MICROBIOLOGY SOCIETY ANNUAL MEETING IN IRELAND

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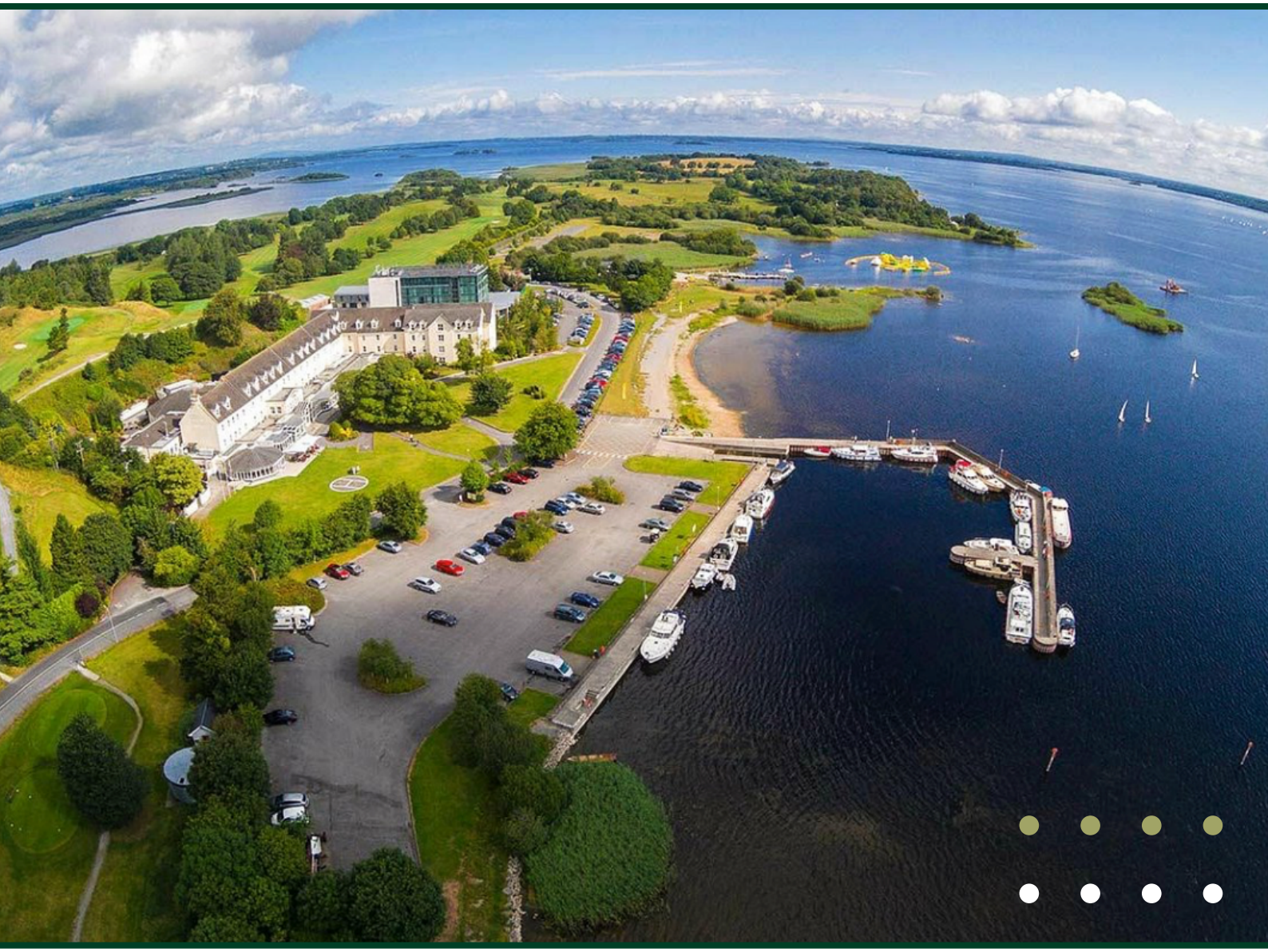
POSTER ABSTRACT BOOK



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P001

ISOLATION AND SCREENING OF ELECTROCHEMICALLY ACTIVE BACTERIA FROM SOIL DUG FROM THE SEA FLOOR OF LAGOS LAGOON, LAGOS STATE, NIGERIA

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Abstract

This study is aimed at isolating and screening electrochemically active bacteria in the soil. The soil sample was collected from sand dug from the sea floor of Lagos lagoon in a sterile polythene bag at Okobaba, Ebute-Metta, Lagos on the western piece of Lagos lagoon, Lagos state, Nigeria. Microbial Fuel Cell was constructed and electricity generated from the soil was recorded to be 213mV, 222mV and 230mV at 24, 48 and 72 hours respectively with the aid of a digital multimeter. The bacteria were isolated from the soil sample. Bacterial isolates were characterized on the basis of their cultural characteristics, colonial morphology, microscopy and biochemical profile. Molecular characterization such as DNA extraction, PCR purification and sequencing were carried out. The PCR analysis of amplified, full-length bacterial 16SrRNA gene fragments from isolates is shown in figure 1. The result obtained for amplification of 16SrRNA gene from Bacillus spp was identified at 1500bp marker. After molecular characterization, four species of Bacillus were identified namely; Bacillus cereus partial 16S rRNA gene, Bacillus pumilus XJSL5-7, Bacillus subtilis HSB-7 and Bacillus amyloliquefaciens DHA55. This study revealed that Bacillus species have the potential of electricity generation with the advantage of being eco-friendly and economical. Key Words: Microbial Fuel Cell, Electrochemically active bacteria, Bacillus spp, Molecular characterization

P002

Clinical isolate of *Listeria monocytogenes* fine tunes SigB activity to optimize fitness by using rare start codons.

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Abstract

Listeria monocytogenes is a food-borne pathogen that causes life-threatening disease when infection happens in immune-compromised individuals. We have previously isolated *L. monocytogenes* strain MQ140025 from a patient in Ireland. This strain displayed reduced SigB activity and compromised acid resistance likely due to the loss-of-function mutations in SigB regulators (RsbU Q317*, RsbX N77K). While SigB regulators mutations are shown prevalent among wild isolates and they confer selective advantages under mildly stressful conditions, it is intriguing to observe this in a clinical isolate as SigB activity is required for stomach survival and intestinal epithelial attachment. We hypothesize that the SigB activities can be lost and regained in *L. monocytogenes* to favour the survival or growth of this bacterium when selective pressures occur. To test this, cultures of strain MQ140025 were exposed to four cycles of acid inactivation and growth recovery. All three cultures rapidly restored both acid resistance and SigB activity. Whole genome sequencing revealed that the evolved strains all acquired mutations in *rsbW*, which encodes a highly conserved SigB antagonist and was considered essential in the presence of SigB. Interestingly, these mutations either resulted in premature stop codons or rare alternative start codons. Examination of >60,000 *L. monocytogenes* genomes from NCBI uncovered usage of alternative start codons in the genes involved in stress response and virulence. Taken together, the results suggests that SigB function can be lost and regained under environmental selective pressure and that rare start codons might be a means of modulating gene expression by *L. monocytogenes*.

P003

Validation and optimization of quantitative real time Polymerase Chain Reaction (qPCR) assays for reliable detection and quantification of Cystic Fibrosis pathogens

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Abstract

Background: People with Cystic Fibrosis (PWCF) are prone to chronic respiratory infections by various pathogens. Quantitative real-time Polymerase Chain Reaction (qPCR) is a powerful tool for the sensitive detection and quantification of microbial pathogens in clinical samples. This study aims to validate and optimize qPCR assays for the detection of common CF pathogens *Pseudomonas aeruginosa, Haemophilus influenzae* and *Staphylococcus aureus* in sputum samples of PWCF.

Methods: *P. aeruginosa*, *H. influenzae* and *S. aureus*-specific primers and probes targeting *opr*L, *hpd* and *lepA* genes were used. The limit of detection (LoD) for each assay was determined using known concentrations of each gene target DNA (10^8 to 2 copies/reaction). A standard curve was developed to quantify copies/reaction for each target in a sputum sample according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines. *P. aeruginosa* (n=14), *H. influenzae* (n=31) and *S. aureus* (n=20) clinical isolates were used for assay validation. A respiratory panel of different clinical isolates (n=10) was tested using the optimized qPCR assays.

Results: The LoD for *P. aeruginosa*, *H. influenzae* and *S. aureus* qPCR assays was 2, 3 and 2 copies with efficiencies of 90%, 95%, and 97%; respectively. All specific clinical isolates were detected. Each gene target in the qPCR assays demonstrated unique specificity against respiratory panel clinical isolates.

Conclusion: The optimized qPCR assays showed high sensitivity and efficiency for detecting DNA of pathogens that are common causes of infection in PWCF. Each gene target exhibited unique specificity. Performance on clinical samples will be assessed.

P004

Zoonotic Pathogen Survival in Commercial Compost Products

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Abstract

As we transition towards more sustainable agricultural systems there is an increasing interest in utilisation of organic fertilisers as a mechanism of recycling nutrients, while reducing environmental losses and enhancing soil health. As organic fertilisers typically contain zoonotic pathogens, it is crucial to understand the risks associated with their use. To this end, this study investigated the survival of zoonotic pathogens in three commercially available compost products under conditions representative of Ireland's mean winter (4°C) and summer (14°C) air temperatures.

Two microbial strain cocktails containing either five strains of *Salmonella* or five strains of *Listeria monocytogenes* were inoculated separately into farmyard manure, horse manure-based compost, and chicken manure-based compost at final concentrations of ~6 log CFU/g. The compost was subsequently maintained at the target temperatures until it no longer yielded positive results upon analysis.

Listeria monocytogenes survived longer than *Salmonella* in all compost types and temperature conditions. Survival rates decreased with increased temperature for both pathogens, with *Salmonella* reaching undetectable levels by day 20-30, while *Listeria* stabilized around 3-4 log CFU/g. We observed longer pathogen survivals in farmyard manure compared to those seen in horse manure and chicken manure.

These findings highlights the importance of considering seasonal temperature variations and product type when managing the safety of compost products. This study provides valuable insights into the temperature-dependent dynamics of pathogen survival in compost, highlighting the need for tailored strategies to mitigate contamination risk under different climatic conditions.

P005

The Effect of Devil's Claw and Moringa oleifera on Cold-contact Fermentation Performance of Metschnikowia pulcherrima in the Production of Low-alcohol Marula Fruit Beer.

Edwin Hlangwani, Bhekisisa Dlamini

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Abstract

Edible medicinal plant powders (EMPPs) have gained interest as ingredients in the production of functional beverages, especially fruit-based alcoholic beverages. However, the effect of Moringa oleifera and Devil's Claw on the fermentation performance of M. pulcherrima during the production of low-alcohol marula fruit beer has not been studied. Thus, this study evaluated the effect of Devil's Claw plant root powder and Moringa oleifera plant leaf powder on the fermentation performance of M. pulcherrima during the production of low-alcohol marula fruit beer under cold-contact fermentation. Changes in cell viability, specific gravity (SG), carbon and nitrogen utilisation, pH, acidity, alcohol, and colour were monitored. In addition, the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity were determined. The addition of EMPPs did not affect SG reduction over time but improved overall sugar utilisation, with Moringa oleifera -treated marula fruit beer showing significantly ($p \leq 0.05$) higher sugar utilisation (up to 94.27 %) after 312 h. In contrast, significantly higher FAN and YAN utilisation (up to 71.47 %) were observed in Devil's Claw-treated marula fruit beer at the end of fermentation. The addition of EMPPs resulted in acceptable alcohol concentrations (1.50 %) for low-alcohol fruit beers. Furthermore, EMPPs increased the TFC but resulted in a lower final TFC than the control at the end of fermentation. Overall, EMPPs-treated marula fruit beers showed higher antioxidant activity than the control at the end of the fermentation. However, the consumer acceptability and storage stability of EMPPs-treated marula fruit beers must still be investigated.

P006

The Impact of Devil's Claw Plant Root Powder and Moringa oleifera Plant Leaf Powder on Spoilage Microbes and Sensory Characteristics of Low-Alcohol Marula Fruit Beer

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Abstract

The South African liquor industry is dominated by established global beer brands, leaving limited research focus on local artisanal alcoholic products such as marula fruit beer. Today, marula fruit beer is solely produced for sale in informal markets. The commercialisation of marula fruit beer has been limited by controlled access to marula fruit, processing constraints, and poor post-production quality of the product such as short shelf life. This study investigated the potential of Devil's Claw plant root powder (DC) and Moringa oleifera plant leaf powder (MO) to extend the shelf-life and improve the sensory characteristics of low-alcohol marula fruit beer. The marula fruit juice was treated with each plant powder and fermented using Metschnikowia pulcherrima for 312 h at 8 °C. All samples were flash pasteurised and stored at 4 °C for 21 days. Tryptone soy agar supplemented with 1% glucose was used to determine aerobic spore-forming bacteria, de Man, Rogosa, and Sharpe agar was used to determine total lactic acid bacteria and potato dextrose agar was used to determine total yeast and mould. The 9-point hedonic scale was used to evaluate the sensory quality of the final product. Microbial analysis showed that both plant powders exhibited antimicrobial properties, preventing spoilage during 21 days of storage. However, the MO imparted an unpleasant medicinal flavour, while DC received the highest flavour score of 4.73. The control received the highest score for appearance and mouthfeel. Overall, DC showed promise as a natural preservative for marula fruit beer, potentially improving commercialisation prospects.

P007

Growth Inhibition of the Aetiological Agent of Tenacibaculosis: Multi- 'omics' Discovery of a Putative Novel Antibacterial Secondary Metabolite from a *Streptomyces* sp. Strain Isolated From a Deep-Sea Sponge

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Abstract

Bacterial diseases of fish in aquaculture systems cause significant production losses with an estimated annual economic cost of $6 billion. Overuse of antibiotics in fish farms, as a disease mitigation strategy, results in environmental damage and leads to increased incidences of antimicrobial resistance. *Tenacibaculum maritimum* is a Gram-negative member of the family Flavobacteriaceae and causes an ulcerative disease termed tenacibaculosis in many fish species, particularly in Salmon. As part of a wider screening effort, using an OSMAC approach, extracts of fermentation broth supernatants from a library of marine sponge-associated *Streptomyces* spp. isolates were screened against a panel of known fish pathogens. Extracts from *Streptomyces* sp. B188M101, isolated from the deep-sea sponge *Lissodendoryx diversichela*, sampled from a depth of 1,300m in the Atlantic Ocean inhibited the growth of *T. maritimum* in well- and disc-diffusion assays. Bioactivity was observed from extracts of media M19, M400, SGG and R358. The chemical dereplication of the bioactive fractions from medium R358 fermentations identified unique chlorinated small molecules that were putatively responsible for the observed bioactivity. Genome mining of *Streptomyces sp.* B188M101 allowed for the identification of a 24Kb non-ribosomal peptide synthetase gene cluster suspected to encode the bioactive metabolite. Bioactivity-guided isolation of the specific, putatively novel, chlorinated metabolite responsible for inhibiting the growth of the pathogen is ongoing. *Streptomyces sp.* B188M101 has the potential to be developed as a probiotic in fish farms to mitigate tenacibaculosis-associated losses and reduce the overuse of antibiotics in those systems.

P008

Targeting *Listeria monocytogenes* virulence with Chitin: A Promising Molecule for Mitigation of Infection

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Abstract

Background: Dietary chitin is known to modulate the gut microbiota, influence the immune response, and to attenuate virulence gene expression in *Listeria monocytogenes in vitro.* However, despite these diverse effects, the role of chitin in combating this pathogen remains largely unexplored. Here, we explored the polymer in the context of *L. monocytogenes* infection using both *in vitro* and *in vivo* model systems.

Results: Chitin significantly diminished virulence gene expression in *L. monocytogenes*, impacting adherence to and invasion of epithelial cell lines. Moreover, chitin exhibited immunomodulatory effects in macrophages, triggering IL-6 and TNF-α responses. In an *in vivo* murine infection model, pre-exposure of *Listeria* strains to chitin, coupled with administration of chitin as a dietary supplement, resulted in a notable reduction in bacterial load within organs post-infection. Furthermore, chitin supplementation enhanced the proliferation of gut bacterial taxa that have previously been associated with improved barrier function. Chitin was therefore seen to play a dual role: directly influencing *Listeria* pathogenicity and promoting host defense mechanisms.

Conclusions: Our studies suggest that chitin is a promising candidate for dietary inhibition of *Listeria* infection. Work is continuing in order to identify the signaling mechanisms by which chitin downregulates virulence gene expression in the pathogen.

P009

Detection and Analysis of Hidden Bacteriophages in Food and Animal Systems

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Abstract

Bacteriophages, viruses that infect bacteria, are a persistent threat to food fermentations, causing fermentation inconsistencies or failures. They also modulate bacterial populations in complex communities, including human and animal gastrointestinal tracts. Recent discoveries have highlighted that bacteriophages can exist in an undetected state within bacterial cultures used in manufacturing processes, known as the pseudolysogeny or carrier state life cycle.

This project employs DNA sequencing and synthetic biology approaches to address this challenge. Initial efforts involved isolating bacteriophages from whey samples associated with dairy fermentation failures. Subsequent steps include viromic analysis of these whey samples using shotgun metagenomics, and studying the community diversity of undefined thermophilic starter cultures used in cheese production that are associated with fermentation failures.

Preliminary work has isolated potential bacteriophages from whey samples. The next phase of the project aims to utilize shotgun metagenomics to identify and characterize viral components. This approach will provide insights into the diversity and behaviour of bacteriophages within these microbial communities.

By advancing our knowledge of bacteriophage behaviour and interactions within microbial communities, this research aims to improve detection methods and develop strategies to mitigate the impact of bacteriophages on food fermentations, ultimately enhancing the stability and efficiency of these processes.

P010

Phenotypic and Genotypic Characterisation of Water Kefir Isolates and Genome-Scale Modelling for Consortia Prototype Development.

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Abstract

Water Kefir (WK) is a traditional fermented beverage and has been widely consumed across the globe for millennia. While various health-claims have been linked to its consumption, there is limited scientific evidence relating to the positive impact of WK and its associated microbiome on human health. The overall aim of this project is to develop a novel, healthy, and sustainable plant-based kefir beverage. Genotypic and phenotypic characterization of water kefir-derived microbes are crucial for understanding their potential health benefits, optimizing fermentation processes, developing probiotic and postbiotic products, and ultimately contributing to the development of a novel plant-based kefir beverage. In this study, thirty bacterial isolates and four yeast isolates were identified by Sanger sequencing and phenotypically characterized using various cultivation-based assays. For genotypic characterization, DNA from the isolates was sequenced using Oxford Nanopore long-read and Illumina short-read technologies, followed by hybrid genome assembly. The genomes were screened for genes related to antimicrobial resistance, bacteriocin production, bile salt hydrolase (BSH) activity, and other genes relevant to probiotic features. Although traditional substrates of WK are figs and sucrose water, this study tested fermentation with waste streams as alternative substrates. The metagenomic DNA of the fermentate was extracted and sequenced using Illumina NovaSeq technology. Metagenomics data and annotated genomes of isolates were utilized for genome-scale modelling and consortia development.

P011

Comparative Analysis of Microbial Diversity and Composition in Flooded versus Non-flooded soils in Ireland, Germany and Portugal.

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Abstract

It is a well-known fact that climate change represents one of the most significant threats to global health. As a result of climate change, increased flooding events have been recorded across the globe. Soil flooding may have detrimental effects on soil fertility due to a variety of reasons, yet there is limited research conducted in this area. The main focus of this research is to identify changes in the soil microbiome due to flooding and determine how it disrupts the balance of soil microorganisms, those of which are vital for nutrient cycling and soil fertility. In this study, 16S rRNA and ITS amplicon sequencing are used to identify the effects of flooding on bacterial and fungal communities in Irish, Portuguese and German agricultural soils. Amplicon sequencing, followed by in-depth bioinformatic analysis revealed that there were significant differences between communities in flooded and non-flooded German and Portuguese soils. There were no significant impacts of flooding on the Irish soils. However, specific soil bacteria and fungi known to have plant-growth promoting abilities and/or are involved in soil biochemical processes are shown to be decreased in flooded soil in all three countries. Some of these include *Serratia* (*plymuthica* and *proteamaculans*) in Ireland, *Bacillus amyloliquefacien* and *Pseudomonas putida* in Germany. Genera of soil fungi that aid in soil fertility that are shown to be decreased in flooded soil include *Chaetomium*, *Sebacina* and *Metarhizium*. Proteobacteria and Ascomycota were the dominant bacterial and fungal phyla across all countries and conditions.

P012

Host-Induced Signalling and Warfare in a Polymicrobial Community

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Abstract

The Post-Antibiotic Era describes the rise in antimicrobial resistance (AMR) and the lack of novel antibiotic discovery. Alternative methods are required for effective pathogen control. Small molecules have gained considerable traction in selective pathogen targeting and can be viewed as an anti-infective strategy. The chemical languages evolved in dominant pathogenic organisms could offer a novel strategy for suppression of key virulence phenotypes and competitiveness in co-colonising fungal and bacterial pathogens. Alternatively, efflux inhibition and cross-regulation from anti-viral frameworks are emerging as promising alternatives to growth limiting control.

One major challenge to the clinical development of anti-virulence interventions is that microbes exist in diverse polymicrobial communities that undergo significant genotypic and phenotypic diversification. Co-colonising microbes present in the cystic fibrosis (CF) lung, encompassing bacterial (*Pseudomonas aeruginosa*) and fungal (*Candida albicans*, *C. dubliniensis* and *Aspergillus fumigatus*) lab and clinical strains were studied in response to host/microbial signals including *N*-acetyl-glucosamine (NAG), bile, and bile salts. Fungal and bacterial pathogens were found to alter their virulence through biofilm formation, pigmentation, and toxin production in response to specific host signals, adopting distinct morphological and pigmentation profiles when co-cultured in the presence of competing organisms. Emergence of distinct pigment-secretion and pigment-retention profiles indicated a context-dependent shift in pathogen behaviour.

Selective control of these mixed consortia and their distinctive pigmentation patterns was explored using a range of small molecule frameworks. While some retained activity similar to the individual species studies, others were diminished in their anti-*P. aeruginosa* potency when other ESKAPE or fungal pathogens were present.

P013

Diversifying receptors meet chemical flexibility in *Pseudomonas aeruginosa* virulence, signalling, and host interaction systems

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Abstract

Transcriptional regulators present a tuneable response system for antimicrobial resistance, nutrient availability, metabolic reprogramming, quorum sensing, cell-cell communication, and biotransformation. Acting as receivers and transducers for distinct chemical languages, members of the LysR- and LuxR-families of transcriptional regulators play key roles in the pathophysiology of infection. However, aside from a subset of well characterised members, many of the proteins in these families remain uncharacterised. Less still is understood about the chemical and structural flexibility that underpins their evolutionary trajectory at the strain and species level.

Comparative analysis of *P. aeruginosa* available genomes revealed a wide distribution of LysR-type transcriptional regulators across the species, with core LTTRs present in >90 % of the genomes and accessory LTTRs present in <2 %. Strikingly, AmpR and PqsR/MvfR were found to be amongst the most variable in the dataset. Variant complementation of the PAO1 *pqsR*- mutant suggests a degree of structural promiscuity within the high variance LTTRs. Surprisingly, a large >600 kb region devoid of LTTR encoding genes was identified, with GO analysis revealing a distinct reduction in frequency of transcriptional control systems therein. A similar trend of promiscuous diversification was also seen following comparative genomics analysis of the LuxR-type transcriptional regulator family. Coumarins and Photopyrones, the latter recently identified as a new chemical language operating through the LuxR system, were found to have anti-biofilm and negative growth impacts on *P. aeruginosa* and other ESKAPEE pathogens. Together, these findings suggest that chemical flexibility matches the diversification signatures identified in these keystone regulator systems.

P014

Nucleoside Adjuvants Manipulate MRSA Physiology and Metabolism

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Abstract

The emergence of bacterial infections with significantly reduced susceptibility to antibiotic therapy is rising at an alarming rate leading to a major burden on global healthcare systems and economies. In the case of methicillin resistant *Staphylococcus aureus* (MRSA) widespread resistance to first-choice beta-lactam antibiotics increases the likelihood of antibiotic treatment failure. Moreover MRSA are also becoming resistant to last line antibiotics like vancomycin. highlighting the requirement to maintain the effectiveness of current antibiotics, The sparsity of new antibiotic in the discovery pipeline highlights the importance of maintaining the effectiveness of current antibiotics as part of efforts to tackle the problem of antimicrobial resistance (AMR).

In previous work, we have shown the potential of nucleotide/nucleoside adjuvants that target essential metabolic pathways in MRSA as adjuvants that enhance the activity of beta-lactam antibiotics. To further improve our understanding of nucleotide/nucleoside adjuvants we conducted proteomic, metabolomic and microscopy experiments to identify metabolic and physiological changes occurring in response to adjuvant exposure. New mechanistic insight was gained with visible changes in peptidoglycan thickness identified using transmission electron microscopy which were accompanied by changes in abundances of enzymes responsible for peptidoglycan synthesis. Data generated from these experiments guided the identification of novel antibiotic/adjuvant combinations that had anti-biofilm potential, anti-virulence properties and exhibited synergy with beta-lactam antibiotics. Kill curve, checkboard and biofilm eradication assays revealed the antibacterial potential of the new synergistic combinations and their potential as anti-MRSA therapeutics.

P015

A systems approach to unlocking student potential and the hidden curriculum

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Abstract

Experiential and Immersive learning approaches have significant potential to transform the student experience when engaging with challenging molecular and cellular concepts in life science education. Experiential learning approaches can instil deeper learning of how molecular biology can be applied to address real-life societal challenges. Group learning, applied performances of understanding, cross-disciplinary international collaborations, and offering multiple modes of expression are just some of the ways we strive to connect the hidden curriculum. In tandem with this, immersive virtual reality and 360 degree simulations offer innovative entry points for student learning where spatial understanding can unlock a deeper engagement with molecular concepts.

We sought to take a systems approach to higher education, building inclusive teaching and assessment modalities for each stage of the student journey, viewing each as a piece in a jigsaw, the realisation of which may arrive at different stages in the journey for each student. While there have been many wonderful initiatives undertaken to support the student journey, these have typically either been designed as stage-specific interventions or as inclusive-driven alterations to conventional teaching practices. To achieve a truly inclusive learning experience, access to and performance of knowledge and understanding should be universal by design. Here we present a roadmap for biological sciences as students transition in, through, and out of higher education.

P016

Knowing thine enemy is key to addressing the AMR-ChemoResistance crisis

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Abstract

The importance of microbial communities for ecosystem health across a wide spectrum of domains has received considerable attention in recent decades, not least due to the emergence of next generation sequencing technologies and the thematic area of microbiome analysis. However, why and how 'bad actors' emerge from these diverse communities to cause dysbiosis and infection remains to be established. Understanding this would provide new opportunities for anti-infective development and would help address the challenges faced by the continued onset of antimicrobial resistance (AMR).

The linguistic nuances that exist across geographical and cultural boundaries can significantly impact on how concepts and occurrences are understood and approached. Slight modifications in structure of sentences, coupled with differences in vocal or visual presentation can elicit a different response from the intended targets. Such a phenomenon is also true at a microbial level, where structural modification of signals coupled with the presence of external cues can provide a distinct context to bacterial and fungal behaviour not often accounted for in experimental design. This is of particular importance and consequence with respect to AMR. New anti-infective and anti-virulence strategies based on existing chemical languages evolved in dominant organisms have the potential to target key community-coordinated behaviours. Key targets include biofilm formation, secretion, and toxin production, all of which can be reached without causing dysbiosis of the microbial community. Unlocking the full potential of this approach requires us to understand better the context in which these communities yield diversity to the emergence of dominant species.

P017

Accessing novel biocatalytic solutions through (meta)genomic exploration of the marine ecosystem

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Abstract

The advent of metagenomic technologies, coupled with greater access to genomic sequences and predictive tools, has enabled the research and development community to harness bioactive potential from previously untapped sources. A particularly intriguing example of this is the Porifera phylum (marine sponges), which has delivered novel anti-infectives, anti-cancer, and anti-inflammatory therapeutics as well as biocatalysts with exciting transformation potential. Indeed, the marine sponge ecosystem has been shown to sustain a rich microbial biodiversity, the biocatalytic potential of which is largely unexplored when compared with its terrestrial counterpart. Understanding the ecological role of biotransformation within the polymicrobial communities that sustain the marine sponge ecosystem is key to advancing biocatalytic mining of this and other resources.

Using both metagenomic and genomic (*in silico*) mining tools we have uncovered a suite of biocatalytic activities with industrially relevant properties. These include marine ω-transaminase, lipase/esterase, amylase, nitrilase, and protease activities isolated from *Axinella dissimilis* microbial communities. Remote stereospecificity, rapid biotransformation, and the ability to accept challenging substrates with bulky R groups have been characteristic of the marine enzymes, which have proven to be suitable for heterologous expression and are stable under reaction conditions. Both transaminase and lipase candidates have also proven amenable to scaled-up production in 4 L bioreactors. Substrate profiling and molecular modelling of the lead biocatalysts has provided insights into the molecular structures that underpin these novel activities and current work is focused on genetic engineering towards further enhancement of their biocatalytic properties.

P019

Exploring the Roles of Phosphofructokinase-1 (PFK-1) and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK2-FBP2) in *Cryptococcus neoformans*

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Abstract

Cryptococcus neoformans is an opportunistic fungal pathogen responsible for more than 190,000 deaths annually. *C. neoformans* affects immunocompromised individuals, especially those with AIDS in Sub-Saharan Africa. Primary infection occurs via inhalation into the lungs, and as the infection progresses, *C. neoformans* can cause a life-threatening infection in the brain called cryptococcal meningitis. The current treatments for cryptococcal meningitis are expensive and not always effective, so finding targets for the development of new antifungals is key. Previously, researchers have deemed a functional glycolytic pathway in *C. neoformans* essential for virulence, making enzymes in this pathway possible drug targets. Two enzymes from the glycolytic pathway were examined. Phosphofructokinase (Pfk1) converts fructose-6-phosphate to fructose-1,6-bisphosphate in the third step of glycolysis. 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Pfk2-Fbp2) generates and breaks down fructose-2,6-bisphosphate, a positive regulator of Pfk1 in glycolysis. Mutant strains of ΔPfk1 (CNAG_04676) and ΔPfk2-Fbp2 (CNAG_04221) were obtained from the Madhani Lab at UCSF. Both showcased reduced growth on carbon sources such as glucose and acetate, reduced melanin production, and reduced capsule induction. Melanin production and capsule induction are important virulence factors in *C. neoformans*, suggesting Pfk1 and Pfk-Fbp2 play a role in pathogenesis. The study's results indicate the roles of ΔPfk1 and ΔPfk2-Fbp2 in *C. neoformans* merit further exploration.

P020

Developing a Barnacle Cement Protein as an Adhesive Coating for Biomedical Applications.

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Abstract

BACKGROUND: In wet environments, certain marine organisms are capable of adhering to various natural and man-made materials. Corning® Cell-Tak™ extracted from the marine mussel *M. edulis* is a commercially available adhesive used in tissue culture, but this is both environmentally toxic and expensive. We examine whether the adhesive coating of barnacle cement protein cp19k from *P. pollicipes*, expressed in *E. coli* possesses strong adhesive properties, making it suitable for coating surfaces *in vitro* and/or *in vivo* and a sustainable avenue for various biomedical applications.

METHODOLOGY: cp19k protein expression was optimised in *E. coli BL21DE3* and purification carried out using Metal affinity chromatography and Ion exchange chromatography. Surface adhesion experiments with purified cp19k, with and without hexa-histidine tag, after different incubation periods, were completed on tissue culture polystyrene plate surfaces in different conditions (pH and NaCl concentration) to determine optimal adhesion conditions. Cell viability on cp19k-his coating was performed with MTS assay using suspension cell lines (THP-1 Monocytes).

RESULTS: Optimization of cp19k protein expression and purification procedures resulted a pure and 2.5-3 mg/L of culture yield. Surface adhesion experiments revealed strong adhesion of cp19k protein with hexa-histidine tag under both high and low pH and low salt concentration after 48 hours of incubation. Cell viability assay showed viable THP-1 cells on cp19k-his coating even after 48 hours.

CONCLUSION: The adhesiveness of the cp19k with hexa-histidine tag provides effective surface coating for cell adhesion, suggesting its potential for in vitro biotechnology applications.

P021

INVESTIGATING THE EFFICACY OF INTEGRATED CONSTRUCTED WETLANDS TO REDUCE ANTIMICROBIAL RESISTANT ORGANISMS IN WASTEWATER IN DIFFERENT SECTORS

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Abstract

Antimicrobial resistance (AMR) is acknowledged as one of the greatest challenges to human health globally, with estimates that, by 2050, AMR will cause 10 million deaths a year, unless action is taken. Efforts to tackle AMR have commonly focused on clinical and veterinary practice but recently the role of the environment in transmitting antimicrobial resistant organisms (AROs) has gained greater attention. Conventional wastewater treatment is often considered a hotspot for AMR and release of AROs into the natural environment. However, less is known about the role alternative treatment options such as constructed wetlands may play in the dynamics of AROs transmission in the environment. This project specifically focuses on integrated constructed wetlands (ICWs), a concept that integrates water management, landscape fit and biodiversity. ICW systems are shallow, free surface-water wetlands, which are densely vegetated with appropriate plant species to treat through-flowing waters. The objective of this study was to examine various microbial parameters in influents and effluents in ICWs serving different sectors, including agricultural and food processing sectors. A monthly monitoring program was carried out to isolate specific target bacteria, including extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, fluoroquinolone-resistant Enterobacterales, carbapenemase-producing Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and enumerate enterococci, and *Escherichia coli* as indicators of faecal pollution. MALDI-TOF was used for bacterial identification and confirmation. . *Escherichia coli* was the predominant species isolated in all sample types To date, the study indicates that ICWs have the potential to effectively reduce AROs present in wastewater from different settings.

P022

Characterisation of aggregate formation in isolates of *Enterococcus faecium*

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Abstract

Background Bacteria can exist in one of 3 phenotypic states. They can exist planktonically, in a free-floating state, in a sessile biofilm community, or in a non-surface attached aggregative state. This aggregation represents an advantageous phenotype as, similarly to traditional biofilms, they confer an elevated tolerance to antimicrobials and other stresses, while remaining mobile to be moved to new sources of nutrition.

Methods Two strains of *Enterococcus faecium* were isolated, where one strain demonstrated an aggregating phenotype while the other did not. A comparison of their antimicrobial susceptibility was made, challenging the strains against four antibiotics – trimethoprim, nitrofurantoin, vancomycin and gentamicin – as well as against compounds known to inhibit aggregation.

Results The aggregative strain demonstrated a much lower tolerance to antimicrobials compared to the non-aggregative strain, which showed wide-spread resistance to the antibiotics tested: when challenged with vancomycin, strain 1-1 had an MIC of 1 mg/mL, while strain 1-2 had an MIC of 512 mg/mL. The addition of compounds known to disrupt aggregation, proteinase K and simple sugars, were used to assess if disaggregation of strain 1-1 would result in a change in the sensitivity to antimicrobials.

Conclusion Aggregation is a protein-mediated interaction which can easily be reversed. By not using a high enough concentration of proteinase K, the bacteria were found to aggregate more due to the stress imposed. While one strain of E. faecium was unable to aggregate, this strain proved more resistant in the planktonic form than the aggregating strain.

P023

Elucidating the potential of a novel pyrimidine nucleotide permease as an antimicrobial drug target in device-associated isolates of *Staphylococcus epidermidis*

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Abstract

Staphylococcus epidermidis is an opportunistic pathogen associated with a range of chronic healthcare-associated infections often involving biofilm. As part of efforts to understand the regulation of biofilm in clinical isolates of *S. epidermidis*, we identified a cerebrospinal fluid isolate (CSF41498) from Beaumont Hospital Dublin that exhibited a growth defect in chemically defined media with glucose (CDMG). Suppressor mutants of CSF41498 that exhibited normal patterns of growth in CDMG were isolated and whole genome sequencing analysis identified SNPs or frameshift mutations in a nucleotide permease in all mutants examined. Here the role of this permease was examined under differing growth conditions, as well as its role in resistance to nucleotide analogues such as 5-fluorouracil (5FU) and 6-thioguanine (6TG). Bioinformatic analysis was performed to predict the substrate of this permease, and homologues were identified in *Staphylococcus aureus*. Using Blastp, Clustal Omega, Alphafold, Protter, I-TASSER, and CB-Dock2, we predict that the permease, AXE41790, is one of the uracil transporters in *S. epidermidis*, that is active during growth in CDMG. Competition experiments were performed using uracil vs 5FU and guanine vs 6TG, to identify substrate specificity and the affinity of this permease for nucleotide transport. These experiments provide novel insights on a potential new drug target in *S. epidermidis* and the relationship between nucleotide metabolism and nucleotide analogue drug resistance.

P024

Evaluating the microbial quality of roof-harvested rainwater as an alternative and sustainable water source for fresh produce irrigation

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Abstract

Irrigation water is increasingly being relied upon within horticultural production, to ensure product yield and quality. Seasonal droughts and alterations in weather patterns caused by climate change have become a serious threat to water availability; hence, assessing the potential of alternative water sources such as rainwater harvesting has gained considerable prominence in recent years. Despite the advantages of harvested rainwater, it is at risk of microbial contamination arising from a range of sources. Contaminated irrigation water is widely recognised as an important source of contamination for fresh produce and therefore it is imperative to ensure that safe irrigation water sources are identified.

The objective of this study was to investigate the microbial quality of roof-harvested rainwater from different roof surfaces sampled from selected rural and urban sites on the east coast of Ireland. Over a six-week period for two sampling periods (August – September 2023) and (March – April 2024), 84 rainwater samples, collected from 7 different sites were assessed for the presence of indicator bacteria: total viable counts (TVC), *Escherichia coli* and enterococci using standard culture based methods. DNA were extracted from rainwater samples and shotgun sequencing was performed.

Results indicated that 100%, 90% and 90% of the rainwater samples were positive for TVC, *E. coli* and enterococci respectively for the first sampling period. The concentration for the tested organisms in the second sampling period was 1 – 2 log less compared to the first sampling season. Variations in the concentrations of these bacteria across the sampling weeks and seasons were observed for all sites.. Metagenomic based analysis of the harvested rainwater samples provided more in-depth information on the dominant microbial communities from each roof type and sampling site; information which can be utilised in the assessment of treatment options.

The detection of bacteria in the harvested rainwater supports the need for effective treatment before irrigation use as it may pose a risk of crop contamination if used untreated.

P025

Potential of silage microbial inoculants to mitigate methane production from the rumen. A systematic review.

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Abstract

Background: Methane originating from enteric fermentation in ruminants is the single largest source of anthropogenic agricultural emissions and has a significant impact on global methane levels. Enteric methane mitigation strategies are being investigated to address its detrimental effects on climate change and ruminant production characteristics.

Objective: This systematic review aimed to investigate whether microbial silage inoculants could reduce methane formation from the rumen microbiome both *ex vivo* and *in vivo*, based on available literature.

Methods: Two independent reviewers conducted a comprehensive search on Google Scholar for peer-reviewed articles, with no year restrictions, up to January 31, 2024. The search focused on experiments reporting methane gas production both *in vitro* and *in vivo*.

Results: A total of 434 articles were found, but only 10 met the quality criteria and were selected. Of these, nine studies measured methane production using *in vitro* assays, while one study reported an *in vivo* trial. Ten bacterial species were used as inoculants across the selected studies. *Lactobacillus buchneri* achieved the highest methane reduction at 83.17%, being the only study that combined a microbial inoculant with additives. *Lactobacillus plantarum* also showed a significant reduction in methane output, achieving a 48.11% decrease.

Conclusions: The use of silage microbial inoculants is a promising approach for reducing methane emissions in livestock, with 80% of the studies reviewed showing a reduction in methane production by ruminant microorganisms. However, further research is needed to validate these findings, given the limited number of published studies in this area.

P026

A S_{180}F mutation in D-alanine aminotransferase increases resistance to β-chloro-D-alanine in *Staphylococcus aureus*

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Abstract

D-alanine is a critical amino acid essential for synthesis of the bacterial cell wall. Key enzymes responsible for the synthesis of D-alanine include alanine racemase (Alr), which converts L-alanine to D-alanine and D-alanine aminotransferase (Dat), which converts pyruvate to D-alanine in a reaction dependent on D-glutamate. We previously reported that impaired alanine transport heightens the susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) to the β-lactam antibiotic oxacillin and D-cycloserine (DCS), an alanine analogue drug that inhibits Alr. To further investigate the therapeutic potential of targeting D-alanine biosynthesis, we used directed evolution to generate a mutant resistant to β-chloro-D-alanine (BCDA), another alanine analogue drug reported to target Alr and Dat. Genome sequencing revealed a single point mutation in *dat*, resulting in a predicted S_{180}F substitution in the BCDA-resistant strain. Expression of the *pepV-dat*$_{S180F}$ operon significantly increased BCDA resistance in wild-type MRSA, whereas wild-type *pepV-dat* had no effect. Enzyme assays revealed that the Dat$_{S180F}$ allele was non-functional. Consistent with this, a *dat*$_{S180F}$*/alr1* double mutant was auxotrophic for D-alanine further indicating that the Dat$_{S180F}$ allele cannot catalyse the conversion of pyruvate to D-alanine. Protein structure modeling followed by superimposition analysis suggests that the S_{180}F substitution alters the conformation of the Dat protein. Bioinformatic docking analysis revealed a higher affinity of BCDA for Dat$_{S180F}$. Our working hypothesis is that the higher affinity of Dat$_{S180F}$ for BCDA protects Alr from inhibition by this antibiotic suggesting that combinations of BCDA and DCS to ensure inhibition of both Alr and Dat may have significant therapeutic potential against MRSA.

P027

Detection and characterisation of antimicrobial resistant Enterobacterales from dairy production environment in low and high zinc containing regions

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Abstract

Antimicrobial resistance (AMR) is a critical public health concern. Limited information is available on the dissemination of AMR in the primary food production environment, where the presence of heavy metals may play a role in influence the antimicrobial resistance gene profile present. In Ireland, soils are very well mapped in relation to the levels of heavy metals they contain, including zinc. The objective of this study was to evaluating the presence of AMR Enterobacterales in dairy pasture soil and bovine milk filters on farms in high and low zinc areas across Ireland.

Fifty soil samples and 29 milk filters were collected from two distinct regions across Ireland, with varying zinc concentrations. Enterobacterales were enumerated and the presence of ESBL- producing Enterobacterales, carbapenem-resistant Enterobacterales, and ciprofloxacin-resistant Enterobacterales were assessed on selective agars. Species were identified by Maldi-TOF, and phenotypic and genotypic profiles were determined by disk diffusion testing and whole genome sequencing, respectively. Additionally, chemical analysis was conducted on soil samples.

Forty AMR Enterobacterales were isolated from both sample types. These isolates had a range of antimicrobial resistance patterns, and ten *Escherichia coli* isolates from high zinc-containing region were resistant to all antimicrobial classes tested. The soil chemical analysis confirmed a significant difference in zinc concentration between the two areas investigated.

This study identified a range of phenotypic resistance profiles among the resistant Enterobacterales isolates identified in low and high zinc containing regions across Ireland. Moreover, this study demonstrated the importance of using milk filters to assess AMR in dairy production.

P028

Metagenomic analysis of microbial composition and AMR in anthropogenically impacted recreational waters

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Abstract

Background: Antimicrobial resistance (AMR) is a major public health issue. Recreational waters, particularly those impacted by untreated wastewater discharges, may represent a reservoir for AMR transmission to humans. This study aimed to utilise shotgun metagenomic sequencing to evaluate the effects of anthropogenic contamination on these environments.

Methodology: Water samples (n=12) were collected across 4 dates between March-April 2021, from a coastal location receiving untreated wastewater on the West coast of Ireland. The sampling points included: 1) seawater at the wastewater discharge point (DP) 2) seawater west of the DP, and 3) an adjacent freshwater stream. Samples were filtered and DNA extracted for shotgun metagenomic sequencing (150Gb/sample, PE150). Modular bioinformatic pipelines were used to evaluate microbial diversity, composition, and AMR profiles.

Results: Overall, genomic signatures for over 1,200 bacterial genera and 6,000 bacterial species were identified. Freshwater and seawater microbiomes were primarily composed of bacteria native to soil, plant roots and algae, while human enteric bacteria, including opportunistic pathogens, dominated the DP. The DP was identified as a source of pathogens and antimicrobial resistant organisms into the environment as all ESKAPE pathogens were detected in significantly higher abundance at the DP and in the adjacent communal seawater. Additionally, genomic signatures of pathogens known to harbour the carbapenemase-encoding gene (*bla*$_{\text{NDM-1}}$) were detected at the DP.

Conclusions: The results offer valuable insights into how untreated wastewater discharges can disrupt microbial communities in recreational waters and potentially pose a risk to human health, while also supporting the use of culture-independent sequencing for environmental surveillance.

P029

Towards One-Health Water Surveillance for Tracking and Understanding Influenza A Virus Threats

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Abstract

Influenza A virus (IAV) is a global threat to the health of humans, animals, and their shared environment, causing seasonal epidemics and recurrent pandemics. The pandemic threat of IAV has increased recently with the continuous spread of a novel variant of highly pathogenic avian IAV H5N1 between wild and domestic mammals across the Americas, including several human infections. To prevent and control IAV infections, 'One-Health' surveillance strategies to monitor and understand its circulation and evolution in animal, human and environmental reservoirs are necessary.

Recent studies in our lab and beyond highlight the potential for wastewater-based epidemiology (WBE) to detect and sequence genomes of IAV from human and wild and livestock animal sources. However, the applicability and integration of these novel methods to other environmental samples, such as natural water sites, and existing clinical data, remains poorly understood. Thus, this study aims to modify WBE methods to improve IAV recovery rates and genomic data quality in a water-sample agnostic manner, to track IAV levels, diversity, and risk, across time and space.

Preliminary results indicate strategies to strengthen the sensitivity and robustness of IAV environmental characterisation through targeting differential phase partitioning of viral RNA IAV; enhancing the stability of viral RNA; and exploiting improved molecular approaches. We will present the potential application of this to monitoring of wild bird habits in Ireland. Altogether, this study suggests the usefulness of environmental genomic surveillance in preventing and controlling IAV epidemic and pandemic threats.

P030

Unravelling Species-Specific Differences in Mucosal Antiviral Immune Signalling in Protection from Emerging Respiratory Viral Infections

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Abstract

Emerging and endemic respiratory viruses, such as influenza A viruses (IAV), coronaviruses, and paramyxoviruses, are major threats to humans and animals in the absence of effective broad-spectrum clinical interventions. The outcomes of infection vary from asymptomatic to fatal both within and between host populations and species. Understanding the mechanism behind such divergent consequences could help mitigate the burden of endemic and emerging diseases.

By inducing the expression of antiviral genes, type III interferon (IFN) 'lambdas/λ', play a critical role in fighting infections in barrier epithelial tissues like the lung targeted by respiratory viruses. *In silico* screening revealed the diversity of IFNλs, with mammals having two distinct lineages: IFNλA (IFNλ4-like) and a diverse IFNλB group (IFNλ-3-like). However, the phenotypic consequences of this diversity are poorly described but could shed light on infection outcomes and facilitate development of interventions. Therefore, our work focuses on *in vitro* investigations comparing diverse IFNλs in antiviral assays.

In initial work, the antiviral activity of IFNλs from three placental mammals (humans, pigs and bats) were compared in a range of cell lines and virus infection models, including IAV in lung epithelial cells. These results revealed that on top of genetic diversity, there is also a considerable functional diversity in their abundance and potency. Future work will focus on understanding the molecular, genetic and cellular determinants of species-specific differences, which could be exploited for the development of the next-generation of broad-spectrum antiviral interventions for humans, livestock and wildlife.

P031

Carbohydrate-Based Coatings for Slow Release of Calcium Peroxide in Cow Rumen Fluid

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Abstract

The oxidising agent calcium peroxide (CaO_2) is a promising feed additive for reducing methane emissions in cattle. Through its oxidising action in the rumen, the production of methane by methanogens is inhibited. However, its effectiveness in pasture-based grazing systems depends on its controlled release in the rumen. This study aims to identify suitable coating materials for CaO_2 to ensure stability and controlled release, with a focus on carbohydrate-based coatings.

The release profile and effectiveness of various coating materials, including sodium alginate and cellulose derivatives were evaluated. CaO_2 was coated in each material, and these additives were introduced to sealed bottles with rumen fluid which simulated the rumen environment. Methane inhibition was tested by measuring the oxidative reduction potential (ORP), with methane production occurring between -200 and -300 mV.

The results showed a sustained increase in ORP values for bottles containing the coated CaO_2 compared to the uncoated control, indicating enhanced oxidative conditions which correlate with methane inhibition. Among the coatings tested, sodium alginate, ethyl cellulose, and carboxymethyl cellulose demonstrated the best performance compared to the uncoated CaO_2. These coatings resulted in an elevated ORP for a longer time compared to that of the uncoated control.

These findings suggest that carbohydrate-based coatings are promising materials for encapsulating CaO_2 to ensure the controlled release of CaO_2 in the rumen as well as sustained inhibition of methane production by methanogens. Further research will focus on optimising these coatings for commercial applications, contributing to sustainable livestock nutrition and Ireland's sustainability goals.

P032

HUMID: Honing Our Understanding of Microbial Diversity in Tropical Peatlands

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Abstract

The overarching goal of the HUMID Project is to increase our scientific understanding of contemporary microbial diversity across different plant ecotones within two peatlands from the South Pacific region and identify the key environmental (abiotic and biotic) controls that drive changes in microbial community structure. The project aims to achieve this through testate amoebae analysis using traditional microscopy methods to create a novel inference-based model to reconstruct past environmental changes in these peatlands. Using NMDS (Nonmetric multidimensional scaling) we aim to form potential links between microbial diversity and dominant abiotic variables such as vegetation, depth to water-table and pH. We aim to identify the prokaryotic diversity and functional traits of these two sites using molecular tools, multi-dimensional sequencing, and community profiling. Our results so far have shown a significant difference in testate amoebae diversity between the two sites, driven by dominant vegetation and depth to water-table. Regarding prokaryotic diversity, DNA has been successfully isolated from both sites and molecular techniques have indicated distinct prokaryotic diversity present in both sites. Research in microbial diversity in tropical peatlands is still in its infancy and more work is needed to understand the microbial significance of these ecosystems.

P033

Developing a protein secretion toolbox for the yeast *Kluyveromyces marxianus* based on α-galactosidase activity

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Abstract

In the context of sustainable biotechnology there is great interest in the industrial production of heterologous proteins. Secreted proteins are preferred since their recovery during downstream processes is easier. In many microorganisms, including yeasts, secretion is mediated by short amino acid sequences that precede a protein, namely secretion tags. Different molecular biology tools allow the heterologous expression of different proteins in yeasts. In particular Molecular Cloning (MoClo) and the Yeast Tool Kit (YTK) have experienced a dramatic increase in their use and expansions to the YTK are being developed, especially for *Saccharomyces cerevisiae*. However, this yeast presents limitations for protein production. In contrast, the non-conventional yeast *Kluyveromyces marxianus* is a valuable candidate as microbial platform for industrial applications due to its unique features: thermotolerance, substrate versatility, GRAS (Generally Regarded as Safe) and QPS (Qualified Presumption of Safety) status, and being phylogenetically similar to *S. cerevisiae.* In this research, the YTK was expanded with *K. marxianus*' secretion tags and implemented in this yeast. Due to the little information about these tags, a detection system that could help in rating their secretion efficiency would prove to be useful. To achieve this, the *S. cerevisiae MEL1*, encoding an extracellular α-galactosidase, was used. A set of new strains of *K. marxianus* expressing *MEL1* fused to these secretion tags were constructed and their secretion efficiency was evaluated.

P034

Synergistic Enhancement of Antibiotic Efficacy Against Staphylococcus aureus Biofilms Using Cold Plasma

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Abstract

The rise of antibiotic-resistant infections necessitates novel therapeutic approaches. Non-thermal cold atmospheric plasma presents a promising adjunctive therapy against biofilm-associated infections, such as those caused by methicillin-resistant Staphylococcus aureus (MRSA). This study evaluates the synergistic effects of the J-Plasma Bovie device combined with antibiotics to enhance antimicrobial efficacy against biofilms.

Staphylococcus aureus biofilms were cultivated and subjected to sub-lethal plasma treatments prior to antibiotic application. The effects were quantified using minimum inhibitory concentrations (MICs), minimum biofilm eradication concentrations (MBECs), and rapid evaporative ionization mass spectrometry (REIMS). Transcriptomic analyses were conducted to examine oxidative impacts on bacterial cell structures, gene expression changes, and stress responses.

Results indicate that cold plasma pre-treatment significantly enhances the efficacy of antibiotics like tetracycline, reducing MICs and MBECs notably. Enhanced disruption of metabolic activity suggests that combined plasma and antibiotic therapy elicits a distinct biofilm response compared to individual treatments. Transcriptomic data corroborates that plasma exposure induces an oxidative stress response, potentially compromising membrane integrity and facilitating increased drug uptake.

This study supports integrating cold plasma into treatment protocols, potentially transforming the management of antibiotic-resistant infections. Understanding device-specific plasma interactions is crucial for optimizing this intervention against resilient biofilm-related infections.

P035

Unraveling the Pseudo-Auxotrophy for Isoleucine in *Listeria monocytogenes* through *In Vitro* Evolution Experiments

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Abstract

The General Stress Response (GSR) in *Listeria monocytogenes* enhances its ability to overcome stress conditions. This robust stress response mechanism, which protects *Listeria* against commonly used food preservation techniques like refrigeration and acidification, is regulated by an alternative sigma factor called Sigma B (SigB). Previous work in our lab has shown that the SigB regulated sRNA *Rli47* might contribute to bacterial adaptation to harsh environments by restricting the growth of *L. monocytogenes* through a block on isoleucine biosynthesis (Marinho et al., 2019). Other factors also contribute to the pseudo-autotrophy for isoleucine that *L. monocytogenes* exhibits, despite encoding all the genes needed for isoleucine biosynthesis. To fully understand the underlying mechanism of this counterintuitive phenotype, we set up an in vitro evolution experiment in a chemically defined medium without isoleucine to isolate suppressors with elevated growth advantages.

First, we reconfirmed that *sigB* and *codY* deletion mutants don't display the isoleucine pseudo-auxotrophy seen in the wild-type EGD-e. Based on high-throughput screening and growth measurement of the mutants, we have observed at least four distinct colony morphologies associated with various suppressors of the isoleucine pseudo-auxotrophy. We then investigated whether mutations that inactivated the *sigB* operon contributed to their growth advantages through Congo red staining and acid survival experiments. These results allowed us to systematically classify the suppressor mutants. The whole-genome-sequencing data show that the pseudo-auxotrophy for isoleucine can be overcome by suppressor mutations that arise when cells are deprived of isoleucine. Both SigB and CodY contribute to the regulation of this phenotype.

P036

Biomining Atlantic Salmon Skin Microbiome in Search of Eco-friendly Bioprotective Strains

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Abstract

The identification of novel antimicrobial compounds has become a priority with the emergence of multiresistant microbial strains. Bioprospecting is the exploration of natural sources in search of new bioactive compounds and this approach can provide solutions to the problem. In the context of MARBLES, an EU project involving 14 partners across Europe, we have speculated that commensal microorganisms isolated from marine ecosystems may have a key role in their hosts' defence against pathogens. Therefore, we have focused on bioprospecting the skin of healthy Atlantic salmon (Salmon salar) as a potential source of novel antimicrobial compounds that might be useful in sustainable aquaculture. Swabs taken from the skin of 3 healthy salmon in Donegal, Ireland, were cultured on LB and Marine Agar incubated at various temperatures (4°C, 28°C, room temperature), and resulting microbial isolates were identified by 16S rDNA sequencing. In total, we identified 302 culturable bacterial isolates belonging to 38 separate genera from 4 different phyla (Actinomycetota, Bacillota, Bacteroidota, Pseudomonadota). Deferred antagonism assays were carried out against a panel of 8 commerciallyrelevant Gram-negative fish pathogens. While no antimicrobial activity was detected using this specific approach, in order to unravel the encoded potential for novel bioactive molecule production associated with bacteria from this environmental niche, 18 bacterial isolates were further selected for genome sequencing and mining. The true potential of the healthy fish microbiota to provide a sustainable alternative to the high levels of antibiotics currently used in aquaculture has not yet been fully assessed, with further studies ongoing.

P038

Identification of Foodborne Pathogens in Mastitic Milk from Irish Dairy Farms

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Abstract

Background: Mastitic milk could pose a significant source of bacterial foodborne contamination in dairy chain. *Staphylococus aureus* and *Esherichia coli* are known to cause mastitis and dairy-borne foodborne illness. Some studies highlighted the presence of other foodborne pathogens such as *Listeria monocytogenes* and *Bacillus cereus* in raw milk, indicating potential contamination risks. This study aims to identify the presence of foodborne pathogens in mastitic milk from Irish dairy farms.

Methods: 146 quarter samples from 49 Irish dairy farms were collected and tested for pathogen identification following the standard method. 10 µL of milk was plated on blood agar, assessed based on morphology and haemolytic activity. Samples exhibiting ≤2 distinct colony types, each presenting >5 colonies, were tested on matrix of 10 agars for identification of presumptive foodborne pathogens.

Results: Among the samples tested, 86 (52.5%) were identified having one pathogen causing infection, while 7 samples (4.2%) had two pathogens. *Escherichia coli* was the most prevalent pathogen at 26.0%, followed by *Staphylococcus aureus* at 17.8%. *Bacillus cereus* was present at 0.7%, while *Listeria monocytogenes* and *Cronobacter sakazakii* were absent.

Conclusion: While standard mastitis detection methods effectively identify primary mastitis pathogens, it may fail to capture a broader spectrum of foodborne pathogens. Additional pathogen, *Bacillus cereus,* was detected in this study, which highlights the uncertainty in prevalence of rare mastitis-related foodborne pathogens. Therefore, there is a need for comprehensive testing and diagnostic techniques to ensure a systematic risk assessment of mastitic raw milk in the dairy supply chain.

P039

Exploring the proteomic effect of secreted fungal natural products on *Klebsiella pneumoniae*

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Abstract

Antimicrobial resistance (AMR) in pathogenic bacteria has been well established as denoted by the World Health Organisation classifying five bacterial strains and one family as the highest priority for research into AMR in bacteria and novel means to bypass AMR. *Klebsiella pneumoniae* has been observed to not only develop resistance to multiple classes of antibiotics but also form novel phenotypes associated with hypervirulence (hvKp). In this study, we assessed the effects of two fungal natural products, triacetylfusarinine C (TAFC) and gliotoxin (GT), produced by the human pulmonary pathogen *Aspergillus fumigatus*, on the intracellular proteome of *K. pneumoniae*. Comparative quantitative proteomic analysis revealed that both TAFC and GT have differential effects on the proteome of *K. pneumoniae*, with changes observed in metal homeostasis pathways, cellular function such as protein synthesis and electron transfer pathways. Exploitation of these two fungal metabolites which have metal sequestering properties may provide new insights into alternative approaches for antibiotic targeting.

P040

Beefing Up Safety: Breaking Down Bacterial Barriers to Reusable Meat Packaging

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Abstract

By promoting sustainability through legislation, the EU is encouraging various industries to adopt sustainable packaging solutions to reduce single-use plastic waste, such as reusable packaging. However, there is a lack of research around the impact of reuse on microbial attachment to plastic packaging. Increasing the knowledge around microbial interactions with reusable plastic packaging (RPP) can mitigate the risks of foodborne illness and foster innovation in reusable systems geared towards long shelf-life applications.

This study investigated the effect of reuse on different plastics to determine if surface weathering impacted microbial adhesion. To do this, 5 plastics were repeatedly exposed to *E. coli* or *B. cereus* which were allowed to form biofilms on the surface before washing to HACCP standards. This process was repeated for 15 cycles. To validate the sanitisation of the washing process, sampling was done before and after washing to quantify bacterial viability. Total inhibition of bacterial attachment was observed after one cycle for all plastic types tested. The cause of this inhibition was not readily obvious so further investigations were taken to determine what intrinsic change in the plastics effected the attachment behaviour of the bacteria. To determine if this change was chemical or physical, a number of avenues were explored to investigate changes in surface texture, wettability, and accumulation or migration of chemicals.

The ultimate aim of this study was to address a gap in the research regarding the microbial safety of RPP and guide the design and development of a novel RPP for fresh beef.

P041

Screening and characterization of poly-ɣ-glutamic acid (ɣ-PGA) producing Bacillus species for application in lignocellulosic biomass valorisation

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Abstract

Poly-ɣ-glutamic acid (ɣ-PGA) is a versatile yet expensive biopolymer that is produced via fermentation by several species of bacteria belonging to the *Bacillus* genus. The identification of new and efficient ɣ-PGA producers, the development of bioprocesses for optimal yield and the use of low cost starting materials such as lignocellulosic biomass have been suggested as some of the strategies towards reducing the cost of ɣ-PGA production. In this study, eight *Bacillus* strains from the species *subtilis* and *licheniformis* from the Teagasc DPC Culture collection were screened for their ɣ-PGA producing ability by cultivating the strains on a ɣ-PGA production medium. All eight strains produced an ultra-high molecular weight (MW > 500 kDa) ɣ-PGA product, with yields ranging from 1 – 18 g/L. *Bacillus licheniformis* DPC6338 had the highest PGA yield (18 g/L). Molecular characterisation via whole genome sequencing and the optimisation of ɣ-PGA fermentation conditions including temperature (25 – 50 °C), pH (5 – 9), inoculum concentration (1 – 10%), fermentation time (24 -120 hrs), stirring speed (150 – 450 rpm) are underway. The effect of fermentation conditions on the microbial growth as well as the yield and characteristics of the ɣ-PGA produced is also being assessed. *Bacillus licheniformis* DPC6338 will be used to ferment pretreated rye grass hydrolysates to ascertain its applicability for biorefinery scenarios.

P042

Baseline assessment of bacterial spores in skim milk powder production: spore population during processing stages of skim milk powder over a lactating season in Ireland

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Abstract

Spore-forming bacteria, resilient to harsh conditions can survive thermal treatments in dairy processing. Moreover they can cause spoilage and foodborne illnesses. This study aims to monitor spore-forming and vegetative bacteria in Skim Milk Powder (SMP) produced by an Irish dairy facility to assess the variation in lactating season.

Samples were aseptically collected and tested using standard enumeration spore-forming bacterial and vegetative microbial analysis for four months in the high lactation season (HLS) and in the low lactation season (LLS). From the microbial tests, 951 selected spore-forming bacterial plate colonies were purified and isolated. Isolates were tested for growth potential at 7, 15, 37, and 55°C.

Bacterial counts in raw milk ranged from 2-6 logs, reduced by 1-2 logs after heat treatment, and final counts of 2-3 logs in SMP. Thermophilic counts remained at 2-3 logs, sometimes exceeding 4 logs post-treatment. Thermoduric counts in raw milk were higher during LLS, decreasing by 1-2 logs after heating. B. cereus counts (0-3 logs) originated from the raw milk silo. Mesophilic spore counts decreased by 1 log during HLS but not LLS, with a 1-log increase in SMP. Thermophilic spore counts increased during processing. LLS thermophilic isolates grew at 37°C and 55°C, while HLS isolates preferred 37°C

Standard vegetative and spore methods assess final product safety, but seasonal in-process sampling showed variations in bacterial and spore counts. Isolate testing shows that the temperature range in which the spore formers grew varied more in the low lactating season for each test.

P043

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

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Abstract

Background: The enzyme *bla*$_{\text{CTX-M-15}}$, an extended spectrum beta lactamase, has potent hydrolytic activity against the third generation cephalosporin cefotaxime. By using Mass Spectrometry, the proteomic response of a *bla*$_{\text{CTX-M-15}}$ producing strain of *Escherichia coli* to cefotaxime can be studied, comparing to a susceptible strain of the same species.

Methods: A cefotaxime resistant and *bla*$_{\text{CTX-M-15}}$ producing strain *E. coli* DSM 22664, and a cefotaxime susceptible strain *E. coli* MG1655, were grown in x2 their MIC of cefotaxime for 2 hours. The proteins were extracted and digested with trypsin, before being analysed on a Q-Exactive Mass Spectrometer.

Results: Proteins increased and decreased in *E. coli* DSM 22664, compared to *E. coli* MG1655 were analysed using Max Quant and Perseus Software. Proteins increased in the resistant strain included ribosomal assembly proteins such as 50S ribosomal protein L16, and 30S ribosomal protein S10 as well as cell wall synthesis proteins such as D-alanyl-D-alanine endopeptidase and penicillin-binding protein 1B. Proteins increased in the susceptible strain, and thus decreased in the resistant strain, included shock and stress proteins such as acid stress chaperone HdeB, and cold shock-like protein CspE,

Conclusion: By studying the proteomic response of a resistant and susceptible *E. coli* to cefotaxime, key proteins and pathways can be identified, which could be potential targets for novel antimicrobial treatments.

P044

Machine learning approaches to improve antibiotic selection for urinary tract infection in renal transplant patients

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Abstract

Urinary tract infection (UTI) poses a significant risk to kidney transplant patients, causing considerable morbidity, prolonged hospital stays and antibiotic use, and increased risk of acute graft loss. One issue in UTI management is the time required to select the most appropriate antibiotic therapy, which can often take several days. Current methods involve culture-dependent identification and antimicrobial susceptibility testing of the causative microorganism, during which time ineffective empiric therapy may be administered, resulting in suboptimal treatment and the emergence of antimicrobial resistance (AMR).

This study aimed to utilise machine learning (ML) approaches to predict antibiotic susceptibility of bacterial isolates from post-kidney transplant patients, using their genome sequence alone. Antimicrobial susceptibility predictions were obtained for over 24 isolates, cultured from ureteric stents and urinary catheters. Predictions were compared to phenotypic resistance data, determined using MIC broth dilution and paper disk diffusions tests.

ML models predicted phenotypic susceptibility to a high degree of accuracy against antibiotics commonly prescribed for UTI. ML approaches achieved high levels of consensus with phenotypic tests for amoxicillin (92%), nitrofurantoin (100%), ciprofloxacin (83%), and gentamicin (92%). ML outperformed predictions based on conventional AMR gene identification tools (ML 92% vs. RGI 65%), and revealed genes not usually associated with the AMR phenotype. These data show the potential of applying ML approaches to expedite targeted antibiotic selection. Fast and accurate predictions would enable vastly improved UTI management in kidney transplant patients, reducing morbidity, AMR emergence, and infection-mediated organ rejection.

P045

Characterisation of Human Milk Oligosaccharide utilisation by Commercial Probiotics using Genotype-Phenotype analysis

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Abstract

Objective:

Human Milk Oligosaccharides (HMOs) are complex sugars found in breastmilk with proven health benefits for the developing infant. HMOs are resistant to gastrointestinal digestion and instead supply metabolic substrates necessary for beneficial bacteria in the intestinal tract. Several members of the infant associated *Bifidobacterium* genus have been shown to utilise HMOs as their sole carbon source, however, utilisation by other early colonizers is less explored. This study aimed to genotypically and phenotypically characterise the HMO utilisation of 23 clinically documented, commercial probiotic strains, including *Bifidobacterium*, *Lactobacillus* and *Pediococcus* strains, using a blend of 8 commercially available HMOs.

Methods:

Genome profiling was used to predict HMO utilisation capabilities by identifying glyco-genes including enzymes and transporters that may be involved in probiotic HMO utilisation. Phenotypic profiling included growth analyses, preferential HMO utilisation through the use of High pH Anion Exchange Chromatography with Pulsed Amperometric Detection and metabolomics analysis using High Pressure Liquid Chromatography with Refractive Index detection.

Results:

Our findings highlighted the growth of several commercial probiotic strains on the HMO blend with preferential utilisation and strain-specific superior growth observed for four infant-associated *Bifidobacterium* species. Individual HMO consumption revealed inter-species preferential utilisation of structures as well as strain-specific variation among members of the same species. HMO utilisation varied from >95% of available HMOs by *B. bifidum* and *B. infantis* strains, to <5% for several *Lactobacillus* species and the *Pediococcus* strain.

Conclusions:

This research gives insight into the complex relationship between HMOs and infant associated bacteria in the early stage of life.

P046

"COMPARATIVE GENOMIC AND PHENOTYPIC INVESTIGATION OF CLINICALLY PROVEN PROBIOTICS TO SUPPORT A HEALTHY GUT MICROBIOME AT ALL LIFE STAGES."

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Abstract

Objective: We aimed to perform comparative genomics on 23 commercial probiotic strains of *Bifidobacterium, Lactobacillus,* and *Pediococcus,* combined with phenotypic analyses, to identify functional signatures supporting probiotic applications.

Methods: We evaluated probiotic traits including carbohydrate utilisation, bacteriocin production, exopolysaccharide (EPS) production, and bile salt hydrolase (BSH) activity. Carbohydrate utilization was assessed using BLAST KOALA, CAZy database, a diamond search and growth curve analysis. BAGEL4 was employed for in silico bacteriocin production analysis alongside agar well-diffusion assays. Target probiotic and BSH proteins were identified through diamond searches and BSH activity was phenotypically investigated using the bile acid, taurodeoxycholic acid. EPS cluster analysis was performed using cblaster and clinker as well as evaluating production phenotypically.

Results: CAZy database searches revealed 130 CAZy families across genomes with CAZy repertoires largely consistent within species. Sixteen putative bacteriocin-producing regions were identified across fourteen strains, with *B. longum* subs*p. infantis*, and *L. acidophilus*, exhibiting highest numbers of associated gene clusters. Consistent putative probiotic proteins were observed within species, while *L. paracasei* strains displayed the highest number of survival-associated proteins. All genomes, excluding *L. paracasei,* featured at least one putative BSH protein. Phenotypic analysis revealed considerable BSH activity in nine strains. EPS clusters varied notably between Bifidobacterium species, with *B. lactis* strains displaying the most consistent EPS-related gene clusters.

Conclusion: This study underscores similarities and disparities between i*n silico* predictions and phenotypic observations, emphasizing the need for experimental validation. The study highlights the potential for *in silico* identification to expedite *in vitro* screening of specific probiotic traits.

P047

The Oral Placenta Infant Microbiome Study (OPIuM):Perinatal factors affect early life microbiome establishment

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Abstract

Objective: While extensive research exists on the human microbiome, a number of outstanding questions remain regarding the infant microbiome in the initial stages of life. The aims of this study were to determine the timing of microbial colonization in humans, assess the contribution of maternal microbial sources to their offspring and examine the effect of perinatal factors on the maternal and infant microbiota in early life.

Methods: Using a cohort of 18 healthy mother-infant dyads, maternal saliva (within 24h postpartum), vaginal (1h prepartum) and placental (1h postpartum) samples were collected. From their corresponding infants, saliva (within 24h postpartum) and meconium (within 96h postpartum) samples were collected. 16S rRNA amplicon sequencing was utilized to assess the taxonomic and inferred functional compositions of the bacterial communities from both mothers and infants.

Results: Our results consolidate and corroborate recent findings surrounding the existence of a meconium microbiome and the absence of a placental microbiome. We show that significant vertical transfer, primarily from the maternal oral cavity to the infant oral cavity occurs in early life. Moreover, we were able to provide further evidence on how perinatal factors influence the microbial bond between mother and infant and the establishment of the infant microbiome.

Conclusions: This study provides information on the relationship between health and delivery factors and establishment of the infant microbiota. These findings could offer valuable guidance to clinicians and mothers in optimizing the infant microbiota towards health during infancy and later life.

P048

Surveillance and detection of SARS-CoV-2 in four national University campuses , the UniCoV Project - Apps, Antigens, Saliva, Wastewater and participation

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Abstract

Detection of SARS-CoV-2 infection during the COVID-19 pandemic was key to disease prevention. Here we present the UniCoV project, a multi-university initiative, aimed to evaluate screening and surveillance of SARS-CoV-2 on University campuses using various detection platforms. Self-reported surveillance and symptom checking was performed using a secure bespoke phone App . Participants using the App could log symptoms, perform self-antigen tests, log results ,barcode scan self-sampled saliva tubes and receive a health status within minutes and Saliva RT-PCR results within hours. Campus wastewater surveillance was used as a sentinel of the entire campus community SARS-CoV-2.

4,726 volunteers were recruited across the four university sites from September 2021 to May 2022. Over 700 positive cases of SARS-CoV-2 were detected correlating with national rates; including the Irish infection surge of Dec 2021 – Jan 2022. While Saliva RT- PCR was 25% more sensitive for detection, all individuals testing by RT-PCR were positive by Antigen testes within 24 hours. Artificial Intelligence (AI) scoring of longitudinal Antigen test band intensity correlated with RT-PCR detection levels revealing an average infection timeline of 10 days, with a notable increase in shedding on day 5-7. Wastewater detection on campus sites allowed detection from non-study participants and correlated with study positive RT-PCR levels. Genomic sequencing revealing changes of the dominant SARS-CoV-2 variant to Omicron over the 2021-2022 period.

On campus surveillance utilising App based solutions, self-testing, laboratory confirmation , and wastewater surveillance has proven to be a highly effective method of disease surveillance with potential for future common and seasonal infections.

P049

Plasma activated water pre-treatment substantially enhances phage activity against Proteus mirabilis biofilms.

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Abstract

The integration of cold plasma technology and bacteriophage therapy is a formidable strategy against the biofilm-forming *Proteus mirabilis,* especially pertinent in urinary tract infections (UTIs) linked with long-term urinary catheter use. Our study investigates the synergistic effects of bacteriophages and plasma activated water (PAW) in targeting the biofilms of *P. mirabilis*.

Generated through a specialised non-thermal or cold plasma discharge setup, PAW is composed of an array of reactive oxygen and nitrogen species (ROS/RNS), well described for their antimicrobial capabilities. Additionally, bacteriophages have gained a recent resurgence, with their bacterial strain specificity, present as viable bio-control agents. Our study explored the interaction between bacteriophages and ROS/RNS on biofilm eradication and assessed the impact of different discharge setups on the antimicrobial efficacy of PAW. The stability of phage vB_PmiS_PM-CJR in PAW, alongside the susceptibility of both planktonic and biofilm cultures to PAW, was assessed, offering critical insights for enhanced antimicrobial strategies.

The sequential application of PAW followed by phage significantly reduced biofilm biomass and bacterial load; the reverse order (phage followed by PAW) did not show better antibacterial effects compared to using PAW or phage alone. We hypothesise that PAW can disrupt biofilm structures, thus enhancing phage penetration and bactericidal action. The synergy unveiled between cold plasma and bacteriophages offers a broader view for tackling biofilm-associated infections in clinical settings and is a promising pathway towards not only managing UTIs associated with *P. mirabilis* but also mitigating the broader antibiotic resistance quandary.

P050

Phenotypic changes and virulence in Campylobacter jejuni following fluoroquinolone adaptation

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Abstract

Campylobacter jejuni is a zoonotic pathogen and a leading cause of bacterial gastroenteritis. The majority of cases are linked to consumption of contaminated poultry, and high rates of resistance to fluoroquinolone antibiotics is a persistent problem despite reductions in the usage of fluoroquinolones in agriculture. Resistance is often conferred through point mutations in the *gyrA* gene, which are known to play a role in DNA supercoiling. Previous studies have shown that fluoroquinolone resistance is associated with increased virulence, and changes in phenotypes linked to environmental persistence, including biofilm formation and aerotolerance. In this study, laboratory and freshly isolated *C. jejuni* strains were adapted to fluoroquinolones through passaging on increasing concentrations of ciprofloxacin. A panel of isolates with low (up to 10µg/mL), medium (up to 40µg/mL), and high levels (>40µg/mL) of ciprofloxacin resistance was obtained for each strain. PCR confirmed the T86I mutation in the *gyrA* gene of resistant isolates, commonly associated with fluoroquinolone resistance. Fluorescence microscopy showed increases in biofilm formation by resistant isolates under both aerobic and microaerobic conditions. Motility testing revealed strain-dependent differences between susceptible and resistant isolates, and some resistant isolates exhibited increased catalase activity and aerotolerance. *Galleria mellonella* larvae were used as an infection model to evaluate changes in virulence between susceptible and resistant isolates. These results build on previously observed connections between fluoroquinolone resistance and clinically relevant phenotypes in *C. jejuni.*

P051

Composition of Campylobacter Biofilms Can be Altered in the Presence of Different Stressors

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Abstract

Campylobacter jejuni and *Campylobacter coli* are commensal intestinal bacteria of chickens and the leading cause of bacterial gastroenteritis in humans worldwide. During the preparation of chicken products, *Campylobacter* species are exposed to compounds designed to prevent bacterial spread. It is hypothesized that *Campylobacter* forms biofilms on the surface of chicken products for protection from these stressors. However, some compounds, like salt, can increase biofilm formation in other foodborne pathogens. The aim of this study was to determine how different stressors impact biofilm formation of *Campylobacter* and if the stressors change the composition of the biofilms. Four strains were assessed for their ability to form biofilms in the presence of sodium chloride and hydrogen peroxide. Immunofluorescent staining of biofilms demonstrated that salt decreased biofilm formation in high biofilm forming strains and increased biofilm in low biofilm forming strains. Hydrogen peroxide caused a clumping biofilm morphology to form. Mature biofilms were treated with sodium metaperiodate, DNAseI, and proteinase k to establish the constituents of biofilms formed in the presence of stressors. Immunofluorescent staining revealed that sodium metaperiodate treatment did not result in disruption of biofilm. Proteinase k treatment caused more disruption in biofilms induced by salt compared to biofilms formed without salt, suggesting these biofilms are more proteinaceous in nature. In conclusion, sodium chloride and hydrogen peroxide promote changes in *Campylobacter* biofilm formation.

P052

Exploring the Efficacy of Lipids as Antimicrobial and Antibiofilm Agents against Streptococcus mutans

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Abstract

Biofilms play a crucial role in the development of oral diseases, particularly dental caries and are known to exhibit increased resistant to conventional treatments.

Screening of long chain fatty acids (FAs) was conducted to assess their antimicrobial activity against *Streptococcus mutans* ATCC 25175. The effects of FAs on *S. mutans* growth was determined at concentrations of 250, 100, 50, 25 and 10 µg/ml. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) were determined, and resazurin assay was carried out. Synergistic activity of FAs was evaluated, and Minimum Biofilm Inhibition Concentration (MBIC) was investigated using crystal violet staining and effects of artificial saliva on FAs was assessed. Assays measuring lactate dehydrogenase (LDH) and cell proliferation (XTT) were employed to investigate cell toxicity and proliferation.

Results indicated that unsaturated long chain FAs such as Oleic (C18:1), Linoleic (C18:2), y-Linoleic (C18:3), Eicosapentaenoic acid (EPA, C20:5), reduced bacterial growth at 10 µg/ml. Docosahexaenoic acid (DHA, C22:6) demonstrated potent antimicrobial effects, inhibiting bacteria growth, reducing metabolic activity, preventing biofilm formation, and displayed a log reduction value of 6 at 10 µg/ml. Combining most effective FAs at this concentration resulted in bacteriostatic effects and enhanced log reduction. Notably, EPA and DHA antimicrobial activity was reduced to 25 µg/ml in the presence of artificial saliva. At 10 µg/ml y-Linoleic, EPA and DHA displayed less cytotoxic effects than chlorohexidine.

This highlights the potential of unsaturated long chain FAs, particularly Omega 3; DHA, against *S. mutans* biofilms. Synergistic effects observed suggests a promising alternative for reducing cytotoxicity while enhancing antimicrobial efficacy. These finding demonstrate the potential of FAs as novel antibiofilm agents in combating *S. mutans* related oral diseases.

P055

The evaluation of the protective efficacy of novel verotoxin-producing E.coli (VTEC) antigens as potential vaccine candidates

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Abstract

Verotoxin-producing E. coli (VTEC) are a group of zoonotic foodborne pathogenic *E. coli* strains that are associated with causing bloody diarrhoea and Haemolytic Uraemic Syndrome (HUS) in more severe cases where young children under the age of 5 are particularly susceptible. Currently, the VTEC notification rate in Ireland is amongst the highest in Europe (19.0 cases per 100,000). While antibiotic treatment is contradicted due to many patients experiencing severe symptoms and complications, there is an unmet need for a vaccine to reduce mortality and reduce the greatest risk of kidney failure in children. As VTEC colonises gastrointestinal epithelial cells, bacterial proteins involved in host-cell attachment represent promising vaccine candidates. Using a proteomic approach, we identified 14 proteins involved in the attachment of VTEC to two independent gastrointestinal cell lines, HT29 or Caco-2. To date, one, GlnH, has shown potential as a vaccine candidate, reducing bacterial colonisation in the colon and caecum by 1.25-log CFU.

Three additional proteins, antigens A, P, G were selected for investigation as potential candidates. These were successfully cloned, expressed, and purified prior to immunisation in mice with the T-cell inducing, SAS adjuvant. All antigens were highly immunogenic and stimulated a mixed Th1/Th2 response in immunised mice. Antigen A showed the greatest level of protection and reduced bacterial colonisation in the colon and caecum by 1.3-log relative to vehicle only control ($p=0.0252$). It is evident that future VTEC vaccine developments should consider the combined use of adjuvants with antigens to provide greater protection against such an infection.

P056

Prolonged exposure to hypoxia promotes adaptations in Pseudomonas aeruginosa which may drive chronic infection.

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Abstract

Pseudomonas aeruginosa is an opportunistic bacterial pathogen which causes chronic lung infections in people with cystic fibrosis (CF) and is a major contributor to morbidity and mortality. Despite this, the mechanism driving its adaptation towards chronic colonisation in the CF lung is not fully understood. Hypoxia is one of the important environmental pressures present in the CF lung. This work focuses on the adaptations of *P. aeruginosa* to long-term hypoxia to investigate whether it drives the development of persistence in CF patients.

We examined how an early CF isolate adapted to 6% oxygen over 28 days. Two distinct small colony variants (SCVs) emerged, one exclusively under low-oxygen conditions. Importantly, SCVs were more prevalent in hypoxia-adapted cultures, comprising up to 98% of the population, while never exceeding 35% in normoxia-adapted cultures. Proteomic analysis revealed significant changes in the abundance of >200 proteins within 28 days, including those involved in antibiotic resistance, stress response and biofilm formation. Two hypoxia-adapted cultures developed higher resistance to eight out of 13 antibiotics tested and showed increased biofilm (4.08-fold and 1.80-fold ($pV<0.0001$)) and exopolysaccharide production. The third population displayed resistance to only two antibiotics and showed decreased biofilm-forming capability. All hypoxia-adapted cultures developed higher resistance to osmotic stress, while two hypoxia-adapted populations also showed increased resistance to oxidative stress but decreased resistance to high temperature.

These findings confirm that exposure to hypoxia alone promotes the development of phenotypes in *P. aeruginosa* associated with persistence in the lung and poor patient outcomes.

P057

Novel vaccine candidates targeting antibiotic-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

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Abstract

Acinetobacter baumannii and *Pseudomonas aeruginosa* are highly antibiotic-resistant pathogens that are major causes of hospital-acquired infections. They are classified by the World Health Organization (WHO) as “critical” and “high” priority pathogens, respectively, requiring immediate action to develop novel treatments. Vaccines are promising tools for combating these pathogens; however, no approved vaccines for these pathogens are currently available.

We have identified several novel proteins involved in the host cell attachment of *P. aeruginosa* and *A. baumannii* to lung epithelial cells, some of which are homologous in both pathogens. Therefore, this project aims to compare the protective efficacy and cross-reactivity of two homologous antigens (AgN) against *A. baumannii* and *P. aeruginosa*.

Cellular immune responses, such as Th1 and Th17 responses, may contribute to protection against these pathogens. T-cell epitope prediction showed that the *A. baumannii* AgN possesses seven predicted CD4+ T-cell epitopes, while *P. aeruginosa* AgN possesses nine, suggesting the potential of these antigens to induce cellular immune responses.

Immunisation of mice with purified and endotoxin-free *A. baumannii* AgN adjuvanted with Sigma Adjuvant System (SAS) and subsequently challenged with *A. baumannii* resulted in significant reductions in bacterial lung bioburden (0.74-$\log_{10}$ reduction, $p<0.0102$) and dissemination to the spleen (0.9-$\log_{10}$, $p<0.0426$). Moreover, high total IgG and IgG1 antibody responses were observed (end-point titres of 1: 62,500 and 1:78,125, respectively). Similarly, mice immunised with SAS-adjuvanted *P. aeruginosa* AgN and subsequently challenged with *P. aeruginosa* showed a significant reduction in lung bioburden (0.96-$\log_{10}$ reduction, $p=0.0268$) and robust total IgG, IgG1, and IgG2a antibody responses (endpoint titres of 1: 312,500, 1: 1,562,500, and 1: 12,500, respectively)

ELISpot analysis revealed that both *A. baumannii* AgN and *P. aeruginosa* AgN elicited substantial IFN-γ- and IL-17 responses, indicating strong T-cell stimulation.

Overall, these findings highlight the potential of these novel antigens as promising vaccine candidates for the development of a multivalent vaccine to combat the antibiotic-resistant *P. aeruginosa* and *A. baumannii*, contributing to the urgent need for effective vaccines against antibiotic-resistant infections.

Funded by the HEA North-South Research programme.

P058

Metagenome analysis of soils in Ireland: a comparative study across diverse grasslands with cultivated and uncultivated zones

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Abstract

This study is a part of the Northern Ireland's Department of Agriculture, Environment and Rural Affairs (DAERA) Soil Nutrient Health Scheme project. We initiated a detailed analysis of microbial composition focusing on the soil samples from four soil types within Northern Ireland agricultural soils. Our methods include a range of genomic initiative to gauge the presence of soil microbiome (metagenomics) inclusive of pathogens that will help understand how grassland management (e.g., nutrient fertilization, liming) might affect soil Carbon storage, N-, P-cycling overarching the generic soil health. Our preliminary data indicates that the relationship between the microbial community in soil and anthropogenic activities such as the application of fertilizers and other agricultural methods yielded a better understanding to improved agronomy practice and/or positive environmental impacts. Shotgun metagenomic profiling used in this cultivated and uncultivated cohort of grassland soil sets are an ideal tool to facilitate the analysis of complex soil matrix inhabiting microbial communities. Our group have previously used similar molecular and bioinformatics approaches to explore the soil horizon stratigraphy effects on microbial communities found in Alpine paleosols. Both phylogenetic and functional gene profiles obtained for example may allow us to identify limiting elements in communities that can facilitate carbon capture, nitrogen fixation and/or phosphorous sequestration. Our data offer a novel insight that compliments other soil health analytical approaches.

P059

Wood-derived xylooligosaccharides as a targeted next-generation prebiotic for human and bovine-associated gut commensals

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Abstract

In Ireland, 11% of the total land area is covered by forest, and around 8.2 million tonnes of wood waste are produced annually in Ireland from construction, demolition, and deforestation. This secondary raw material can provide a sustainable source of wood-derived xylan and xylooligosaccharides (XOS) which are proposed as next-generation prebiotics for human health and animal feed supplements. However, it is unknown whether human- and bovine-associated commensals demonstrate different preferences and utilisation of these prebiotics and associated anti-microbial properties. In this study, four lactobacilli species, human-associated (*Lacticaseibacillus rhamnosus*, *Lacticaseibacillus paracasei*) and human- and bovine-associated *(Lactobacillus acidophilus*, *Levilactobacillus brevis*), were investigated for their ability to utilise xylan and XOS of varying lengths. The growth of lactobacilli was monitored for 16 h in nutrient-rich media supplemented with different concentrations of xylan and XOS. Bacterial supernatant was analysed for XOS degradation enzyme using enzymatic assay and XOS breakdown products using HPLC analysis. XOS improved the growth of l*actobacilli* strains from 5% to 30%, particularly by mixing different concentrations and lengths of XOS (cocktail mix-CTM). Degradation profiles of xylan and XOS CTM indicated that bacterial utilisation mechanisms were not restricted by host species and that xylanase was produced within 2 h for *L. brevis*. Our research will provide insights into the different utilisation mechanisms in human and bovine digestion. Therefore, tailored combinations of xylan and XOS could be targeted for different outcomes in animal and human health.

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