signalling. In-depth structural analysis revealed that actifensin belongs to a trans-kingdom conserved subfamily of CSαβ-defensins, named GXGCP, found across bacteria, fungi, and invertebrates, sharing key lipid-II binding residues. This group appears distinct from another subgroup, XTCD, present in more recently evolved insect phyla. These findings present actifensin as a promising antimicrobial agent, highlighting its conserved structure-function relationship with defensins and suggesting a shared evolutionary history for these diverse antimicrobials.

P048

Feline oral squamous cell carcinoma: expression of toll-like receptors 2 and 4 and the impact of feline oral bacteria on tumour cell growth (SIMBA)

Alison Lee[1,2], Hanne Jahns[2], Ann Hopkins[3], Gary Moran[1]

[1]Dublin Dental University Hospital, Dublin, Ireland. [2]University College Dublin School of Veterinary Medicine, Dublin, Ireland. [3]Royal College of Surgeons Ireland, Dublin, Ireland

Abstract

Feline oral squamous cell carcinoma (FOSCC) is the most common oral neoplasm in cats and generally has a poor prognosis. While the cause(s) of this tumour remains unknown, FOSCC has been proposed as a comparative model for human oral squamous cell carcinoma (HOSCC), for which periodontal disease is a risk factor. Therefore, this project investigates the role of oral Gram-negative bacteria and toll-like receptors (TLRs) in FOSCC.

RNA in-situ hybridization was used to demonstrate expression of TLR2 and TLR4 mRNA in normal and inflamed feline gingiva and FOSCC. Oral swabs were taken from cats without oral disease (n=8), cultured anaerobically, and putative colonies of *Fusobacterium* and *Porphyromonas* species underwent sequencing of the 16S rRNA gene for speciation. FOSCC cell lines (SCCF2, SCCF3) were co-cultured with two isolated bacterial strains. Cell proliferation was assessed using the Alamar blue and scratch wound assays.

Normal, inflamed and neoplastic oral epithelium expressed TLR2 and TLR4 mRNA. *Fusobacterium canifelinum*, *Porphyromonas gulae* and *P. macacae* were isolated from three, five and one cats respectively. Co-culture of either cell line with *P. gulae* (alone or with *F. canifelinum*) resulted in a tendency towards increased proliferation and caused significantly increased growth of SCCF3 at 72 hours compared with earlier time points (using the Alamar blue assay).

This demonstrates that non-neoplastic and neoplastic oral epithelial cells have the capacity to recognize bacteria via TLRs. Additionally, *P. gulae* affected the growth of FOSCC cell lines, thus highlighting the possible influence of the oral microbiome on feline oral cancer.

P053

Novel insights into *Listeria monocytogenes* Motility on Eiken Agar: Evidence for Strain-Specific Swarming Motility Patterns

Monica Cazzaniga[1,2], Peiyao Shen[3,2], Paul D. Cotter[4,2], Cormac G.M. Gahan[5,1,6], Jerry F. Reen[7,1]

[1]School of Microbiology, Cork, Ireland. [2]APC Microbiome Ireland, cork, Ireland. [3]University College Cork, Cork, Ireland. [4]Teagasc Food Research Centre and VistaMilk SFI Research Centre, Fermoy, Ireland. [5]APC Microbiome Ireland, Cork, Ireland. [6]School of Pharmacy, Cork, Ireland. [7]SSPC, the Research Ireland Centre for Pharmaceuticals, Cork, Ireland

Abstract

Introduction: Motility is a key adaptive trait that allows bacteria to navigate diverse environments, access nutrients, and colonise surfaces. While typically *Listeria monocytogenes* is motile at 23–30°C on soft BHI agar, at 37°C (mammalian body-temperature), motility is repressed. This study explored motility on Eiken agar, a medium known to support swarming in other species, and for the first time, we observed dendritic, swarming-like patterns in *L. monocytogenes*.

Materials and Methods: Isolates from clinical, food, and environmental sources were tested on soft Eiken agar at 23°C, 30°C, and 37°C, with various carbon sources. Motility was quantified by measuring the area of expansion. Microscopy, time-lapse imaging, RNA sequencing, and pangenome analysis were used to study swarming dynamics and identify associated genes and molecular markers.

Results: Dendritic motility patterns were observed in Lineage II strains at 37°C, and only in the presence of monosaccharides (glucose, fructose, and mannose). Complex carbon sources such as lactose and sucrose did not support motility. Microscopy revealed a surfactant-like layer in motile strains, suggesting a potential mechanism facilitating surface spreading. Pangenome analysis uncovered a hypothetical protein differing between swarming/non-swarming strains, with pathway enrichment in starch and sucrose metabolism.

Conclusion: This study provides the first description of swarming-like motility in *L. monocytogenes* on Eiken agar. The phenomenon is specific to individual isolates, is predominant in Lineage II strains and is dependent on temperature and nutrient conditions. Ongoing transcriptomic and genomic analyses aim to further elucidate the molecular basis of this behaviour.

P054

Salivary antibodies mirror systemic humoral immunity to SARS CoV-2 and reveal sex-specific differences in salivary anti-spike IgA and IgG levels.

Joshua Flynn[1], Eolann Dinneen[2], Niall O'Leary[2], Aideen Long[1], John MacSharry[2]

[1]TCD, Dublin, Ireland. [2]UCC, Cork, Ireland

Abstract

Durable antibody responses are essential for long-term protection against SARS-CoV-2. We assessed the utility of point-of-care lateral flow tests (LFTs) versus ELISA in monitoring systemic and mucosal immunity and explored saliva as a surrogate for serum antibody measurement. 450 participants were recruited in a university and serum and saliva samples were analysed for anti-Spike IgA and IgG antibodies using ELISA, and for nucleocapsid (N) and neutralising Spike antibodies via LFT. Despite only 46% self-reporting prior infection, 95% had detectable N antibodies. Serum demonstrated higher anti-Spike IgA and IgG positivity than saliva. Moderate correlations between serum and salivary IgA and IgG were observed. LFT band intensity significantly correlated with ELISA-derived antibody levels. Sex-specific differences in salivary anti-Spike IgA and IgG levels were identified, with males displaying higher levels in early post-exposure periods. These findings support LFTs and saliva sampling as accessible tools for monitoring SARS-CoV-2 immunity and guiding booster vaccination strategies.

P058

Presumptive *Bacillus cereus* genomic confirmation of in-process skim milk powder samples using whole genome sequencing

Vincenzo Fiore[1,2], Francis Butler[2], Raffaele Magliulo[3], Geraldine Duffy[4], Triona O'Brien[1]

[1]Food Safety Department, Teagasc Food Research Centre, Moorepark, Fermoy, Ireland. [2]University College Dublin School of Biosystems and Food Engineering, Belfield, Dublin, Ireland. [3]Department of Agricultural Sciences, University of Naples Federico II, Napoli, Italy. [4]Food Safety Department, Teagasc Food Research Centre, Ashtown, Dublin, Ireland

Abstract

Bacillus cereus sensu lato (s.l.) is a group of spore-forming microorganisms that produce heat-resistant endospores, allowing survival in harsh conditions, which can also be pathogenic. Toxins produced by the bacteria cause foodborne illnesses. This study aimed to determine the specificity of several commercial chromogenic agars for detecting *B. cereus* from in-process samples collected during a skim milk powder production process.

In-process samples were aseptically collected from an Irish dairy facility and tested for *B. cereus* presence using different commercial selective media. From each positive sample, three colonies were selected from the commercial agar plates and screened for hemolysis on blood agar. From the isolates, DNA was extracted, and whole genome sequencing (WGS) was performed for taxonomic classification.

From a total of 77 presumptive *B. cereus* isolates from commercial agars, nine were confirmed as members of the *B. cereus* s.l. group using WGS. From the presumptive isolates, identifications included other species of *Bacillus* (*n* = 3), *Enterococcus* (*n* = 11), *Lactococcus* (*n* = 10), *Cellulosimicrobium* (*n* = 6), *Staphylococcus* (*n* = 4), and 32 isolates unclassified. Ongoing analyses is focused on characterising toxin genes.

Commercial agars for presumptive *B. cereus* showed low specificity. Although some media performed better than others, reliance solely on selective media may lead to inaccurate identification. WGS provides a more specific method for confirming species level identification of presumptive *B. cereus* isolates.

P060

Proteomic response of *bla*$_{\text{CTX-M-15}}$ producing *Klebsiella pneumoniae* in cefotaxime

Chloe McKenna, Monika Subanovic, Fiona Walsh

Maynooth University, Maynooth, Ireland

Abstract

Background: The enzyme *bla*$_{\textit{CTX-M-15}}$, an extended spectrum beta lactamase, has potent hydrolytic activity against the third generation cephalosporin cefotaxime. The proteomic response of CTX-M-15 producing, cefotaxime resistant *K. pneumoniae* can be studied using Mass Spectrometry, in order to understand the whole cell response to the antibiotic

Methods: A plasmid containing the CTX-M-15 gene was conjugated into *K. pneumoniae NCTC 418*. This strain, containing the plasmid, as well as the wild type strain were grown in X2 MIC cefotaxime for 2 hours. The proteins were extracted and digested with trypsin, before cleaning with C18 Zip-Tip filters. Peptides were loaded onto a timsTOF Mass Spectrometer. Raw data files were analysed using MaxQuant and AMICA.

Results: Over 400 proteins were differentially expressed when *K. pneumoniae* + CTX-M-15 plasmid was compared to *K. pneumoniae* WT: 167 proteins were increased in abundance, and 236 proteins were decreased in abundance in the resistant strain when compared to the susceptible. Of the proteins increased in abundance 73 were involved in metabolic processes, and 35 were involved in biosynthesis of secondary metabolites. Proteins that were increased in abundance were seen to be involved in pathways such as biosynthesis of co-factors, biosynthesis of amino acids, and purine metabolism. Proteins that were decreased in abundance were seen to be involved pathways such as ABC transporters, oxidative phosphorylation, and peptidoglycan biosynthesis.

Conclusion: Key pathways and proteins identified in this study are important in understanding the whole bacterial cell response to antibiotic treatment, which is useful in tackling the fight against antimicrobial resistance.

P062

A gelatine coating for calcium peroxide to prolong methane inhibition in simulated cow rumen conditions

Alison Hall, Vincent O'Flaherty

University of Galway, Galway, Ireland

Abstract

The deadline for reaching our Global Methane Pledge commitment is fast approaching and pressure is mounting for Irish farmers to reduce methane emissions. There is demand for a safe and effective methane inhibitor for ruminant livestock, which are responsible for 19% of Ireland's total greenhouse gas emissions. Thus, the aim of this research is to develop a novel slow-release methane inhibitor for cattle using calcium peroxide (CaO_2), which has previously been shown to supress methane production.

To simulate the environment within a cow's rumen, a trial was carried out using dual-flow fermenters with a working volume of 800 mL (1:1 rumen fluid and buffer). Two fermenters received no treatment and two received a treatment of gelatine-coated CaO_2. The oxidative reduction potential (ORP) was measured using *in situ* ORP probes which allowed the release of CaO_2 from the gelatine to be monitored.

The untreated fermenters maintained negative ORP values around -450 mV, indicating active methane production. For the fermenters which received the coated CaO_2, a significant increase in ORP to +200 mV was observed which remained elevated for ~2 hours. These preliminary findings indicate both the successful release of CaO_2 from its coating and the suppression of methane production.

In conclusion, CaO_2 was successfully coated in gelatine and maintained its oxidising power upon release from this coating in simulated rumen conditions. Further research will be conducted to enhance and refine this novel treatment. Future work will investigate any potential impact of this treatment on the microbial community present in the fermenters.

P063

Accumulation of intracellular sucrose activates the cell wall stress response in *Staphylococcus aureus*

Kate Kearney[1,2], Elizabeth Ledger[1,2], Mario Recker[3], Ruth Massey[1,2,4]

[1]1. School of Microbiology, University College Cork, Cork, Ireland. [2]2. APC Microbiome Ireland, University College Cork, Cork, Ireland. [3]3. Centre for Ecology and Conservation, University of Exeter, Exeter, United Kingdom. [4]4. School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom

Abstract

Staphylococcus aureus bacteraemia is among the most prevalent bacterial infections worldwide, with a mortality rate of approximately 30%. To survive in the bloodstream, *S. aureus* must overcome the hostile conditions of this environment, in particular, cell wall-targeting antibiotics. *S. aureus* responds to these by inducing the cell wall stress response (CWSR), however, how this response is activated is not fully understood. Our work aims to elucidate this, to provide insights into how *S. aureus* survives cell wall damage and causes persistent infections. To do this, we measured the rate and magnitude of induction of the CWSR in a panel of clinical isolates in response to daptomycin, an antibiotic known to cause cell wall damage. We observed that both susceptibility to daptomycin and induction of the CWSR in response to daptomycin exposure varied across the clinical isolates. To understand the mechanism behind this, we did a genome-wide association study which identified a link between *scrB*, a gene encoding a sucrose-6-phosphate hydrolase, and the CWSR. We found that sucrose induced the CWSR in the wild-type strain and led to significantly increased induction in a *scrB* mutant, which is unable to break down sucrose. This suggests that increased intracellular sucrose concentration activates the CWSR. This effect was further enhanced on exposure to daptomycin, indicating *scrB* may play a role in responding to daptomycin stress. In conclusion, *scrB* is a crucial link between carbon metabolism, osmotic imbalances, and the CWSR of *S. aureus*.

P067

Herbicolin A, an antifungal lipopeptide produced by Pantoea agglomerans APC 4211 is a potential biocontrol agent against food spoilage fungi

Elena Kamilari[1], Paula M. O'Connor[2], Jerry Reen[1], Promi Das[1], Aiswariya Deliephan[3], Daragh Hill[3], Oxana Fursenko[3], Jonathan Weise[3], Ay Sha Moore[1], Colin Hill[1], Catherine Stanton[2], Paul Ross[1]

[1]University College Cork, Cork, Ireland. [2]Teagasc, Moorepark Food Research Centre, Fermoy, Cork, Ireland. [3]Kraft Heinz Corporate Headquarters, Chicago, USA

Abstract

Background

Fungal contamination of food leads to significant economic losses and poses serious health risks due to mycotoxin production. In this study, *Pantoea agglomerans* APC 4211 was found to exhibit broad-spectrum antifungal activity against a variety of spoilage fungi.

Methods

Identification and purification of the antifungal compound was performed using a combination of whole genome sequencing analysis with reversed-phase HPLC and matrix-assisted laser desorption ionization–time of flight mass spectrometry analysis. The purified antifungal lipopeptide was assessed for thermal and protease stability, cytotoxicity against epithelial cell lines, and solubility and stability in 10% skim milk. The minimum inhibitory concentration against spoilage fungi was also determined.

Results

Genomic analysis revealed a novel biosynthetic gene cluster responsible for the production of the antifungal lipopeptide herbicolin A. Matrix-assisted laser desorption ionization–time of flight mass spectrometry analysis of the cell-free supernatant confirmed the presence of a mass corresponding to herbicolin A (1300.8 Da). Purified herbicolin A exhibited desirable properties for biotechnological application, including thermal and protease stability, low cytotoxicity against epithelial cell lines, and minimum inhibitory concentrations lower than those of some commercial antifungal agents (0.2 to 1.5 µg/ml). In a dairy model herbicolin A demonstrated excellent solubility and stability, effectively eliminating *Aspergillus niger* and *Penicillium notatum* at a concentration of just 5 µg/mL.

Conclusion

These findings highlight the potential of herbicolin A as natural antifungal agent suitable for use as biopreservative in food systems, offering an effective, safe, and clean-label alternative to synthetic preservatives for controlling fungal spoilage.

P070

Novel rhamnose-glucose polysaccharide structure elucidation and experimental confirmation of phage receptor specificity in *Streptococcus thermophilus*

Zoe Kampff[1], Irina Sadovskaya[2], Evgeny Vinogradov[3], Douwe van Sinderen[1], Jennifer Mahony[1]

[1]University College Cork, Cork, Ireland. [2]Univ. Littoral Côte d'Opale, Lille, France. [3]National Research Council Canada, Ottawa, Canada

Abstract

Streptococcus thermophilus is one of the most widely applied starter culture bacterial species in the dairy industry globally, where bacteriophages pose a significant threat to production efficiency. A key feature that underpins phage-host interactions in this species are the host-encoded cell wall polysaccharides (CWPS), namely exopolysaccharides (EPS) and rhamnose-glucose polysaccharides (RGP). These structures serve as receptors for infecting phages and play a critical role in dictating phage specificity and host range. While previous studies have linked certain phage genera to their respective polysaccharide receptors, insights into the structural diversity of RGPs and their correlation with phage-encoded receptor-binding proteins (RBPs) across the five *S. thermophilus* infecting phage genera remain unclear.

In this study, we elucidated five novel *S. thermophilus* RGP structures, each comprising unique combinations of backbone (Bt) and variable (Vt) region genotypes, thereby significantly expanding the current structural repertoire of *S. thermophilus* RGPs. Based on these structures, we predicted a novel RGP structure, further extending the framework for establishing a genotype to structure relationship. Furthermore, we explored the binding specificities of phage RBPs across all five *S. thermophilus* infecting phage genera by generating GFP-tagged RBP fusion proteins and performing binding assays on a diverse panel of *S. thermophilus* strains. The findings of this study significantly expand the understanding of RGP structural diversity and establish phage-host receptor specificities. This work has significant implications for the development of phage-robust starter culture strategies in dairy fermentation processes.

P082

Novel Workflow for Serotyping Porcine Pathogens *Actinobacillus pleuropneumoniae* and *Streptococcus suis*

Christina Killian[1], Seán Storey[1], Brian Byrne[2], Montserrat Gutierrez[2], Kevin Kenny[2], Séamus Fanning[3,4], Guerrino Macori[1,3]

[1]UCD School of Biology and Environmental Science, University College Dublin, Dublin, Ireland. [2]Department of Agriculture, Food and the Marine, Celbridge, Ireland. [3]UCD-Centre for Food Safety, Dublin, Ireland. [4]UCD School of Public Health, Physiotherapy and Sports Science, University College Dublin, Dublin, Ireland

Abstract

Actinobacillus pleuropneumoniae (*APP*) and *Streptococcus suis* (*S. suis*) are major porcine pathogens causing severe disease and mortality, leading to high antimicrobial compound use and economic losses on Irish pig farms. Access to serotype-specific autogenous vaccines would allow veterinary practitioners to target *APP* and *S. suis* more precisely, reducing reliance on broad-spectrum antimicrobial agents and supporting antimicrobial resistance mitigation.

A cost-effective workflow for serotyping *APP* and *S. suis* was developed using Oxford Nanopore Technologies (ONT). Eight serotypes (four per pathogen) were used to evaluate DNA extraction methods, library preparation, and *in-silico* serotype prediction. Of five methods tested, the Zymo Quick-DNA HMW MagBead Kit consistently yielded high-integrity DNA (DIN >9.0). Isolates were sequenced on a MinION platform using the Ligation Sequencing and Rapid Barcoding Kits, with protocol refinements improving DNA yield and sequencing performance.

In parallel, direct-colony sequencing using ONT's RBK114-24 rapid workflow was assessed. Due to cell wall differences, *S. suis* required pre-lysis with lysozyme, mutanolysin, and proteinase K, while *APP* was processed using proteinase K and enhanced buffer. Broth amplification was used where colony biomass was insufficient. Species-specific digestion and barcoding multiplexing were implemented.

Bioinformatic serotyping pipelines included Kaptive and Serovar detector for *APP*, and SsuisSero with Flye assemblies and cps locus BLAST for *S. suis*. This workflow enables rapid, accurate *in-silico* serotyping directly from colonies or extracted DNA, with applicability in outbreak response and vaccine design.

P093

Advancing the Understanding of Microbial Signature Species in Chronic Non-Communicable Diseases through Multi-Omics Approaches

Liam Walsh[1], Monica Hill[2], Aygul Dagbasi[2], Baichen Lu[2], Isabel Garcia Perez[2], Gary Frost[2], Sara Arranz Martinez[3], Itziar Tueros[3], Paul Cotter[4,5,6,7]

[1]Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland. [2]Section of Nutrition, Department of Metabolism, Digestion and Reproduction, Imperial College London, Hammersmith Campus, London, United Kingdom. [3]AZTI, Food Research, Basque Research and Technology Alliance (BRTA), Bilbao, Spain. [4]Teagasc Food Research Centre, Moorepark, Cork, Ireland. [5]School of Microbiology, Cork, Ireland. [6]APC Microbiome Ireland SFI Research Centre, University College Cork, Cork, Ireland. [7]VistaMilk SFI Research Centre, Teagasc, Moorepark, Cork, Ireland

Abstract

Non-communicable diseases (NCDs) are increasingly linked to disruptions in gut microbiome composition and function, yet systematic evaluations of microbiome health across at-risk populations remain scarce. We performed shotgun metagenomic sequencing on stool samples from 285 adults with elevated metabolic risk recruited across four European countries. Microbiome health was quantified using the Gut Microbiome Wellness Index (GMWI), a validated taxonomy-based score stratifying individuals as Healthy (GMWI ≥ 0) or Unhealthy (GMWI < 0). The majority of participants (n = 241) were classified as Healthy, with significant compositional differences observed between health strata. Longitudinal profiling revealed dynamic but reversible shifts in microbiome health, with 26 participants transitioning from Healthy to Unhealthy and 22 showing improvement over time. Global comparisons of clinical and microbial profiles via Procrustes and Mantel tests demonstrated significant concordance between host metabolic status and microbiome structure. We further curated a reference panel of reproducible microbial signatures from public meta-analyses and our cohort data, categorizing taxa as health-associated, disease-associated, or of conflicting association. Community association analyses indicated that health-associated species were more ecologically connected and prevalent than disease-associated taxa. Most high-prevalence signature species were not linked to temporal instability, suggesting robust compositional cores within the gut microbiome, whereas several low-prevalence taxa were significantly associated with instability and may act as potential drivers of microbiome state transitions

P095

How to choose the best differential abundance analysis method for your data

Calum Bridson, Rachel Connolly, Xiaowei Duan, Aaron Casey, Shane Maher, Tommy Boland, John O'Doherty, Stafford Vigors

UCD, Dublin, Ireland

Abstract

Identifying taxa that differ in abundance between conditions or treatments is a fundamental goal of microbiome analysis. It is important to choose a differential abundance analysis (DAA) method that can detect differences, but also has a low false positive rate. However, microbiome data provides several challenges, including excessive zeros and compositionality, that make DAA difficult. A confusing array of tools have arisen to attempt to deal with these issues, which makes choosing an appropriate method challenging. Here we compared 10 commonly used DAA methods across 5 real datasets, as well as across simulated datasets with different signal strengths, to determine the variability of the methods in the differential taxa found, the ability to detect differences, and the false positive rate. We found that there were large differences between the methods in the number of differentially abundant ASVs found, and the degree of overlap between methods varied considerably. In general, ANCOM-BC2 and ALDEx2 were more conservative, with a low false positive rate, but sometimes also a low power to detect differences, while DESeq2 often found the highest number of differentially abundant ASVs. However, the results were not consistent across datasets, especially when the data was more complex, such as longitudinal data or data with multiple covariates. Therefore, we provide novel insight into the factors that influence the suitability of DAA methods for different datasets, and provide recommendations for choosing DAA methods based on your data and research question.

P106

Effect of waste-derived biochar to enhance Methylomicrobium alcaliphilum 20Z growth and ectoine production

Damini ., Dr. Gustavo Sambrano, Dr. John McGinley, Dr. Alma Siggins

School of Biological and Chemical Sciences, and Ryan Institute, University of Galway, Ireland, Galway, Ireland

Abstract

The halotolerant alkaliphilic methanotroph, *Methylomicrobium alcaliphilum* 20Z, uses methane as a carbon source for growth, and can produce ectoine, a high value osmoprotectant. However, low methane solubility prevents the commercial scale-up of this process. Low-cost adsorbents like biochar were investigated to provide a solution by improving methane-microbe contact and enhancing microbial growth.

The effect of a range of concentrations of biochars on *M. alcaliphilum* 20Z growth was assessed using replicated batch assays, with a 1:1 v/v methane:air, at 30 ℃. After 48 hours, growth was quantified gravimetrically to determine cell dry weight. Bacterial growth on biochars was spatially visualised using Scanning Electron Microscopy (SEM). Following sample fractionation to separate suspended, loosely attached and tightly attached cells, total DNA and ectoine in each fraction were extracted and quantified using Promega QuantiFluor^{R} ONE dsDNA kit, and HPLC-UV, respectively.

Lower concentrations of biochar (0.25%) typically resulted in increased *M. alcaliphilum* 20Z growth compared to a no biochar control. SEM showed more bacterial cells associated with biochars at 0.25% compared to 15%. At 0.25% biochar, DNA was higher in the loosely and tightly attached fractions than in the suspended fraction, indicating the bacteria preferred to grow attached to the biochar. However, ectoine concentration was higher at 15% biochar, despite the reduced microbial growth, potentially indicating a role of stressors other than salinity in ectoine production.

This study is the first to report the potential for using pyrolysed waste as a low-cost platform for improving *M. alcaliphilum* 20Z growth and ectoine yield.

P110

Predictive modelling optimises reactive oxygen species generation by cold atmospheric plasma and enhances bactericidal activity

Conn Ritchie, Thomas Thompson, Elizabeth Hartley, Thomas Field, Brendan Gilmore

Queen's University Belfast, Belfast, United Kingdom

Abstract

INTRODUCTION: Cold plasma can destroy cancer cells and bacterial biofilms, whilst promoting beneficial inflammatory responses. Reactive oxygen species, like hydrogen peroxide (H2O2), produced by cold plasma are the main mediators of these effects. Adjusting the cold plasma parameters affects the concentration of H2O2 produced and its cellular effects.

METHODS: This study seeks to optimise the H2O2 concentration generated by a cold plasma jet and observe the impact on bactericidal activity. The Taguchi design of experiments (DoE) approach was used to investigate the effect and relative importance of four plasma jet operating parameters on the hydrogen peroxide (H2O2) concentration: the treatment time, the treatment distance, the helium gas flow rate and the frequency of the power supply.

RESULTS: The DoE analysis identified the percentage contributions from each of the parameters above to be 48.8%, 23.3%, 19.9% and 8.0% respectively. The Taguchi model was able to predict the optimum combination of parameters to maximise and minimise the H2O2 concentration and this was validated by experiment with acceptable accuracy. The plasma with maximal H2O2 was able to completely eradicate the bacteria within 5 min, whereas the plasma with minimal H2O2 caused less than 1 log reduction in colony forming units.

IMPORTANCE: This result highlights the importance of H2O2 for bactericidal activity and confirms the utility of the Taguchi DoE to characterise and optimise plasma operating parameters. Furthermore, the model could predict RONS concentrations with biologically relevant implications and could be used to optimise cold plasma treatment.

P111

Metagenomic Discovery of Novel antimicrobial peptides in oil-contaminated Nigerian soil/ sediments.

ZAINAB AHMAD BINJI

QUEEN'S UNIVERSITY BELFAST, BELFAST, United Kingdom

Abstract

Names: Zainab Ahmad Binji

Supervisory Team: Prof Brendan Gilmore, Dr Stephen Kelly, and Dr Thomas Thompson

Antimicrobial resistance (AMR) poses a global health threat, driving an urgent search for novel antimicrobial agents. This study explores a hydrocarbon-contaminated environments in Nigeria as a potential reservoir of antimicrobial peptides (AMPs), leveraging microbial adaptations to oil pollution to uncover novel compounds for combating AMR. Hydrocarbon contamination has significantly altered microbial community compositions, enhancing production of bioactive compounds including AMPs and secondary metabolite. AMPs typically under 100 amino acids exhibit broad spectrum antibacterial activity, attracting research interest. Fifteen soil/sediment samples from five oil contaminated sites under went both culture dependent and metagenomic methods. Culture dependent methods identify 56 isolates, with 14 demonstrating antimicrobial activity against ESKAPE pathogens. Metagenomic methods involving DNA extractions and Illumina sequencing, revealed microbial community of *Proteobacteria* and *Actinobacteria*. Contigs were assembled from Metagenomic data and open reading frames (ORFs) were predicted, yielding 73 AMPs via AMPA tool and 95 via Macrel software. Further filtering based on physicochemical properties from the Antimicrobial Database 3 (APD3), and ≤ 22 amino acids, refined them to 12(AMPA) and 28(Macrel) AMP candidates for synthesis. Additionally, 38 Metagenomic-Assembled Genomes (MAGs) were generated, with 10,543 (AMPA) and 111,060 (Macrel) AMPs streamlined to 358 and 3, respectively for synthesis. These findings confirm that hydro-carbon contaminated sites in Nigeria are sources of unique AMPs for novel antibiotic development to combat AMR. Future research will focus on synthesizing and validating these peptides invitro to access their therapeutic potential.

The Microbiology Society is a membership charity for scientists interested in microbes, their effects and their practical uses. It has a worldwide membership based in universities, industry, hospitals, research institutes, schools, and other organisations. Our members have a unique depth and breadth of knowledge about the discipline. The Society's role is to help unlock and harness the potential of that knowledge.

Microbiology Society @microbiosoc @microbiosoc @MicrobiologySocietyOrg1945 Microbiology Society MS Microbiology

We are now on Bluesky @microbiologysociety.org

Microbiology Society 14–16 Meredith Street, London EC1R 0AB, UK +44 (0)20 3034 4870 microbiologysociety.org