#Biofilms11

BIOFILMS 11

13–15 May 2025

Mercure Cardiff Holland House
Hotel & Spa, Cardiff, UK

POSTER ABSTRACT BOOK

National Biofilms Innovation Centre

MICROBIOLOGY SOCIETY

Flash poster : P002

Rapid Non-Contact Approach for Real-Time Characterization of Biofilm Mechanics

Jack Dawson, Jinju Chen

Loughborough University, Loughborough, United Kingdom

Abstract

Understanding biofilm mechanics is critical for tackling challenges ranging from persistent infections to industrial fouling. However, existing methods for characterizing these properties often require invasive or time-consuming techniques, limiting their applicability across scales and dynamic environments.

This study presents an innovative, rapid, and non-invasive air jet indentation system integrated with high-speed Optical Coherence Tomography (OCT) for real-time characterization of biofilm mechanical properties. Unlike traditional methods, this approach eliminates the need for direct contact, allowing precise measurements across multiple length scales without disrupting the biofilm structure.

The system underwent rigorous calibration with benchmark materials such as Polydimethylsiloxane (PDMS) and agarose hydrogels, demonstrating excellent agreement with conventional mechanical testing techniques. Validation results confirmed its accuracy and reliability, setting a new standard for non-destructive mechanical characterization.

When applied to biofilms, the system unveiled novel insights into the role of pili and flagella, revealing their significant impact on biofilm mechanical properties. These findings underscore the potential of this technology to decode the complex interplay of bacterial motility, biofilm structure and their mechanical behavior.

This groundbreaking, non-contact method offers an unprecedented tool for studying biofilm mechanics in situ, enabling researchers and industries to better understand biofilm behavior and optimize intervention strategies. It paves the way for advancing biofilm management in medical, environmental, and industrial contexts.

P006

Critical analysis of methods to determine growth, control and analysis of biofilms for potential non-submerged antibiofilm surfaces and coatings

James Redfern[1], Alexander Cunliffe[1], Darla Goeres[2], Nuno Azevedo[3], Joanna Verran[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]Montana State University, Bozeman, USA. [3]University of Porto, Porto, Portugal

Abstract

Background. The potential uses for antibiofilm surfaces reach across different sectors with significant resultant economic, societal and health impact. For those interested in using antibiofilm surfaces in the built environment, it is important that efficacy testing methods are relevant, reproducible and standardised where possible, to ensure data outputs are applicable to end-use, and comparable across the literature.

Methods. Using pre-defined keywords, a review of literature reporting on antimicrobial surfaces (78 articles), within which a potential application was described as non-submerged/non-medical surface or coating with antibiofilm action, was undertaken.

Results. The most used methods utilized the growth of biofilm in submerged and static systems. Quantification varied (from most to least commonly used) across colony forming unit counts, non-microscopy fluorescence or spectroscopy, microscopy analysis, direct agar-contact, sequencing, and ELISA. Selection of growth media, microbial species, and incubation temperature also varied. In many cases, definitions of biofilm and attempts to quantify antibiofilm activity were absent or vague. Assessing a surface after biofilm recovery or assessing potential regrowth of a biofilm after initial analysis was almost entirely absent.

Conclusion. It is clear that the field would benefit from widely agreed and adopted approaches or guidance on how to select and incorporate end-use specific conditions, alongside minimum reporting guidelines to improve reproducibility may benefit the literature. In 2024, international (Biofilm Regulatory Toolbox - COST Action) and national (Biofilm Alliance – InnovateUK) networks have launched to further these activities.

P007

AC electro-osmosis in bacterial biofilms - a cautionary tale for electrophysiology experiments

Thomas Waigh, Victor Martorelli, Rok Krasovec, Ian Roberts, Raveen Tank

University of Manchester, Manchester, United Kingdom

Abstract

Synthetic cationic fluorophores are used widely as probes to measure the membrane potentials of bacterial cells, eukaryotic cells and organelles, such as mitochondria. An external oscillating electric field was applied to *Escherichia coli* cells using microelectrodes and AC electro-osmosis was observed for the fluorophores, independent of the electrophysiology of the bacteria, giving rise to phantom action potentials. The fluorophores migrate around the microfluidic device in vortices modulating their concentration. We show that the fluorescent dips are universally present when using cationic fluorophores, such as thioflavin-T, propidium iodide, Syto9 and Sytox Green, with or without *E. coli* cells in the inoculum, when stimulated with AC voltages. This is in contrast to the study of Stratford et al (PNAS, 2019) who claim the existence of action potentials. Furthermore, *E. coli* biofilms also demonstrated similar phenomena with dips in the fluorescence. We measured the relaxation times of the fluorophores experiencing AC electro-osmosis, which depended on the biofilm, the cells and the fluorophores used. PI had the smallest relaxation time and Syto9 the highest. Furthermore, fluorescently labelled DNA and fluorescent colloidal beads also demonstrate fluorescent dips through AC electro-osmosis, showing that these particles can be driven through biofilms. This is the first study of AC electro-osmosis in bacterial biofilms, indicating a surprisingly high mobility of charged molecules within the extracellular polymeric substance, which could be used to treat biofilms i.e. to increase the kinetics of delivery of antibiotics or to introduce horizontal gene transfer by driving DNA through biofilms.

P011

Burkholderia cenocepacia diffusible signalling factor eliminates *Candida auris* biofilms by suppressing fungal ER- associated protein degradation pathway

Gayan Kanchana Wijesinghe, Angela Nobbs, Nihal Bandara

University of Bristol, Bristol, United Kingdom

Abstract

Candida auris, a pan-drug resistant fungal pathogen, causes life-threatening infections with longer hospital stays, poor quality of life and increased medical care costs. Cis-2-dodecenoic acid (BDSF), a quorum sensing molecules synthesised by *Burkholderia cenocepacia* have shown promising antifungal effects on *Candida albicans*, however, its impact on *C. auris* is largely unknown.

The effects of BDSF on *C. auris* NCPF8977 (BDSF-sensitive) and CBS12372 (BDSF-resistant) were assessed quantitatively by minimum inhibitory/biofilm inhibitory concentrations (MIC/MBIC), biofilm viability and biomass assays and qualitatively via Scanning Electron Microscopy (SEM) and Confocal Microscopy (CLSM). The molecular basis of BDSF action on 24h *C. auris* biofilms was determined using Tandem mass tagging and nano-liquid chromatography. Differentially expressed proteins were analysed using DAVID Functional Annotation Bioinformatics Microarray Analysis.

MIC_{80} and $MBIC_{50}$ for *C. auris* NCPF8977 and CBS12372 were 125µM, >1000µM and 500µM, 250µM respectively. SEM and CLSM observations confirm viability and biomass data. There were 292 upregulated and 14 downregulated proteins in *C. auris* NCPF8977 biofilms whereas 140 upregulated and 98 downregulated proteins in *C. auris* CBS12372 biofilms compared to controls. Several energy synthesis associated pathways were upregulated in both strains. Heat-shock proteins and co-chaperones associated with Endoplasmic Reticulum-Associated Degradation (ERAD) pathway that eliminate misfolded, unassembled, or toxic proteins in the ER lumen/membrane were exclusively downregulated in CBS12372.

BDSF possesses strain dependent anti-biofilm activity against *C. auris* likely by suppressing ERAD pathway that leads to the accumulation of toxic and/or misfolded proteins in ER lumen driving ER stress and subsequent *C. auris* apoptosis.

P012

Disruption of *Pseudomonas aeruginosa* quorum-sensing influences biofilm formation without affecting antibiotic tolerance

Elvina Smith, Edze Westra, Rafael Custodio

University of Exeter, Penryn, United Kingdom

Abstract

The opportunistic bacterial pathogen *Pseudomonas aeruginosa* is a leading cause of antimicrobial resistance-related deaths. *P. aeruginosa* infections are especially difficult to treat due to the bacterium's propensity to form biofilms. Autolysis, the self-killing of bacterial cells, and the bacterial cell-to-cell communication system, quorum-sensing (QS), play essential roles in biofilm formation. Strains of *P. aeruginosa* that have lost the LasI/R QS system commonly develop in patients, and previous studies have characterised distinctive autolysis phenotypes in these strains. Yet, the underlying causes and implications of these autolysis phenotypes remain unknown. This study confirmed these autolysis phenotypes in the PA14 QS mutant strains, Δ*lasI* and Δ*lasR*, and investigated the consequences of QS loss and associated autolysis on biofilm formation and antibiotic susceptibility. QS mutants exhibited delayed biofilm formation but ultimately surpassed the wild-type (WT) in biofilm mass, despite containing fewer numbers of live-cells. Nevertheless, QS mutant biofilms were not more susceptible to antibiotics than the WT. Artificial supplementation of Δ*lasI* with QS signal molecule (autoinducer) restored the strain's QS system without the associated costs of QS, enabling Δ*lasI* to achieve higher pre-treatment and post-treatment live-cell numbers. Overall, the lack of QS-functioning was not detrimental to biofilm antibiotic tolerance, though the artificial disruption of QS may reduce the advantages of QS mutants within *in vivo* mixed strain populations.

P013

Monitoring biofilm productivity 24/7 reveals surprising correlation between biofilm age and productivity

Hannah Berreth, Johannes Lambrecht, Katja Buehler

Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

Abstract

The structure and gradients in continuously operated phototrophic biofilm reactors make detailed system analysis challenging. We present a toolset for real – time assessment of biofilm productivity, analyzing structures, population dynamics, and carbon balance.

Our model biofilm consists of a mixed culture of *R. palustris* DSM127, which produces H_2 during nitrogen fixation, and *Pseudomonas taiwanensis* VLB120 eGFP, which enhances biofilm stability. It is cultivated in capillary biofilm reactors (CBR) optimized for anaerobic cultivation and gaseous product capture. Product concentrations and gas compositions are continuously monitored using membrane inlet mass spectrometry (MIMS), and gas chromatography (GC). MIMS enables direct, non-invasive quantification of gaseous products within the biofilm. Biofilm development and population dynamics are analyzed via flow cytometry and gravimetry, while the organic carbon source is quantified via HPLC. Based on these data, a system balance is calculated.

MIMS probes were integrated into the CBR to quantify gases directly in the biofilm, minimizing diffusive and biological losses. Combined with flow cytometry, this provided valuable insights into the consortium. Notably, bioH_2 production appears linked to biofilm growth phases. While the biofilm remains stable throughout the frost-free period even in outdoor conditions, product formation declines in the mature consortium. Biofilm renewal through induced wash-out events can restore product formation.

To achieve sustained H_2 production, a carefully managed regime of dynamic biofilm renewal is essential, ensuring consistent performance and long-term productivity.

P020

A Unique Biofilm Morphology from Detached Aggregates

Ciarán Dunne, Dibyangshee Singh, Jacobus Brink, Dishon Hiebner, Eoin Casey

University College Dublin, Dublin, Ireland

Abstract

Suspended bacterial aggregates are becoming increasingly relevant in the context of the biofilm developmental cycle and represent a third lifestyle distinct from both planktonic single cells and surface associated biofilm. Few studies have investigated aggregates that arise from the mechanical detachment of biofilm material. Here we developed a method for isolating detached aggregates from *Pseudomonas fluorescence* biofilms and investigating their secondary colonization capacity using confocal microscopy complemented by molecular assays. Aggregate-derived biofilms exhibited a more complex morphology with a 2 fold increase in thickness, a 2.2 fold biomass increase and a 1.3 fold reduction in roughness when compared to planktonic-derived biofilms. Aggregate-derived biofilms also demonstrated a unique dome-shaped microcolony structure that formed in clusters with a polysaccharide-based network of peripheral linking structures. These clusters were absent in biofilms inoculated with planktonic cells, regardless of inoculum density or physiological state. These findings demonstrate that aggregates detached from established biofilms are capable of biofilm development with a unique architecture that could convey heightened robustness. This has implications for biofilm removal and control strategies, the mechanical dissociation of biofilm material can release aggregate 'seeds' harbouring cells primed to produce altered secondary biofilms that dominate during surface recolonisation.

P022

The potential of urinary extracellular vesicles against biofilm-associated urinary tract infections

Ashraf Zarkan, Aziz Ur Rehman, Tim Williams

University of Cambridge, Cambridge, United Kingdom

Abstract

Urinary Tract Infections (UTIs) are the most common bacterial infections and up to 80% of infections are caused by *E. coli*. Various other bacterial species are responsible for the remainder of infections such as *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, and *S. aureus.* Recurrent UTIs (rUTIs) is common and linked to the formation of biofilms. Extracellular vesicles (EVs) are small (30-1000nm) membrane-bound particles released by all cells into extracellular space. EVs can be isolated from all body fluids, including urine. Urinary EVs (UEVs) are predominantly derived from cells in the kidney, although some UEVs can originate from cells lining the lower urinary tract. UEVs have been previously shown to be enriched in antimicrobial proteins and have *in vitro* bactericidal activity against *E. coli,* but their effect on biofilms is less understood. To investigate the activity of UEVs against biofilm-associated UTIs, UEVs were isolated from human urine by ultracentrifugation, characterised by transmission electron microscopy and proteomic analysis, and quantified by nanoparticle tracking analysis. Predetermined quantities of UEVs were then incubated with biofilms of UTI pathogens, and biofilm inhibition was monitored. We show that UEVs from some healthy volunteers prevent the formation of biofilms in a dose-dependant manner, thereby highlighting the importance of UEVs in combatting biofilm-associated UTIs. We hypothesise that UEVs form part of the innate immune defence system against UTIs.

P023

Fragmented but Functional: Post-Dispersion Dynamics and Phenotypic Variation in Biofilm-Associated Cells

Dibyangshee Singh, Jacobus Brink, Ciarán Dunne, Dishon Wayne Hiebner, Raghu Krishna Moorthy, Eoin Casey

University College Dublin, Dublin, Ireland

Abstract

Background

Biofilm dispersion leads to its fragmentation into an extracellular polymeric substance (EPS) matrix, biofilm-associated cells, and clusters, yet the dynamics of this process remain poorly understood. Limited research exists on how dispersed cells differ phenotypically during different stages of biofilm development and their secondary relocalization efficiency. The current study investigates dispersion dynamics by characterizing dispersed biofilm-associated cells and clusters while assessing biofilm matrix alterations.

Methods

To characterize phenotypic differences in dispersed cells, samples were collected at different time points for qualitative and quantitative characterization using confocal laser scanning microscopy (CLSM), live/dead assays, size analysis, and growth kinetics assays. Advanced image analysis tools were employed to monitor the discrepancy in dispersed cell viability, morphological characteristics, spatial distribution, relocalization efficiency, and the integrity of the biofilm matrix.

Results

Dispersed cells exhibited distinct phenotypic traits, with significant differences in cell viability and growth rates. CLSM image analysis revealed structural variations in the biofilm matrix at different stages. Dispersion kinetics varied across the time points, influencing overall biofilm stability and integrity. Differences in relocalization efficiency were observed between early and late-dispersed cells, suggesting a time-dependent adaptation mechanism influencing secondary relocalization potential.

Conclusion

Dispersion events influence the growth kinetics, relocalization efficiency, and biofilm matrix structure overtime. The variations in phenotypic traits and relocalization efficiency emphasise the dynamic nature of biofilm dispersion events. The current study highlights how dispersion events shape microbial population dynamics, with implications for developing biofilm control strategies.

P026

Alginate exopolymer significantly modulates the viscoelastic properties and resilience of bacterial biofilms

Binu Kundukad[1], Scott Rice[2], Patrick Doyle[3,4], Staffan Kjelleberg[1,5]

[1]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore. [2]CSIRO, Microbiomes for One Systems Health, Agriculture and Food, Westmead NSW, Australia. [3]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, USA. [4]Singapore MIT Alliance for Research and Technology, Singapore, Singapore. [5]School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia

Abstract

Biofilms are microbial communities where the extracellular matrix acts as a protective shield against antimicrobials and microbial invasion. Numerous studies have explored biochemical resistance mechanisms, which work in concert with the physical properties of the matrix. However, the role of biofilm mechanics in preventing bacterial recolonization remains unclear.

In this study, we employed particle-tracking microrheology to investigate the viscoelastic behavior of biofilm matrices from a mucoid strain of *Pseudomonas aeruginosa* (*P. aeruginosa ΔmucA*) and its isogenic wild-type counterpart (*P. aeruginosa* PAO1). By examining bacterial recolonization on both pre-formed biofilms and residual matrices left behind after bacterial eradication with N-acetyl cysteine (NAC), we uncovered key differences in matrix behavior.

Our results reveal that the alginate-rich matrix of *P. aeruginosa ΔmucA* effectively prevents recolonization, through excessive swelling and an increase in elastic modulus of the matrix upon NAC treatment. In contrast, wild-type biofilms exhibited limited swelling, yet their matrix suffered a significant reduction in elasticity post-treatment, suggesting crosslink breakages in the matrix. These results align with polymer physics theories, where the polyelectrolyte nature of alginate drives swelling via the Donnan effect, reinforcing matrix stability even after bacterial death. Conversely, the wild-type matrix demonstrated reduced mechanical strength following treatment due to the dominance of a neutral polysaccharide called Psl (polysaccharide synthesis locus).

By integrating physics with microbiology, this study highlights the critical role of matrix composition in biofilm mechanics and resilience. These insights open new avenues for biofilm-targeted therapies aimed at disrupting the matrix integrity, offering a promising approach to combat biofilm-associated infections.

P033

Cellulose-driven adaptation in a persisting *Listeria monocytogenes* clone

Ana Victoria Gutierrez[1], Oleksii Omelchenko[1,2], Maria Diaz[1], Matthew Gilmour[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

Abstract

Listeria monocytogenes (*Lm*) is a foodborne pathogen found across diverse food categories, reflecting its expanding ecological niche and food safety risks. Comparative genomics of *Lm* sequence type (ST121), commonly associated with foods, has identified a unique prophage (LP-13-6) in an individual strain linked to multi-year persistence at a UK food production facility (Company-X). LP-13-6 is flanked upstream by a gene cluster that includes a putative cellulose synthase, which we hypothesised to contribute to persistence by promoting biofilm formation. This study aimed to evaluate the role of these genetic traits with a functional analysis comparing the wild-type ST121 strain to a deletion mutation of the cellulose synthase gene. Cellulose production by planktonic cells was measured using calcofluor white M2R (CF-M2R), and biofilm structures grown on stainless steel coupons were assessed by widefield fluorescence microscopy using CF-M2R and SYTO9. Additionally, biofilm cell attachment to glass beads under nutrient-limited conditions was quantified after 48 hours by CFU enumeration. Cellulose synthase deletion significantly reduced cellulose production in planktonic cells ($p < 0.0001$ reduction in CF-M2R fluorescence). Biofilms formed by the wild-type strain displayed honeycomb-like matrix structures co-localising with CF-M2R and SYTO9, which were absent in the Δcellulose strain. In preliminary experiments of biofilms cultivated on glass beads, the wild-type strain exhibited a greater CFU enumeration compared to the Δcellulose strain, suggesting that cellulose production plays a critical role in persistence. This study provides novel insights into adaptations driving the persistence of *Lm* and the potential contributions by *Lm* to multi-species biofilm in food production environments.

P039

Factors affecting plasmid transfer in biofilms

Ho Yu Liu[1,2,3], Muhammad Yasir[1,3], Eleftheria Trampari[1,3], Gemma Langridge[1,3], Mark Webber[1,2,3]

[1]Quadram Institute Biosciences, Norwich, United Kingdom. [2]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [3]Centre for Microbial Interactions, Norwich, United Kingdom

Abstract

It has been shown that antibiotic-resistance genes can be transferred within biofilms via horizontal gene transfer, with plasmid conjugation being an important mechanism. However, the factors that influence the rate of plasmid transfer within biofilms remain unclear.

To study this, we established a multispecies biofilm model using *Escherichia coli* and *Salmonella enterica* serovar Typhimurium to monitor the impact of different environmental stressors on the conjugation efficiency of a clinically relevant plasmid encoding *bla*-$_{\text{CTX-M-14}}$. Stress factors investigated include food preservatives such as sodium nitrite and sodium benzoate, as well as antibiotics such as ciprofloxacin.

In parallel, efficiency of transfer of the plasmid into a large (>700,000) *E. coli* transposon mutant library was determined to identify genes involved in host plasmid acceptance.

The data showed changes to the rate of plasmid conjugation within a multispecies biofilm community under different conditions. We have also identified multiple host loci with novel roles in plasmid acceptance. These included genes involved in lipopolysaccharide synthesis and RNA polymerase recycling, that are predicted to have major impacts on plasmid acceptance by *E. coli*. Together, these results provided new information about how plasmids transfer between species in biofilms and suggest key pathways which could be targeted to reduce plasmid-mediated transfer of antibiotic resistance.

P040

The impact of residual chlorination on the presence and incorporation of antibiotic resistance genes into drinking water distribution system biofilms

Sam Walsh[1], Katherine Fish[1], Frances Slater[1], Cindy Smith[2], Joby Boxall[1]

[1]University of Sheffield, Sheffield, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Residual disinfection of drinking water during distribution is employed to mitigate microorganism re-growth, manage contamination and associated water quality parameters in the bulk water, but with little known about its impacts on biofilms. This has been observed to enrich the presence of antibiotic resistance genes (ARGs) within distributed water, but the impact on and the broader implications of ARGs within biofilms is unclear.

Results are presented from experiments which used an internationally renowned, full-scale simulated distribution system to grow (5 months) and then spike (with an environmental contaminant) biofilms under three disinfection regimes (dechlorinated, medium-, and boosted-free chlorine). Thus investigating 'naturally' occurring ARGs, incorporation due to contamination and subsequent persistence within the biofilm, and how these changed as a function of the residual concentration using the quantification capabilities of digital droplet PCR.

The behaviour and response of a range of ARGs were investigated. For example, tetracycline resistance gene, *tetA,* which was ubiquitous within biofilms after 5 months of growth, irrespective of chlorine regime (dechlorinated 43 – 161 copies/cm^2; medium-chlorine 46 – 163 copies/cm^2; boosted-chlorine 41 - 456 copies/cm^2). Contaminant ingress appeared to remove *tetA* from the biofilm population with reduced detection in all conditions. After a further six days, *tetA* reappeared in the medium-chlorine and dechlorinated conditions at concentrations similar to the grown biofilms (dechlorinated 32 – 174 copies/cm^2; medium-chlorine 39 – 110 copies/cm^2; boosted-chlorine 0 – 40 copies/cm^2).

The novel understanding from these experiments adds important insight into the overall balance of risks and benefits of maintaining disinfection residuals within distribution systems.

P046

Investigating *Salmonella* Biofilm Responses to Antibiotic Treatment Using Optical Photothermal Infrared Spectroscopy

Daniel Smaje, Cassio Lima, Xiaojun Zhu, Jay Hinton, Rasmita Raval, Howbeer Muhamadali

University of Liverpool, Liverpool, United Kingdom

Abstract

Biofilms have complex metabolic dynamics due to the diversity of chemicals in their extracellular matrix, formed of polymeric substances including protein, polysaccharides and eDNA, and the highly heterogenous nature of the population of microbial cells. Despite this complexity, an understanding of the metabolic dynamics within a biofilm is critical for development of solutions tackling pathogenic biofilm infections as treatments can be designed to act on critical metabolic pathways. This means the field of metabolomics is well-suited to biofilm research, as it can be used to probe bacterial metabolism with high resolution using vibrational spectroscopy and mass spectrometry.

In this project, a gentamicin-resistant *Salmonella* Typhimurium mutant was produced to allow investigation into how the resistance profile of a biofilm impacts its metabolic response to antibiotic treatments. *Salmonella* Typhimurium biofilms were treated with gentamicin, which they are resistant to, and kanamycin, which they are sensitive to, and cryosectioning was used to allow cross-sectional slices of the biofilms to be imaged. This imaging was performed using optical photothermal infrared (O-PTIR) spectroscopy combined with stable isotope probing (SIP), to allow visualisation of regions with active metabolism within the biofilm. The results showed that biofilms have a core of non-metabolically active cells, and that the pattern and extent of nutrient incorporation is markedly altered when antibiotic-resistant biofilms are treated with an antibiotic. This work helps to improve understanding of biofilm resistance dynamics and can help with the development of methods to disrupt biofilm antibiotic resistance mechanisms.

P050

TomB has a novel species-specific role in biofilm formation in Enterobacteriaceae

Emma Holden[1,2], Muhammad Yasir[1,2], Keith Turner[1,2], John Wain[1,2,3], Ian Charles[1,2,3], Mark Webber[1,2,3]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Centre for Microbial Interactions, Norwich, United Kingdom. [3]Norwich Medical School, Norwich, United Kingdom

Abstract

Progression through the biofilm lifecycle requires controlled temporal gene expression to maximise fitness at each stage. To identify genes involved in different stages we used TraDIS-*Xpress*; a massively parallel transposon mutagenesis approach incorporating transposon-located promoters. This allowed us to measure how gene essentiality and expression affected the fitness of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium growing as a biofilm on glass beads after 12, 24 and 48 hours.

We identified novel roles for multiple genes in biofilm formation, including *tomB* where interruption reduced biofilm formation in *E. coli*. We constructed *tomB* gene deletion mutants in both *E. coli* and *S*. Typhimurium which confirmed biofilm biomass, matrix production and intracellular cyclic-di-GMP levels were reduced in an *E. coli* mutant. However, the opposite was seen in *S*. Typhimurium.

Previous work describes TomB as an antitoxin to co-transcribed toxin Hha, which interacts with H-NS to modulate gene expression. Deletion of *hha* or *hns* alleviated the *tomB* biofilm defect in *E. coli* but had no impact on biofilm hyper-production in *Salmonella*. Current work is exploring how this regulatory circuit differs so profoundly between *E. coli* and *S.* Typhimurium to improve our fundamental understanding of biofilm formation.

P052

Can nanoparticles be a useful tool for treating or preventing bacterial biofilms?

Nicolás Navarro[1], Erlen Cruz[1], María josé González[1], Javier O. Morales[2], Luciana Robino[1], Paola Scavone[1]

[1]Laboratorio de Biofilms Microbianos, IIBCE, Montevideo, Uruguay. [2]Departamento de Ciencias y Tecnología Farmacéuticas, Facultad de Ciencias Químicas y Farmacéuticas, Santiago, Chile

Abstract

Bacterial resistance to antibiotics is a major concern for the WHO, necessitating the development of novel therapeutic strategies. One key resistance mechanism is biofilm formation, which significantly hampers bacterial eradication with conventional antibiotic treatments. Nanoparticles (Nps) represent a promising alternative, including gold (Au-Nps), silver (Ag-Nps), zinc oxide-magnesium (ZnO:MgO-Nps), and poly(n-butyl cyanoacrylate) (PBCA-Nps).

Au-Nps and Ag-Nps were synthesized by a modified Turkevich/Frens seed growth method and ZnO:MgO-Nps and PBCA-Nps were synthesized by nanoprecipitation. Different physicochemical characterization was performed to all NPs. The Nps capability to inhibit or prevent biofilm formation was tested against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 902, *Escherichia Coli* 144 and *Proteus mirabilis* 2921. The *P. mirabilis* and *E. coli* biofilm formation were prevented by Au-Nps, Ag-Nps, ZnO:MgO-Nps, and PBCA-Nps. Furthermore, eradication of pre-formed *P. mirabilis* biofilms were only observed with Au-Nps and Ag-Nps. *E. coli* biofilms were eradicated by Ag-Nps and ZnO:MgO-Nps. *S. aureus* biofilm formation was prevented only for PBCA-Nps and *P. aeruginosa* biofilm was prevented by Au-Nps. The mature biofilms of these two microorganisms could not be eradicated by any of the Nps tested.

The Nps could be a suitable alternative for coating surfaces and/or antibiotic carriers with medical interest to prevent biofilm formation. However, its effectiveness depends on the microorganism, sometimes even leading to an undesired outcome, such as an increase in biofilm.

P054

"Interplay between sRNA RsaE and αPhenol Soluble Modulins (αPSM) toxins: Implications for Biofilm Formation in *Staphylococcus aureus*"

Mehak Chauhan, Sander Granneman

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Background

Staphylococcus aureus causes diverse human diseases through the coordinated expression of multiple virulence factors, including Phenol Soluble Modulins (αPSMs). αPSM1-4 are cytolytic toxins encoded on the *psmα* operon and play crucial roles in bacterial survival by facilitating biofilm formation and structuring.

We recently discovered that the RsaE small RNA base-pairs with Shine-Dalgarno sequences of *psmα3* and *psmα2* in the presence of RNase III, hinting at selective inhibition of the translation. We hypothesise that RsaE-dependent regulation of αPSM toxin production enhances the stability of *S. aureus* biofilms, implying an important role for this sRNA-mRNA interaction in immune evasion.

Aim

We aim to explore the contribution of RsaE - αPSMs interaction in biofilm formation.

Methods

We generated RsaE, RNase III and *psmα* operon deletion mutants. Three different techniques were utilised to investigate how these mutations impacted biofilm formation. For each mutant, three technical and biological replicates were cultivated at three time points (24h, 48h, and 72h). Crystal violet assay quantified total biomass, Combination of fluorescent dyes studied extra-polymeric substances (EPS) and CFU/ml was measured bacterial survival within biofilms.

Result

Significant differences in total biomass were observed between the wild-type and the Δ*psmα* operon at 48 hours, while RNase III mutant had low biomass consistently. The absence of RsaE resulted in increased bacterial survival up to 48 hours.

Conclusion

RsaE plays a role in regulating biofilm persistence, as its absence enhances bacterial survival. RNase III deletion results in low biomass, potentially due to its direct or indirect interaction with the *psmα* operon.

P058

Battle for the Surface: Staphylococcus Biofilms vs. Osteoblast Adhesion on DNA Polyelectrolyte Multilayer Coatings

Carmelo Covato[1], Alina Pilipenco[1,2], Erik Reimhult[1], Guruprakash Subbiahdoss[1]

[1]Institute of Colloid and Biointerface Science, BOKU University, Vienna, Austria. [2]FZU - Institute of Physics of the Czech Academy of Sciences, Prague, Austria

Abstract

Background: Biomaterial-associated infections remain a major challenge in modern medicine. We previously demonstrated that polyanionic DNA multilayer-coated surfaces repel bacterial adhesion and support osteoblast-like cell attachment in monoculture experiments, proposing a suitable candidate for orthopaedic implant coatings. However, monocultures cannot capture the influence of bacteria or bacterial toxins on osteoblast adhesion to biomaterial surfaces. In this study, we studied cocultures of staphylococci and SaOS-2 osteosarcoma cells on chitosan-DNA polyelectrolyte multilayer coated glass based on the concept of the `*race for the surface*`.

Methods: To mimic perioperative contamination, staphylococcus was first deposited onto glass or chitosan-DNA multilayer-coated surfaces in a microfluidic chamber. Subsequently, SaOS-2 cells were seeded and cultured together on the surfaces for 24 h under flow. SaOS-2 cells were evaluated using fluorescence microscopy after nucleus and actin cytoskeleton staining and after vitality staining.

Results: The presence of *S. epidermidis* decreased the number of SaOS-2 cells found on all surfaces after 24 h. However, the SaOS-2 cells that adhered spread equally well in the presence of *S. epidermidis* contact to the highly virulent *S. aureus,* induced cell death of all adherent SaOS-2 cells on chitosan-DNA multilayer-coated glass, a worse outcome than on uncoated glass.

Conclusions: The different outcomes of our monoculture and coculture experiments highlight the limitations of monoculture models. The relative failure of cell-adhesive and bacteria-repelling DNA polyelectrolyte multilayer coatings in cocultures also suggests the need to incorporate bactericidal in addition to non-adhesive functions to protect cell spreading over long periods when zero adhesion of bacteria cannot be ensured.

P068

Hair Follicle Biofilm: Reservoirs Driving Scalp Microbiome Balance and Dysbiosis

Viduthalai Rasheedkhan Regina[1,2,3], Tarun Chopra[4], Weihao Kwok[5], Sreelakshmi Cheruvalli[6], Sabrina Ang[7], Hashmath Fatimah Binte Jamal Mohamed[2], Kesava Priyan Ramasamy[2], Sarah Kay[2], Yik Yan Chong[2], Eganathan Kaliyamoorthi[8], Rohan Williams[8], Xianghui Liu[2], Vedula Krishna[7], Nasrine Bourokba[4], Anjali Jhingan[9], Steven Thng[9], Olivier Da Cruz[10], Sylvie Riu[11], Romain De Dormael[12], Kahina Abed[13], Olivia Touriguine[11], Roland Jourdain[13], Sylvie Cupferman[14], Luc Aguilar[13], Scott A. Rice[2,15]

[1]A*Skin Research Labs, Agency for Science Technology and Research, Singapore, Singapore. [2]Singapore Centre for Environmental Life Sciences and Engineering, Nanyang Technical University, Singapore, Singapore. [3]Skin Research Institute of Singapore, Singapore, Singapore. [4]LOreal Research and Innovation, Singapore, Singapore. [5]Loreal Research and Innovation Singapore, Singapore, Singapore. [6]singapore Centre for Environmental Life Sciences Engineering, Nanyang Technical University, Singapore, Singapore. [7]Loreal Research and Innovation, Singapore, Singapore. [8]Singapore Centre for Environmental Life Sciences and Engineering, National University of Singapore, Singapore, Singapore. [9]National Skin Centre, Singapore, Singapore. [10]LOreal Research and Innovation, Chevilly Larue, France. [11]Loreal Research and Innovation, Saint-Ouen-sur-Seine, France. [12]LOreal Research and Innovation, Aulnay-sous-Bois, France. [13]Loreal Research and Innovation, Aulnay-sous-Bois, France. [14]Loreal Research and Innovation, Chevilly Larue, France. [15]CSIRO Agriculture & Food, Microbiomes for One Systems Health, Sydney, Australia

Abstract

A balanced scalp microbiome is crucial for maintaining healthy scalp, yet the mechanisms driving this balance and the onset of disorders like dandruff and seborrheic dermatitis (D/SD) remain unclear. Here, we show that microbial biofilms colonizing the hair follicles potentially serves as a reservoir for the scalp microbiome, forming an interconnected scalp-follicle-hair ecosystem, and interactions within the hair follicle microbial community is essential for the scalp health. Through an analysis of samples from 65 volunteers (33 healthy, 32 D/SD) using direct imaging by scanning electron microscopy and metagenomic sequencing, we observed microbiome dysbiosis in D/SD characterized by an increased abundance of *Malassezia restricta* and *Staphylococcus spp.* and a decrease in *Cutibacterium acnes*. To understand the mechanisms governing the microbial community structure, we developed a simple three species synthetic microbial communities grown as biofilms to mimic the follicular microbial community. By investigating this model system, we identified propionic acid, a postbiotic metabolite produced by *C. acnes*, as a key factor in regulating microbial balance. Mechanistic studies, including transcriptomics and metabolomics analyses, revealed that propionate modulates the growth and biofilm formation of *S. epidermidis,* and inhibits *M. restricta* by inducing iron starvation. Clinical validation confirmed that propionic acid stabilizes and rebalances the dysbiotic scalp microbiome, reducing D/SD symptoms.

By integrating synthetic microbial communities grown as biofilms with clinical studies, this research provides ecological and mechanistic insights into the interactions and functions of the scalp microbiome, offering a deeper understanding of the processes governing scalp health and dysbiosis.

P072

G-quadruplexes and extracellular RNA co-exist in *Pseudomonas* biofilm matrices

Sudarsan Mugunthan[1], Thomas Seviour[2], Staffan Kjelleberg[3]

[1]SCELSE- NTU, SINGAPORE, Singapore. [2]Aarhus university, Aarhus, Denmark. [3]SCELSE-NTU, SINGAPORE, Singapore

Abstract

While extracellular DNA (eDNA) has been described as critical to biofilm formation across multiple settings, it has been unclear why eDNA behaves so differently to nucleoid DNA. In the extracellular matrix, the eDNA exists as fibers that form the foundational viscoelastic cross-linked structure of biofilms, and inside the cell, DNA is supercoiled, despite no clear differences in their sequences. Understanding what factors contribute to the ability of eDNA to assemble into viscoelastic fibers is key to resolving processes contributing to eDNA release and biofilm formation.

Through 2D solid state NMR, microrheology, and confocal microscopy, our group previously identified two key structural features of *P. aeruginosa* biofilms; G-quadruplexes and extracellular RNA (eRNA) (Seviour et al 2021 and Mugunthan et al 2023).

G-quadruplex formation correlated with biofilm viscoelasticity. Biofilm dissolution involved eRNA transesterification, with specific RNA detected along eDNA fibers. Mild DNase pre-treatment enhanced RNase sensitivity of biofilms, suggesting eDNA:RNA hybrid formation.

R-loops are one of the potential sources of DNA:RNA hybrids. Here we described the presence of extracellular R-loops throughout the extracellular matrix of *Pseudomonas aeruginosa* biofilms. These were found to have a different gene profile to the transcriptome, indicating that they were *in trans* R-loops*,* which are known to be extremely toxic to bacteria. We therefore describe how previous observations of G-quadruplex structures and e-DNA:RNA hybrids can be explained by extracellular R-loops, and whether this is the mechanism that cells achieve viscoelastic eDNA matrix formation. These findings will likely inform on new strategies for managing biofilm growth.

P073

The role of fluid friction in streamer formation and biofilm growth

Cornelius Wittig[1], Michael Wagner[2], Romain Vallon[1,3], Thomas Crouzier[4], Wouter van der Wijngaart[5], Harald Horn[6], Shervin Bagheri[1]

[1]KTH Royal Institute of Technology, FLOW, Stockholm, Sweden. [2]Karlsruhe Institute of Technology, Institute of Biological Interfaces, Eggenstein-Leopoldshafen, Germany. [3]Ecole Centrale de Lyon, CNRS, Universite Claude Bernard Lyon 1, INSA Lyon, LMFA, UMR5509, Ecully, France. [4]DTU, Department of Health Technology, Kongens Lyngby, Denmark. [5]KTH Royal Institute of Technology, Division of Micro and Nanosystems, Stockholm, Sweden. [6]Karlsruhe Institute of Technology, Engler-Bunte-Institut, Karlsruhe, Germany

Abstract

Biofilm growth has long been known to depend on local flow conditions, specifically the wall shear stress. Despite extensive research on biofilm growth, the precise role of fluid friction in shaping biofilm morphology remains poorly understood. Here, we investigate the influence of the wall shear stress on B. subtilis biofilms in millifluidic channels. The biofilms are grown on flat surfaces in laminar flow, with wall shear stress spanning from 0.068 Pa to 0.67 Pa. We use optical coherence tomography to automatically monitor the three-dimensional growth of the biofilms over a duration of eight days.
We observe that the biofilms form microcolonies in the shape of leaning structures, often topped by a streamer that extends up to millimetres downstream. While the size and shape of these structures vary with the shear stress, the overall features remain consistent. The accumulation of biofilm scales linearly in time and inversely proportional to the wall shear stress. We provide a scaling model explaining this relationship. Additionally, we show that streamers form in a location consistent with microfluidic experiments, where a geometric defect is required to create secondary flows. Here, this defect is formed by the biofilm itself creating the leaning structures, showing how even on a featureless surface, streamers can occur within days [1]. This study provides insight into the interplay between flow-induced friction and biofilm developement.

[1] Wittig, C. et al. The role of fluid friction in streamer formation and biofilm growth. npj Biofilms Microbiomes 11, 17 (2025). https://doi.org/10.1038/s41522-024-00633-2

P076

Exploiting microbial interactions for safer food

Noemie Vimont[1,2], Emma Holden[1,2], Justin Abi Assaf[1], Haider Al-Khanaq[1,2], Mark Webber[1,2,3], Eleftheria Trampari[1,2]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Centre for Microbial Interactions, Norwich, United Kingdom. [3]University of East Anglia, Norwich, United Kingdom

Abstract

Fresh produce is implicated in over 30% of outbreaks of bacterial foodborne infection, with *Salmonella* being a leading cause, implicated in a wide range of products, including leafy greens, cucumbers, and alfalfa sprouts. Current methods for controlling pathogens in fresh produce production sites include seed-media sterilisation, and post-harvest sanitisation. These approaches have limitations – they are expensive to run, and often not effective enough to remove strongly attached pathogenic bacterial aggregates (biofilms). Plant ecosystems harbor diverse microbial communities that promote plant health and disease resistance. Some of these microbes serve as biocontrol agents, antagonising harmful pathogens. However, their effectiveness in acting against foodborne pathogens such as *Salmonella* remains poorly understood.

My research bridges this gap by investigating the mechanisms Salmonella employs to colonise fresh produce and developing microbial interventions to control these pathogens in controlled environment agriculture systems. Using genome-wide screening techniques like Transposon Directed Insertion Sequencing (TraDIS-Xpress), I identified specific mechanisms *Salmonella* uses during distinct phases of colonisation. Additionally, I identified and characterised commensal microbes with biocontrol activity against *Salmonella* in various settings (e.g., *in vitro* and *in planta*). Transcriptomic analyses revealed that these biocontrol agents employ environment-specific mechanisms to inhibit *Salmonella*.

This work provides insights into how *Salmonella* establishes on plants and demonstrates the potential of biocontrol agents to mitigate foodborne pathogen colonisation in fresh produce systems. Understanding competitive microbial interactions within plant ecosystems has implications for human health, food safety, and sustainable agriculture practises.

P080

EXPLORING THE INFLUENCE OF HOST ENVIRONMENT ON P. AERUGINOSAVIRULENCE & ANTIBIOTIC SUSCEPTIBILITY USING HOST-MIMICKING MEDIA AND MODELS

Ayomikun Kade, Freya Harrison, Kim Hardie, Dean Walsh

University of Warwick, Coventry, United Kingdom

Abstract

Pseudomonas aeruginosa is a difficult-to-treat pathogen that causes chronic infections in immunocompromised patients. Its virulence factors and biofilm formation confer it with an ability to colonize more than one site in the body including the lungs of people living with cystic fibrosis (pwCF), wounds, and endotracheal tubes in mechanically ventilated patients. Studies have investigated *P. aeruginosa* using routine media, without considering the environment encountered *in vivo* at different sites. We compared the virulence factor production, and antibiotic susceptibility profile of two laboratory strains of *P. aeruginosa* (PAO1 and PA14) in three host-mimicking media (HMM). These were synthetic wound fluid (SWF), synthetic cystic fibrosis medium (SCFM), and synthetic ventilated airway mucus (SVAM) to mimic different physiological secretions. We combined the SVAM with a novel endotracheal tube (ETT) model to test the effect of antimicrobials. There were marked differences in the biofilm structure of PAO1 and PA14 strains grown in different HMM. Both strains formed more biofilm in lysogeny broth than the HMM. Polysaccharide was prevalent in the biofilm matrix formed by both strains in all the media used. Siderophore production was highest in SCFM for both strains, and lowest in SVAM. Increased tolerance to colistin and tobramycin was observed when both strains were cultivated in SVAM while high tolerance to meropenem was observed in SWF. Biofilms that developed on the ETT model were significantly dispersed by the enzyme glycoside hydrolases. This study confirmed that *P. aeruginosa* modulates its biofilm architecture, virulence and antibiotic susceptibility in different physiological sites in the body.

P085

BatR: A Key Regulator of Pseudomonas aeruginosa Biofilm Structure and Antibiotic Tolerance

Ainelen Piazza[1], Freya Harrison[2], Jacob Malone[1]

[1]John Innes Centre, Norwich, United Kingdom. [2]School of Life Sciences, University of Warwick, Coventry, United Kingdom

Abstract

Background: *Pseudomonas aeruginosa* chronic infections involve biofilm formation, complicating treatment. Uncharacterized biofilm genes may play pivotal roles in the biofilm development. Here, we investigate the unknown gene *PA3049* and its role in biofilm formation.

Methods: We assessed *PA3049* impact on *P. aeruginosa* biofilm architecture and antibiotic tolerance using the *ex vivo* pig lung model (EVPL). Proteomic analyses were performed to explore its mechanism of action.

Results: *PA3049* plays a role in *P. aeruginosa* biofilm development and antimicrobial tolerance. WT *P. aeruginosa* formed structured biofilms on EVPL tissue, resembling those observed in cystic fibrosis infections, whereas the *PA3049* mutant produced a biofilm with disrupted architecture and reduced antibiotic tolerance. Proteomic analysis revealed differential expression of pyocins in the WT versus the mutant. Pyocins are multiprotein particles morphologically like phage tails that kill or prevent the growth of related bacteria. Further validation confirmed that the WT PAO1 strain produces self-targeting pyocins, likely in a *PA3049*-dependent manner. We propose that pyocin-mediated lysis of a subpopulation of cells releases cytoplasmic content, such as extracellular DNA, which is essential for biofilm stability.

Conclusions: Renamed as the Biofilm Antibiotic Tolerance Regulator (BatR), *PA3049* is a key determinant of *P. aeruginosa* biofilm structure and antibiotic tolerance. Our findings highlight a potential role for pyocins in biofilm formation and provide new insights into bacterial survival strategies in chronic infections.

P088

Detection, characterisation and early-stage culturing of Magnetospirillum gryphiswaldense biofilms: A previously planktonic-only system used for biogenic magnetic nanoparticle production.

Josh Bond[1], Alfred Fernandez-Castane[1], Tim Overton[2], Vesna Najdanovic[1]

[1]Aston University, Birmingham, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

Magnetotactic bacteria (MTB) are capable of biomineralizing chains of magnetic nanoparticles called magnetosomes under microaerobic conditions. The reference strain *Magnetospirillum gryphiswaldense* MSR1, known for high purity magnetosomes made of magnetite, is exclusively cultivated planktonically. During bioreactor culture of MSR1, biofilm-like morphologies were observed. This is the first observation of biofilm formation in MSR1 and so could open a new avenue for MTB research.

This study aims to identify growth conditions that induce biofilm formation and develop a fully characterised platform for cultivation. Initial experiments confirmed the observation of biofilms by screening various materials for adherence and magnetism. Biofilm formation was optimised through altered media composition and addition of signalling molecules. Next, the structure and EPS composition of MSR1 biofilms were characterised using imaging techniques and elemental analysis including SEM-EDS, TEM-EDS and ICP-OES. Finally, some early attempts to upscale from tube/bottles to bench-top bioreactor scale were performed.

Results showed that magnetic MSR1 biofilms formed robustly on rough surfaces of polypropylene. Additionally, biofilm formation increased significantly with iron and carbon enrichment and indole supplementation. SEM/TEM analysis revealed that the EPS matrix contained high concentrations of iron, potentially aiding magnetite crystal formation. These findings represent the first reliable report of biofilm formation by MSR1 and its applicability for magnetosome production. Bioreactor prototypes robustly produced high biomass serving as a proof of concept for biotechnology applications.

This study confirms MSR1 biofilm cultivation is plausible and offers an alternative to planktonic systems and can be leveraged to biotechnological applications.

P092

Long-term biofilm development on antifouling coatings: community composition, enzymatic activity, and environmental influence

Eveline Diopere[1], Silke Lambert[2], Tineke Vanhalewyn[1], Filip Van Nieuwerburgh[2], Colin Janssen[2], Jana Asselman[1]

[1]Ghent University, Ostend, Belgium. [2]Ghent University, Ghent, Belgium

Abstract

Biofilm formation on marine surfaces presents a complex challenge with both ecological and economic implications. This study investigates the relationship between biofilm communities, their fouling mechanisms, and the chemical properties of antifouling coatings. Uniquely, our test plates were exposed to natural conditions for 18 months, including 6 months offshore under high-energy conditions, followed by 12 months in various harbor locations. Sampling occurred twice—after offshore exposure and after the extended harbor period—allowing us to assess long-term biofilm development in contrasting environments. Using high-throughput Illumina sequencing of both 16S and 18S rRNA genes, we will analyze microbial and eukaryotic community composition, succession, and potential shifts in dominant taxa. Additionally, we will investigate whether specific enzymatic activities play a role in biofilm adhesion and fouling processes. By linking the presence of organisms with known enzymatic capabilities to the chemistry of the different coatings—ranging from biocidal (Micron Extra EU navy, Micron LZ) to biocide-free (B Free Explore navy) and untreated controls—we aim to identify key interactions that drive biofilm formation and persistence. These insights will contribute to the development of more effective and sustainable antifouling strategies.

P093

Biofilm Alliance – A Regulatory Science Network

Paulina Rakowska[1], Geertje Van Keulen[2], Gillian Iredale[3], James Redfern[4], Peter Askew[3]

[1]National Biofilms Innovation Centre, Southampton, United Kingdom. [2]Swansea University, Swansea, United Kingdom. [3]Industrial Microbiological Services Limited, Hartley Wintney, United Kingdom. [4]Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Despite the significant impact of biofilms on health, the environment, and industry, resulting in an estimated economic cost of US$5 trillion annually, progress in biofilm control, management, and prevention is hindered by challenges in regulatory sciences. Outdated guidelines, limited collaboration between researchers, industry, and regulators, and the absence of standardised biofilm methodologies create the urgent need for a systematic approach.

To address these challenges, a collaborative network - the Biofilm Alliance - has been established in the UK, to bring together academic experts, industry professionals, regulatory authorities, and metrology and standardisation bodies. Supported by the Innovate UK, this initiative aims to assess and recommend existing methodologies and models, as well as develop a structured framework for interpreting biofilm data, ultimately leading to a set of recommended regulatory tools.

Focusing on four key areas: food, water, industrial processes, and the built environment, the Biofilm Alliance aims to build a strong network that bridges the gap between scientific advancements and regulatory decision-making. Through dialogue, collaboration, education, knowledge exchange, and capacity-building efforts, we strive to integrate state-of-the-art biofilm research into regulatory science. By informing regulations and advancing the development of validated, standardised biofilm control technologies, the network will play a crucial role in enhancing biofilm-related innovation across multiple sectors.

This presentation will provide an overview of the current landscape of biofilm standardisation, as well as the challenges faced by both innovators and regulators. We will also introduce the Biofilm Alliance initiative and explore opportunities for involvement.

P101

Host-pathogen interactions of polymicrobial biofilm communities within a urine-tolerant human urothelium organoid model (3D-UHU)

Ramon Garcia Maset[1,2], Aaron Crowther[2], Laia Pasquina[3], Victoria Chu[1], Ian White[1], Maria Hadjifrangiskou[4], Dario Carugo[2], Jennifer L Rohn[1]

[1]University College London, London, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]University of Sheffield, Sheffield, United Kingdom. [4]Vanderbilt University Medical Centre, Nashville, TN, USA

Abstract

With 0.4 billion cases annually, urinary tract infection (UTI) is a major driver of the global antimicrobial resistance (AMR) crisis. Although biofilms are associated with diverse chronic infections, resulting in high tolerance against antibiotics, their role in non-catheter associated UTI is poorly understood. Uropathogens deploy key adaptations like attachment, filamentation, and intracellular invasion to survive in the bladder, but biofilm formation on the bladder surface itself might be another virulence and treatment-resistance strategy. Polymicrobial biofilms are present in nearly all chronic infections, but relevant polymicrobial models are scant, especially in the UTI field.

Hence, we wanted to investigate UTI biofilms in a more physiological context. We found that polymicrobial communities triggered more biofilm formation compared with single species, and certain pathogen combinations coexisted in human urine but exhibited competitive dynamics in standard media, highlighting the importance of mimicking the host-microenvironment. We explored biofilm formation in our 3D human urothelial urine-tolerant organoid/microtissue model, where dual and triple species colonization caused an enhanced cytotoxicity and inflammation, alongside a decrease in urothelial barrier function compared with single species infections. We are currently performing metabolomics to understand the mechanisms behind this enhanced virulence. Our study represents the first example of dual- and triple-species biofilms cultivated in 100% urine on an advanced human urothelial model. These findings enhance our understanding of single-species and polymicrobial biofilm dynamics in UTI and underscore the need to understand uropathogenic behaviour in a more physiological context.

P107

Targeted Biofilm Disruption in Bone Infections Using Ultrasound-Activated Microbubbles for Enhanced Antibiotic Delivery

Aya Ben Issa[1], Janos Kanczler[1], Dario Carugo[2], Eleanor Stride[2], Jeremy Webb[3], Nicholas Evans[1]

[1]Bone and Joint Group, Faculty of Medicine, University of Southampton, Southampton, United Kingdom. [2]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom. [3]School of Biological Sciences, University of Southampton, Southampton, United Kingdom

Abstract

Biofilm-associated infections in orthopaedic surgery are highly resistant to antibiotics, necessitating novel therapeutic strategies. This study investigates ultrasound-responsive microbubbles (MBs) to enhance antibiotic penetration and disrupt biofilm structure through generating localised mechanical forces upon stimulation.

Methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms were cultured on fibronectin-coated, acoustically compatible substrates for 72 hours. Lipid-shelled MBs were fabricated using a two-step sonication process and stimulated at 0.5 MPa acoustic pressure with a pulse length of 400 µs and a pulse repetition frequency (PRF) of 500 Hz for 100 seconds, using a 0.9 MHz focused transducer. Biofilms were treated with ultrasound-activated MBs alone or with vancomycin. Biofilm biomass, thickness, and cell viability were assessed using confocal laser scanning microscopy and BiofilmQ. Detached bacteria were collected post-treatment, and CFU/mL were quantified to assess bacterial dispersal.

Treatment with ultrasound-activated MBs significantly reduced biofilm biomass by 75% ($p < 0.005$) and thickness by 40% ($p < 0.05$) compared to untreated controls ($n = 3$). The ultrasound parameters were varied to induce cavitation, which is known to generate localised shear forces that can disrupt the biofilm's structure. When combined with antibiotics, biofilm biomass was further reduced by up to 88% ($p < 0.005$), with a corresponding 68% decrease in cell viability ($p < 0.0005$, $n = 3$). Detached bacteria from treated biofilms showed reduced CFU/mL counts with higher antibiotic concentrations, suggesting effective biofilm disruption and reduced bacterial colonisation potential.

Ultrasound-activated MBs significantly enhance biofilm disruption and antibiotic efficacy, offering a promising, minimally invasive therapeutic strategy for biofilm-associated infections in orthopaedic surgeries.

P111

Spatial Organisation and Transcriptional Responses Reveal Interspecific Interactions in a Model Multispecies Biofilm

Faizan Sadiq[1,2], Nan Yang[3], Koen De Reu[2], Marc Heyndrickx[2], Mette Burmølle[3]

[1]Cardiff University, Cardiff, United Kingdom. [2]The Flanders Research Institute for agriculture, fisheries and food (ILVO), Melle, Belgium. [3]University of Copenhagen, Copenhagen, Denmark

Abstract

Bacterial interspecies interactions within biofilms are directly linked to their tolerance to antimicrobial agents and enzymatic potential. Here, we investigated the spatial organisation and biovolume of a three-species biofilm community comprising *Stenotrophomonas rhizophila* (SR), *Bacillus licheniformis* (BL), and *Microbacterium lacticum* (ML), recovered from a biofilm sample on a dairy pasteuriser following cleaning and disinfection. We previously reported interspecies interactions among these community members in both pairwise and higher-order associations. Using fluorescence *in situ* hybridisation (FISH) and confocal laser scanning microscopy (CLSM), we quantified species-specific biovolume and analysed 3D biofilm dynamics across all layers over 24 hours. SR was the most abundant species, whereas ML had the lowest relative abundance. Using RNA-Seq, we compared SR gene expression in monoculture to its expression when co-cultured in biofilms with ML and BL in pairwise combinations, where it exhibited a commensal and a neutral interaction with ML and BL, respectively. SR selectively upregulated TonB-dependent receptors (btuB, cirA, fpvA) and associated proteins (exbB), which facilitate substrate-specific transport (e.g., vitamin B12 and siderophores) across the outer membrane when co-cultured with ML. In contrast, these proteins were downregulated in biofilms with BL. Similarly, genes linked to flagella production, chemotaxis, cell division, cellular respiration, energy production, peptidoglycan synthesis, amino acid biosynthesis, and fatty acid metabolism were upregulated in the SR-ML combination. Our findings reveal the dynamic growth adaptation strategies of SR within mixed-species biofilms and demonstrate that interspecies interactions within a spatially structured biofilm community shape its architecture and organisation.

P116

Antimicrobial Coatings on Glass: An imaging and Spectroscopic Approach

Abbie Johnson[1], Sam Haq[1], Verity Piercy[2], Erica Li[1], Su Varma[2], Rasmita Raval[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]NSG Group Ltd, Pilkington Technology Centre, Ormskirk, United Kingdom

Abstract

Antimicrobial glass coatings are an important technology for combatting microbial transmission and protecting the built environment from degeneration by biofilms. This project focusses on a novel nanoparticle-based glass coating, developed by Pilkington Technology Management Limited (PTML), part of NSG Group, which can be applied to windows, furniture and electronic device screens and is suitable to be used in a range of areas including hospitals and public transport.

To guide the development of more efficient antimicrobial coatings, it is important to study the relationship between the bacteria and the coating structure. Optimal nanoparticle loading and film thickness was obtained by varying the coating formulation and application conditions to prepare a robust and transparent coating. X-ray photoelectron spectroscopy (XPS) was used to characterise the elemental composition and chemical state at the surface and within the coating through argon ion depth profiling. The size and spatial distribution of nanoparticles across the coating was mapped using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). This was combined with a series of microbial assessments including fluorescence microscopy live/dead staining, SEM and antibacterial surface testing adapted from ISO 22196:2011 to assess the biocidal efficiency against *Escherichia coli* and*Staphylococcus aureus* and study the interaction with the coating surface. This revealed an interesting relationship between the living system of bacteria and the structure of the coating, probed by focused ion beam (FIB) to observe the effect above and below the coating surface.

P119

Monitoring Biofilm Penetration and Disruption Using Fluorescent Antibiotic-loaded Nanoparticles

Maria Odyniec[1], Daniel Bell[1], Grace Ball[1], Rininta Firdaus[1], Damien Walmsley[1], Sarah Kuehne[2], Zoe Pikramenou[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Nottingham Trent University, Nottingham, United Kingdom

Abstract

Antimicrobial resistance is an ever-increasing concern, posing significant challenges in treating infections. Bacterial biofilms, in particular, are difficult to eradicate due to their multi- layered structure, which hinders antibiotic penetration. Therefore, localised treatment modalities with a stimuli-responsive drug release mechanism are preferential. Additionally, mechanisms assuring penetration of all biofilm layers are required.

Here, we report the encapsulation of rifampicin into hybrid organically modified silica-silica ($PhSiO_2$-SiO_2) nanoparticles. Low frequency ultrasound (LFUS) is used to release the antibiotic from the nanoparticles and increase penetration into biofilms by disrupting the extracellular polymeric substance. To confirm the latter, a luminophore was introduced inside the nanoparticles, allowing monitoring of their location within the biofilm.

Ultrasound-induced release of rifampicin was confirmed by LC-MS. MIC studies showed that the antimicrobial efficacy of rifampicin-doped nanoparticles against planktonic *Staphylococcus aureus* increased upon sonication. Biofilm studies exhibited significant increases in bactericidal activity from the dual treatment of rifampicin-doped nanoparticles and LFUS against 72-hour *S. aureus* biofilms compared to free rifampicin and LFUS. This was quantified by live bacteria counting and LIVE/DEAD confocal imaging. Without LFUS, nanoparticles remained towards the top of the biofilms. However, after ultrasound application, nanoparticles penetrated more effectively, reaching the bottom of the biofilms within 30 minutes of sonication.

In conclusion, fluorescent, ultrasound-responsive silica nanoparticles enable localised drug delivery throughout all biofilm layers, achieving significant biofilm disruption and bacterial killing.

P125

Composition and Function of the Extracellular Polymeric Substances Produced by the Cyanobacterium *Synechocystis sp. PCC 6803*

Sivan Luz Nir, Jessica I. Wilson, Jenny Z. Zhang

The Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

Abstract

Cyanobacteria exhibit multicellular organisation, forming surface-attached communities called biofilms in which a complex matrix of extracellular polymeric substances (EPS) is secreted. The EPS provides a structured microenvironment and enhances resilience against environmental stressors. This has allowed cyanobacteria to proliferate in many environmental niches and contribute around 30% of the Earth's photosynthetic oxygen production, forming a vital part of global biogeochemical cycles. Several studies have reported stronger biofilm formation in cyanobacterial mutants deficient in EPS, contradicting the expected adhesive function of the EPS. However, the physico-chemical nature and physiological implications of this effect remain unexplored.

Here, we examine the role of EPS in biofilm formation and organisation by comparing the physiology and EPS composition of ***Synechocystis sp. PCC 6803*** wild-type (WT) and an EPS-deficient mutant. Through quantitative analyses of surface adsorption and biofilm morphology, we confirmed that EPSmutant cells indeed adhere more strongly and form denser biofilms. Compositional analysis of EPS revealed a decreased production of sulfated exopolysaccharides and elevated protein content in the EPS-mutant. Characterisation of the physico-chemical properties of EPS revealed that sulfated exopolysaccharides create a gel-like matrix, which reduces direct cell-cell and cell-surface interactions. This results in a sparse biofilm, highlighting the role of sulfated EPS in modulating biofilm architecture. This work provides a comprehensive study of the adhesive properties of Synechocystis EPS, demonstrating that it facilitates a sparsely distributed biofilm structure. These findings may contribute to building comprehensive mechanistic understandings of cyanobacterial biofilm formation, offering manifold implications across fields such as cyanobacterial ecology, biosynthetic technologies and the development of new biomaterials.

P133

Breaking Biofilms: Discovering Biofilm Modifying Enzymes to target Staphylococcal Pathogens

Connor Munro[1], Marjan van der Woude[2], Gavin Thomas[1]

[1]University of York, York, United Kingdom. [2]Hull York Medical School, York, United Kingdom

Abstract

Staphylococcus species are among the most clinically significant pathogens, causing a wide range of infections. Their virulence is often exacerbated by biofilm formation, which enhances immune evasion and antibiotic resistance. In *ica*-dependent *Staphylococcus* biofilms, the primary polysaccharide component of the extracellular matrix is poly-*N*-acetyl-β-(1-6)-glucosamine (PNAG). Despite the promise of enzymatic therapies for biofilm disruption, few glycoside hydrolases targeting PNAG have been characterised, and none have been identified in *Staphylococcus* species. This research investigates a group of putative glycoside hydrolase family 20 (GH20) proteins identified in several *Staphylococcus* species, hypothesised to degrade PNAG. Protein sequence analysis revealed conserved catalytic residues essential for GH20 enzymatic activity, as shown in Dispersin B – a PNAG-degrading enzyme from the Gram-negative pathogen *Aggregatibacter actinomycetemcomitans*. Notably, genes encoding these putative GH20 enzymes are situated near the operon responsible for the production of PNAG, the *icaADBC* operon, often replacing its transcriptional repressor, *icaR*. This genomic positioning suggests a potential role in PNAG synthesis and degradation. While Gram-negative bacteria produce PNAG via the *pgaABCD* operon, which encodes the GH20-containing enzyme PgaB for PNAG modification, the *icaADBC* operon in Gram-positive bacteria lacks such an enzyme. The presence of GH20 proteins in *Staphylococci* suggests a previously unrecognised role in PNAG processing or biofilm regulation. Through protein expression, biochemical assays, biofilm degradation studies, and structural characterisation, we aim to assess these enzymes' biofilm-dispersing potential and role in biofilm development. Identifying biofilm degrading enzymes could lead to novel anti-biofilm strategies, offering preventive, adjunctive, or alternative approaches to antibiotic therapies.

P137

Characterising and disrupting biofilms in endotracheal tubes

Jeffrey Cheng[1], Dean Walsh[1,2], Chris Parmenter[3], Ana Kotowska[3], Keegan Neave[4], Saskia E Bakker[1], Freya Harrison[1]

[1]University of Warwick, Coventry, United Kingdom. [2]UK Health Security Agency, London, United Kingdom. [3]University of Nottingham, Nottingham, United Kingdom. [4]Neave Engineering Ltd, Leamington Spa, United Kingdom

Abstract

Endotracheal tubes (ETTs) are used to connect hospital patients to mechanical ventilators. ETTs provide a nidus for biofilm formation, and fragments of this biofilm can detach and disperse to the lungs. This leads to ventilator-associated pneumonia, which is costly to treat and often fatal. Treating & preventing ETT biofilm is a major unmet healthcare need.

We have developed synthetic ventilated airway surface mucus (SVAM), which can be used in combination with ETTs in the lab to explore biofilm biology and potential methods of biofilm prevention and removal. We present results demonstrating the successful culture of single-species and polymicrobial biofilm on sections of ETT in SVAM, and show that SVAM cues alterations in biofilm matrix composition and antimicrobial tolerance compared with standard lab medium. We show that certain adjuvants can enhance antimicrobial biofilm clearance in our model. Using cryo methods to preserve the native, hydrated state of biofilm, we are able to visualise ETT biofilm and map the location of key microbial exoproducts within it. We have also employed 3D printing and microcontrollers to present a tractable and affordable ventilator for use in biofilm experiments.

By building a high-validity lab growth platform, we provide new insights into the biology of clinically important biofilm, and demonstrate a pipeline for identifying candidate antimicrobials and adjuvants for use in the prevention and management of ETT biofilm.

Poster: P001

Preventing Biofilms: Leveraging Ultra-Low Liquid-Solid Friction for Non-Bactericidal Antibiofilm Surfaces

Yufeng Zhu[1], Glen McHale[2], Hernan Barrio-Zhang[2], Gary Wells[2], Rodrigo Ledesma-Aguilar[2], Rui Han[3], Hongzhong Liu[4], Waldemar Vollmer[5], Nicholas Jakubovics[6], Jinju Vicky Chen[3]

[1]Shanghai Jiao Tong University, Shanghai, China. [2]University of Edinburgh,, Edinburgh, United Kingdom. [3]Loughborough University, Loughborough, United Kingdom. [4]Xi'an Jiaotong University, Xi'an, China. [5]The University of Queensland, Brisbane, Australia. [6]newcastle university, newcastle, United Kingdom

Abstract

Microbial biofilms, sessile communities encased in self-produced extracellular polymeric substances (EPS), pose significant challenges across diverse fields. Traditional understanding links surface energy to initial bacterial attachment and biofilm formation. This study presents a paradigm shift, suggesting an ultra-low static liquid-substrate friction ("slipperiness") is dominant over the influence of surface energy and normal liquid adhesion in suppressing biofilm development. This implies that surfaces with exceptionally high slipperiness, regardless of their hydrophobicity/hydrophilicity, may resist biofilm formation by both hydrophobic and hydrophilic bacteria. We demonstrated permanently bound liquid-like solid surfaces with either hydrophobic or hydrophilic properties, but exceptionally high slipperiness, could effectively reduce biofilm formation by 3-5 orders of magnitude against two nosocomial pathogens (Pseudomonas aeruginosa, PAO1 and Staphylococcus epidermidis, FH8) compared to Polydimethylsiloxane (PDMS) under both static and dynamic culture conditions for 14 days. Remarkably, these slippery-liquid-like solid surfaces outperform commercially used antimicrobial coatings consisting of silver particles. This work offers a new perspective on surface design for superior anti-biofilm materials.

P003

Influence of non-coding small RNA00203 in biofilm cells of *Acinetobacter baumannii*: Transcriptomic and phenotypic

Daniel Gebrelibanos, Abebe Shenkutie, Franklin Chow, Polly Leung

The Hong Kong Polytechnic University, Hong Kong, Hong Kong

Abstract

Background: Our previous work demonstrated that a novel non-coding small RNA00203 plays a major role in biofilm formation in *A. baumannii* ST1894. However, the comprehensive genome-wide consequences of the sRNA are unexplored.

Methods: Total RNA sequencing was performed on the RNA samples prepared from the biofilm cells of sRNA00203-deleted and wild-type strains. A combination of bioinformatics pipelines were employed to identify the differentially expressed genes (DEGs) and pathways. IntaRNA and CopraRNA prediction tools were used to predict the binding sites of sRNA00203 on the DEGs.

Results: The comparative transcriptomic analysis identified 816 genes significantly influenced by sRNA00203 in biofilm cells (absolute value of $\log_2$ [fold change] ≥ 1, $p < 0.05$). Of the DEGs in the sRNA00203-deleted strain, 406 were upregulated and 410 were downregulated. The functional annotation showed that genes related to AdeIJK efflux pumps and porins (CarO type IV and OmpH) were induced, whereas major facilitator superfamily efflux pump, type IV pili (T4P), quorum sensing and iron acquisition systems were repressed. Phenotypically, the sRNA-deleted strain displayed a significant reduction of T4P under an electron microscope. Altered expression levels of 16 influenced genes as measured by RT-qPCR, showed a strong correlation with RNA-seq data ($r^2 = 0.951$). It is also predicted that presence of binding sites for sRNA00203 is confined to either the 5' UTR and/or the coding regions of these candidate target genes.

Conclusion: The study has demonstrated that the sRNA00203 deletion impairs biofilm formation in *A. baumannii* by suppressing the pili formation, quorum sensing, and iron acquisition pathways.

P004

Role of V. cholerae biofilm formation in Glycan- Host interaction

Bassam Elgamoudi[1], Ronald A. Coetzee[2], Fitnat Yildiz[3], Victoria Korolik[2]

[1]Institute for Biomedicine and Glycomics , Griffith University, Southport, Australia. [2]Griffith University, Southport, Australia. [3]University of California, Santa Cruz, USA

Abstract

In many bacterial and viral illnesses, the interactions between the pathogen and host based on glycans are crucial. Glycan interactions cover a wide spectrum of activities, from molecular mimicry to initial receptor-based adhesion shielding the infectious agent from the host's immunological response. Due to its ability to cause severe and frequently fatal diarrheal illnesses in humans, *Vibrio cholerae* is a major global public health concern. *V. cholerae* biofilms significantly aid the growth and spread of cholera. In this work, we looked into the function of glycans in host-pathogen interactions and the roles of lipopolysaccharide (LPS) and Vibrio polysaccharide (VPS), two components of biofilms, in infection. Glycan array study has demonstrated that *V. cholerae* interacts with various host glycoconjugates, including ganglioside (GM1), blood group antigens, and Lewis antigens. Interestingly, only rugose and VPS mutant strains were able to bind to H and P blood group antigens, while matrix and LPS lost these bindings. This suggests that overproduction of VPS may lead to additional interactions with host cells. We also studied the role of VPS, LPS, and matrix proteins for biofilm formation in the presence of several monosaccharides. Results showed that rugose (Wildtype) significantly increased biofilm production due to overexpression of VPS, while the levels of biofilm were significantly reduced in VPS, LPS, and matrix mutants compared to rugose. Galactose was found to be very effective in restoring some of the functionality of the truncated LPS. Finally, we examined the adhesion of those strains to Caco-2 cells and found severe adhesion defects in VPS, LPS, and matrix strains.

P005

Inhibition of biofilms formation by *Acinetobacter baumannii* phages

Rasha Jabbar, Ban Abdulsattar

Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

Abstract

One of the most difficult infections to treat is *A.* baumannii infection due to its resistance to most antibiotic therapies. Therefore, a bacteriophage infecting *A. baumannii* was isolated from wastewater in order to offer an alternate approach to inhibit the biofilm developed by *A.* baumannii. During the period from October 2023 to February 2024, 26 *A. baumannii* clinical isolates were collected from the laboratory of Ghazi Al-Hariri Hospital, Baghdad, Iraq, from different sources and identified via the automated VITEK®2 system phenotypically and for antimicrobial susceptibility profiles. All the isolates were multidrug resistant (MDR). The microtiter plate test and scanning electron microscopy (SEM) showed that among the 26 isolates, 31% were moderate biofilm formers, 54% were weak biofilm formers, and only 4 isolates were non-biofilm formers (15%). The presence of the biofilm-forming genes *OmpA* and bla_{PER-1} was evaluated. The occurrence rates of the biofilm-related genes *OmpA* and bla_{PER-1} were 16 (61.5%) and 1 (3.8%), respectively. A phage designated RKL was isolated from sewage water. Based on transmission electron microscopy, phage RKL was from the *Siphoviridae* family of the *Caudovirales* order. The optimal multiplicity of infection was at 10. For the host range assay, phage RKL exhibited antibacterial activity against 12 out of 26 *A.* baumannii clinical isolates, with no effect against other tested species. For anti-biofilm activity, phage RKL was able to reduce biofilm formation in 10/22 biofilm-producing isolates. The results suggest that RKL phage has a potential impact in inhibiting MDR *A. baumannii* biofilm producer infections.

P008

Biological clogging applied to developing landfill liners

Alice Morita[1,2], Mercedes Regadio[1]

[1]Universidad Autónoma de Madrid, Madrid, Spain. [2]Universidad Tecnológica del Uruguay, Durazno, Uruguay

Abstract

Biological clogging causes a reduction in the permeability of materials, which can be used in the design of barriers for retaining contaminants, avoiding their leaching into the environment. The present study evaluated adopting waste as support material for developing biological barriers within the landfill design. The experimental setup consisted of vertically positioned PVC columns (50 mm in diameter, 600 mm in length), through which leachate flow ascended from bottom to top. The columns were filled with construction and demolition fine aggregates (CDW), their mixture with tire waste (CDW/TW), or with anaerobic biomass (IN CDW). The columns were fed with raw leachate collected from an active municipal landfill in Madrid, Spain (dissolved organic carbon 278±150 mg/L, pH 7.4±0.4, electrical conductivity 14±5 mS/cm, oxidation-reduction potential –126±100 mV). Head loss and flow measurements enabled to calculate the permeability of the materials over time (Darcy's law). The results showed a reduction in hydraulic conductivities of 2-3 orders of magnitude, achieving better results for the CDW/TW columns (10^{-7} m/s in 170 days), possibly due to the hydrophobicity of the tire, enhancing biofilm adhesion. An exponential correlation showed that hydraulic conductivities of about 10^{-9}m/s (the minimum legal limit for compacted clay liners under non-hazardous and inert waste landfills) can be reached before 420 days of operation. Wastes containing hydrophobic materials can be used for the attachment of biofilms and the development of bio-barriers, protecting landfill clay liners, increasing their lifetime, and potentially reducing their thickness.

P009

Battling Biofilms: Investigating the role of *maoP* in Enterobacteriaceae biofilm formation

Joshua Horton[1,2,3], Emma Holden[1,3], Eleftheria Trampari[1,3], Jacob Malone[3,4,5], Mark Webber[1,2,3]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [3]Centre for Microbial Interactions, Norwich, United Kingdom. [4]John Innes Centre, Norwich, United Kingdom. [5]School of Biosciences, University of East Anglia, Norwich, United Kingdom

Abstract

Biofilm-producing bacteria show higher tolerance to many sanitisers and antibiotics. Understanding how biofilm formation can be manipulated in Enterobacteriaceae, which cause foodborne illnesses, is therefore crucial for healthcare and food production. Given its common use in bioengineering and the robustness of biofilms, understanding into *E. coli* biofilm formation is key for its potential in bioremediation applications. Despite substantial research into the energy-intensive and hence complex and tightly regulated pathways of biofilm formation, much remains to be understood.

maoP has recently been found to be important in biofilm formation in the key foodborne pathogens *Escherichia coli* and *Salmonella* Typhimurium. Originally identified to be involved in the organisation of the chromosomal origin of replication macrodomain, it has since been found to be important in numerous biofilm-related phenotypes in Enterobacteriaceae and suggested to be an RNA-binding protein in *E. coli*; the RNA species are however yet to be described.

This work aims to characterise the mechanism of action of MaoP in biofilm formation, using *Salmonella* Typhimurium as a model. Here we present the genetic context of *maoP* in Enterobacteriaceae and how it may allude to its role in biofilm formation. We also show the phenotype of a *maoP* deletion mutant: a reduction in biofilm biomass and curli production, both of which can be complemented back to wild type via reinsertion elsewhere in the chromosome. Finally, initial investigation has started to discover the binding targets that MaoP interacts with to play its role in biofilm formation.

P010

Chemical composition and integrity of oral biofilm extracellular matrix: Insights from Microarray Polymer Profiling

Alina Goldberg[1,2], Adam Dawson[1], Ilieva Lelenaite[1], Charlotte Fletcher[1], Nick Jakubovics[1], Hamish Yau[2], William Willats[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]Procter & Gamble, Newcastle upon Tyne, United Kingdom

Abstract

Oral biofilms have significant clinical implications, as they not only cause oral diseases such as caries and periodontitis but also facilitate microbial translocation to the gut and bloodstream, contributing to systemic conditions like cardiovascular and kidney diseases. Tooth-associated oral biofilms are categorized into supragingival biofilms, found on exposed enamel surfaces, and subgingival biofilms, located below the gumline and within periodontal pockets. These spatial variations influence the biofilm's microbial community composition and extracellular matrix composition. Utilizing high-throughput Microarray Polymer Profiling (MAPP), a method combining molecular probe specificity with robotic microarray technology, we analysed the extracellular polymeric substances (EPS) from *in vitro* biofilms developed from saliva and clinical plaque samples collected *in vivo*. Biofilms were grown under conditions designed to simulate different environments, and various methods were tested to extract their matrix components. The analysis identified potential rate limiting substrates within the biofilm matrix. Further validation methods were employed to confirm the presence of certain matrix components. Future research will focus on investigating strategies to disrupt biofilm structure, which could pave the way for innovative therapeutic applications

P014

Ferulic acid and sinapic acid attenuate *Pseudomonas aeruginosa* biofilm formation and virulence by targeting the *pqs* quorum sensing system

Miguel M. Leitão[1,2,3], Manuel Simões[1,2,4], Anabela Borges[1,2,4]

[1]LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal. [2]ALICE—Associate Laboratory for Innovation in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465, Porto, Portugal. [3]CIQUP-IMS—Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Porto, Portugal. [4]DEQB—Department of Chemical and Biological Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465, Porto, Portugal

Abstract

Background: Quorum sensing (QS) is a bacterial intercellular communication mechanism mediated by extracellular signalling molecules that regulate gene expression. Pseudomonas quinolone signal (PQS) QS system, which is mediated by autoinducers of the quinolone family, is a fundamental QS component in *Pseudomonas aeruginosa*. It plays a crucial role in the formation and maintenance of biofilms and in the regulation of virulence factors. Phenolic acids, such as ferulic and sinapic acids, are plant secondary metabolites well known for their biological properties and have shown promise in modulating bacterial communication. The aim of this study was to evaluate the potential of ferulic and sinapic acids to inhibit the *P. aeruginosa pqs* QS system and underlying effects on biofilm structure and virulence factor production. **Methods:** The inhibitory effect on the *pqs* system was evaluated using bioreporter strains and bioluminescence-based assays. Biofilm architecture was analysed using optical coherence tomography, while virulence factors (pyoverdine, pyocyanin, total proteases, lipases, gelatinases and siderophores) production and motility was analysed by absorbance measurement and plate agar method. **Results:** Ferulic and sinapic acids inhibited *pqs* QS activity by 90 % at a concentration of 1000 µg mL^{-1}. These compounds significantly changed biofilm architecture, reducing thickness from 96 µm-11 µm. They also markedly reduced the production of key virulence factors and impaired swarming motility. **Conclusion:** Ferulic and sinapic acids demonstrated strong inhibitory effects on the *pqs* QS system, leading to altered biofilm structure and reduced virulence. These findings support their potential as antipathogenic/antivirulence agents for prevention/treatment of *P. aeruginosa* biofilm-associated infections.

P015

Biofilm formation of poultry-associated *Salmonella* evaluated in standardised laboratory models

Emma Brook, Jonathan Lewis, Muhammad Ali, Claire Oastler, Shaun Cawthraw

Animal and Plant Health Agency, New Haw, United Kingdom

Abstract

Salmonella is continually the second most reported foodborne zoonosis in Great Britain and other countries. *Salmonella* contamination on poultry premises poses a significant risk to human and animal health. Isolation of poultry-associated *Salmonella* can be isolated across all areas of the poultry industry, including poultry farms, feed mills and hatcheries. Environmental persistence is partly attributed to ineffective cleaning and disinfection (C&D) measures, such as inappropriate concentrations of biocide, improper application and limited implementation of protocols effective on bacteria in the biofilm state. Bacteria in biofilm are known to be more tolerant to biocides than in the planktonic state and tolerance can vary depending on bacterial species and strain. However, data gaps exist about the biofilm forming ability for *Salmonella* serovars commonly isolated from poultry.

This study assesses biofilm formation of 240 *Salmonella* isolates across 11 serovars of significance in the poultry industry, isolated from meat or the environment of poultry farms, feed mills and hatcheries. The crystal violet assay will be used to assess biofilm formation at 20°C and 30°C following 48- and 120-hours incubation.

It is anticipated that biofilm formation will vary between serovars and source of isolation. Understanding the role of serovar and source of isolation on biofilm formation is crucial for controlling persistence of *Salmonella* and establishing effective C&D protocols for different areas of the poultry industry. Further work will explore biocide tolerance of biofilms in comparison to their planktonic state.

P016

Biofilm formation of bacteria harbouring the plasmid of emerging *Salmonella* Infantis

Emma Brook[1,2], Sian-Marie Frosini[2], Lucy Brunton[2], Ben Swift[2], Francesca Martelli[1]

[1]Animal and Plant Health Agency, New Haw, United Kingdom. [2]Royal Veterinary College, London, United Kingdom

Abstract

Salmonella serovar Infantis is one of the top five most reported *Salmonella* serovars in the EU. It is the most prevalent serovar in chicken broilers and can be highly multi-drug resistant (MDR). Co- and cross-resistance between antibiotics and disinfectants, thought to be encoded on a MDR plasmid, contributes to its persistence in the environment. Additionally, biofilm formation potentially influences the serovars tolerance to biocides.

This study aimed to establish whether biofilm formation differed between antibiotic and disinfectant susceptible isolates (i.e. without plasmid of Emerging *Salmonella* Infantis (pESI)) and conjugated counterparts (i.e. pESI conjugated *in vitro*). *Salmonella* Infantis, *S.* Enteritidis and *Escherichia coli* were assessed. The crystal violet assay was used to evaluate biofilm formation at 20°C and 37°C, for 24, 48, 72, 96 and 168 hours, in various media types.

Biofilm formation varied widely depending on experimental condition. Media heavily influenced biofilm formation. Biofilm production was poor at 24 hours, increased after 48 hours, and varied between 48 and 168 hours depending on media, temperature and isolate. Generally, biofilm formation was stronger at 20°C then 37°C. In some cases, the isolate harbouring pESI produced weaker biofilm than the paired isolate without the plasmid. In very few cases the isolate harbouring pESI produced a stronger biofilm.

Future studies will evaluate the role of different aged biofilms of pESI and non-pESI isolates on disinfectant tolerance. A larger isolate panel should be studied to gain a better understanding of the role of pESI on biofilm production and biocide tolerance.

P017

Evaluating the role of zinc in oral dentifrices to combat volatile sulphur compound production by oral biofilms

Kelly Jane Robertson[1], Paige Lockett[1], Katie Grey[1], Alex Platts[1], Panagiota Yiasemi[1], Rob Howlin[1], Abish Stephen[2]

[1]Haleon, Weybridge, United Kingdom. [2]Queen Mary University, London, United Kingdom

Abstract

Oral malodour results from the production of volatile compounds, especially volatile sulphur compounds (VSCs) including hydrogen sulphide (H_2S) by oral biofilms. Beyond the social issues, these compounds are also associated with the inflammatory response. This study aimed to evaluate the role of zinc to combat VSCs produced by oral biofilms.

Chemical neutralisation by zinc within a toothpaste formulation was tested via a gas generator producing H2S at 1.25ppm which was 'bubbled' through samples and measured on a gas chromatograph (Agilent 8890). Additionally, biofilms were grown on hydroxyapatite discs in a CDC Bioreactor using a mixed species oral inoculum and stained using a H2S Fluorescent probe- P3 (Sigma-Aldrich) to visualise bacterial metabolic production of hydrogen sulphide and quantify their reduction. Finally, biofilms grown in a sorbarod-based perfusion model were treated with either a standard sodium fluoride dentifrice (SFD) or a zinc containing dentifrice (ZnD) The expression of VSC-producing genes cysteine desulfhydrases (cdh*)* and methionine gamma lyase (mgl) was evaluated by qPCR.

There was a decrease in hydrogen sulphide output with the zinc containing toothpaste relative to the concentration of zinc (0% Zinc in dentifrice: 12807µV*s and 1% Zinc in the same formulation: 69.667µV*s). Staining identified H2S produced by the biofilm which was reduced following treatment with a zinc containing toothpaste. Zinc containing toothpaste was also able to reduce VSCs produced by oral biofilm in the sorbarod model and qPCR showed a reduction in VSC producing genes.

The present study confirms that zinc is effective in reducing VSCs produced by oral biofilms.

P018

Investigation of quorum sensing inhibition in diverse biofilm models of *Pseudomonas aeruginosa*

Dimitra Panagiotopoulou[1], Carey Lambert[1], Sam Fenn[2,1], Shaun Robertson[3,1], Fadi Soukarieh[1,4], Brogan Richards[1], Manuel Romeron[5], Miguel Cámara[1], Kim Hardie[1]

[1]University of Nottingham, Nottingham, United Kingdom. [2]University College Cork, Cork, Ireland. [3]MiDX LTD, Nottingham, United Kingdom. [4]University of Sheffield, Sheffield, United Kingdom. [5]University of Santiago de Compostela, Santiago de Compostela, Spain

Abstract

Chronic lung infections in hospitalised patients caused by *P. aeruginosa* biofilms are persistent and increasingly tolerant to current antibiotic treatments. A promising strategy is to make the cells avirulent and increase antibiotic susceptibility by silencing the Quorum Sensing (QS) cell-cell communication system of *P. aeruginosa.* Our lab has developed SEN089, a Pqs QS system inhibitor that antagonises PQS for binding to the cognate transcriptional regulator PqsR. Clinical strains of *P. aeruginosa* produced less pyocyanin and were more susceptible to ciprofloxacin and tobramycin when treated with SEN089. Here, the effect of SEN089 was explored through transcriptomics in two different biofilm models to understand the impact each model might have on the activity of the inhibitor. Additionally, the models included both single species and polymicrobial biofilms with *Staphylococcus aureus* and *Candida albicans.* Multiple phenotypes were interrogated, including siderophore production, increase of antibiotic resistance and virulence factor production. This study will help identify how the biofilm model along with the presence of a polymicrobial environment, may affect the activity of antivirulent compounds, hence informing the choice of model for the development of future antibacterial strategies.

P019

Regulation of Bacterial Biofilm Dispersal by Multi-Domain Red-Ox Sensing Proteins

Malek Hawela[1,2], Ivo Tews[1,2], Jeremy Webb[1,2]

[1]University of Southampton, School of Biological Sciences, Southampton, United Kingdom.
[2]National Biofilms Innovation Centre, Southampton, United Kingdom

Abstract

Pseudomonas aeruginosa is a gram-negative bacterium ubiquitously present in the environment. It is an opportunistic pathogen and is part of the ESKAPE group, which are the primary sources of nosocomial infections in healthcare environments. *P. aeruginosa* is shown to have a pervasive expression of antibiotic resistance mechanisms and demonstrates a robust capability for biofilm formation. As such, the presence of *P. aeruginosa* has been correlated with a poorer prognosis in persons with cystic fibrosis and immunocompromised patients. Biofilms are a key mechanism in antibiotic resistance. Within biofilms there are redox gradients, occurring due to factors such as oxygen and nutrient availability. Redox activity plays a key role in biofilm dispersal. Examples include production of reactive oxygen species by neutrophils which mediate destruction of biofilm structures or nitric oxide which induces biofilm dispersal.

In this project we focus on the study of several key membrane proteins which sense nitric oxide and have a role in regulating production of Cyclic diguanylate monophosphate (C-di-GMP). C-di-GMP is a signalling molecule which upregulates biofilm formation. We will be utilizing confocal microscopy, flow cell culture, attachment assays, fluorescence staining and crystallography to study the effects of modulating these proteins. Using a combined structural bioinformatic and phylogenetic analysis approach one can better identify target sensory domains within proteins, which can bind nitric oxide and other redox mediating compounds.

More specific targeting of redox sensors is expected to improve the efficacy of antibiotics, through improved targeting of localised biofilm signalling pathways and localised pools of c-di-GMP production.

P021

The Biomass Battle: Super-Resolution Microscopy Reveals Key Differences in Biofilm and Planktonic Cell Kinetics

Dishon Wayne Hiebner, Jacobus Brink, Raghu Krishna Moorthy, Ciaran Dunne, Dibyangshee Singh, Eoin Casey

School of Bioprocess and Chemical Engineering, University College Dublin, Dublin, Ireland

Abstract

Background: Biofilms represent a highly structured and resilient bacterial phenotype, distinct from their planktonic counterparts. While the physiological differences between these states are well recognized, direct quantification of biomass kinetics at the single-cell level remains limited. Understanding how biomass accumulation differs between these phenotypes is critical for refining models of biofilm growth and persistence.

Methods: To address this, we employ single-cell super-resolution confocal imaging to track and compare biomass accumulation in biofilm-associated and planktonic cells over time. By resolving individual cells within structured biofilms and freely suspended populations, we generate high-resolution, quantitative data on biomass kinetics. Our approach enables biomass measurements based on cell count rather than biovolume, providing an enhanced framework for assessing growth dynamics at the single-cell level.

Results: Our findings suggest differences in biomass accumulation between the two phenotypes, with biofilm-associated cells potentially exhibiting distinct growth kinetics compared to planktonic cells. However, further investigation is required to confirm whether biofilms demonstrate slower yet sustained biomass accumulation or follow a different kinetic profile. These findings provide insights into potential metabolic adaptations governing resource allocation, structural reinforcement, and long-term survival in biofilms.

Conclusion: By leveraging super-resolution microscopy for real-time kinetic analysis, this study enhances our ability to quantify biofilm growth kinetics at the cellular level. These insights will contribute to the refinement of mathematical models of biofilm growth and have implications for microbial ecology, biofilm control strategies, and antimicrobial interventions.

P024

Spatial Heterogeneity in Biofilms: Insights from High-Resolution Imaging

Jacobus Brink, Dishon Hiebner, Raghu Moorthy, Ciarán Dunne, Dibyangshee Singh, Eoin Casey

University College Dublin, Dublin, Ireland

Abstract

Biofilms are dense, hydrated cell clusters bound by extracellular polymeric substances (EPS) that enable adaptation across diverse niches. However, cells can disperse from biofilms into a planktonic state depending on conditions like nutrient or oxygen limitation, making biomass quantification challenging due to dynamic changes over time. In this study, we utilized high-resolution single-cell imaging to explore the dynamic processes underlying biofilm formation, maturation and dispersal over time. Using advanced imaging techniques, we followed individual *Pseudomonas fluorescens* or *Escherichia coli* cells as they developed into clusters across diverse environmental conditions, including 96-well plates, glass coverslips and flow cells. This approach allowed us to uncover how nutrient availability and dynamic conditions shape biofilm architecture and drive spatial heterogeneity in bacterial communities.

Our findings demonstrate that biofilm growth, morphology, and dispersal are influenced by nutrient availability, cell-cell interactions, and structural properties within the EPS matrix. Nutrient conditions significantly affected biofilm architecture, with lower nutrient availability promoting three-dimensional microcolonies, with biomass peaking at around 12 hours after incubation, while biomass peaked at 8 hours for nutrient rich conditions and resulted in a decrease in biofilm height, as well as differences in cell shape, spacing and orientations. This study also highlighted how nutrient levels and EPS components, particularly polysaccharides and extracellular DNA (eDNA), play key roles in biofilm growth, morphology, and dispersal, leading to distinct spatial heterogeneity in bacterial colonies. These findings contribute to a deeper understanding of biofilm formation and offer a framework for developing targeted strategies to manage biofilms in various applications.

P025

Testing the efficacy of disinfection procedures on complex sink drain biofilms in a close-to-life assay

Nicole van Leuven[1,2], Ralf Lucassen[1], Jean-Yves Maillard[3], André Lipski[2], Dirk Bockmühl[1]

[1]Rhine-Waal University, Kleve, Germany. [2]University of Bonn, Bonn, Germany. [3]Cardiff University, Cardiff, United Kingdom

Abstract

Drain biofilms are omnipresent in all One Health areas and are able to disseminate resistant bacteria by splashing and cross-contamination of pipes. We used a small-scale complex biofilm model simulating sink traps to grow and test biofilms originated from homes, healthcare settings and farms from three European countries. Cell counts before and after biocide treatments were analysed by agar plating, while the genetic resistance marker *intI*1 was checked for using qPCR. Different types of biocides were tested for their bactericidal efficacy and biofilm regrowth potential. Regardless of the biofilm origin, a formulation based on essential oils achieved the highest mean reduction. Still, reactive oxidizing agents also showed a good efficacy against biofilms, but with higher variations depending on the biofilm. For oxidizing agents, farm biofilms seemed less susceptible to biocide treatment, while the biological disinfectant barely showed differences in the mean reductions of biofilms from the different One Health areas. Slightly elevated values for the *intI1* gene were found for some biofilm-biocide combinations.

In total, our biofilm model allowed to estimate the influence of different biocides on sink biofilms from different settings in a practice-relevant way. Efficacy in cell reduction and influences on genetic resistances of disinfectants varied and showed unique impacts depending on the biofilm.

P027

Imaging and quantifying effective biofilm removal from dentures to reduce the risk of denture stomatitis

Roxane Bonithon[1], Sarah Davis[1], Katerina Karali[1], Marta Roldo[2], Lauren Ashcroft[2], Jason Brown[3], Andy Hunt[4], Sandra Clifford[4], David Bradshaw[4], Behrad Mahmoodi[4], Maria-Teresa Addison[4], Eva Jeffcock[4], Rob Howlin[4]

[1]School of Electronics and Mechanical Engineering, University of Portsmouth, Portsmouth, United Kingdom. [2]School of Pharmacy & Biomedical Sciences, University of Portsmouth, Portsmouth, United Kingdom. [3]Oral Sciences Research Group & Glasgow Biofilm Research Network, University of Glasgow, Glasgow, United Kingdom. [4]Haleon, St. Georges Av., Weybridge, United Kingdom

Abstract

Denture stomatitis (DS), an inflammatory condition associated with biofilm accumulation on dentures, affects ~70% of wearers globally. Effective cleaning is essential to mitigate the associated risks.

This study assessed the effectiveness of a commercially available denture cleanser (DC) tablet in removing biofilm from full palate-less dentures. Biofilms were grown using an 11-species model for 8 days or Klebsiella pneumoniae alone for 4 days. Single-species biofilms were stained with 1% PTA in 70% EtOH and treated with a DC tablet for 3min, with or without brushing. High-resolution X-ray computed tomography (XCT) imaging was conducted using an Xradia 520 Versa system. Mixed-species biofilms were treated daily for 7days with DC tablets for 3 or 15min, with or without brushing, followed by staining with crystal violet (CV) and Live/Dead imaging. Quantitative PCR (qPCR) was performed to measure total microbial counts.

XCT imaging showed DC tablet treatment was effective at removing biofilm from the denture, including hard-to-reach areas (eg. interproximal spaces) showing >85% of biofilm removal from the denture surfaces and >95% from hard-to-reach areas. Effective removal was also observed from all areas of the denture, including hard-to-reach areas using CV and Live/Dead staining. qPCR showed that all treatments reduced the number of viable biofilm cells ($p<0.05$). Time dependent killing effects were observed, with 15min treatment regime more effective (>2-log reduction) than 3min interventions (~1-log reduction).

This in-vitro study demonstrates that DC tablets significantly reduce biofilm burden and viability, particularly in hard-to-clean areas, highlighting their potential for effective denture hygiene.

P028

Towards the Development and Control of Structured, Mixed-Species Biofilms for Biocatalytic Applications

Selina Hanisch[1], Rohan Karande[2], Katja Bühler[1]

[1]Helmholtz Centre of Environmental Research - UFZ, Leipzig, Germany. [2]Leipzig University, Leipzig, Germany

Abstract

Nature-based solutions, such as the microbial mat concept, offer resource-efficient solutions to spatial areas facing environmental and economic challenges. Unlike single-species systems, these complex and robust consortia take optimal advantage of the solar spectrum, thus, concerting the activity of the different locally-associated microorganisms for the design of energy-efficient photo-biocatalytic processes. The aim is to engineer such structured communities on different scales with the target of producing commercially attractive products like biohydrogen via biological pathways.

A model consortium was developed using *Synechocystis* sp. PCC 6803, a photoautotrophic cyanobacterium that absorbs visible light and fixes CO_2, *Pseudomonas taiwanensis* VLB120eGFP, a chemoheterotroph that initiates biofilm formation and consumes O_2, and *Rhodopseudomonas palustris*, a photoheterotroph that can utilize infrared light to generate H_2. Key to controlling these communities is the quantitative understanding of oxygen dynamics, as O_2 directly influences mass and energy flux within the biofilm.

To achieve this, the consortium was cultivated under continuous flow conditions in a custom-designed microfluidic flow-cell set-up. Phosphorescent microbeads embedded within the biofilm enabled in-situ oxygen measurements along the layer thickness. A crucial discovery was that the presence or absence of organic carbon source glucose modulated oxygen concentrations within the biofilm over time, likely due to shifts in *P. taiwanensis* abundance. This demonstrates that intra-biofilm oxygen levels can be actively controlled - a crucial step towards engineering robust, structured phototrophic biofilms for biotechnological applications.

P029

Microbial Profile of Dental Plaque (Biofilm) in Children with Early Childhood Caries

Sarah Alkandari[1,2], James Garnett[1], Marie Therese Hosey[1], David Moyes[1]

[1]King's College London, London, United Kingdom. [2]Kuwait University, Kuwait city, Kuwait

Abstract

Biofilm/plaque formation is an important survival strategy commonly used by oral bacteria and fungi. Early childhood caries (ECC) is a biofilm-mediated disease resulting from an unfavourable ecological shift in the oral microbiota. This study aimed to perform metagenomic microbial analysis of the supragingival dental plaque in children with ECC.

The study protocol was approved by the Health Research Authorities (REC Reference: 22/NW/0403). After obtaining parental informed consent, an oral examination was performed. Supragingival plaque samples were collected from medically-fit patients under six years who had ECC and attended general anaesthesia for dental treatment at Guy's and King's College Hospitals. Control samples were collected from caries-free matched subjects, who attended dental trauma clinics at both Hospitals. Microbial DNA was extracted from the collected plaque samples using the DNeasy PowerSoil Pro kit per the manufacturer's instructions. Qubit fluorometer was used to assess the quantity of the DNA in the purified samples.

A total of 100 paediatric dental patients have participated in the current study. The median total DNA concentration for the ECC and caries-free related samples were 4,140 ng and 1,161 ng, respectively. The purified DNA samples (containing at least 200 ng), frozen on dry ice, were shipped to the DAS MICROBIOME LTD laboratories (Turkey), an external third-party company with appropriate agreements. A comprehensive picture of the microbiota of the clinical samples will be obtained using WGS metagenomics. We will carry out species-level microbial profiling, strain-level microbial profiling, and functional microbial profiling. Both Alpha and Beta diversities will be analyzed.

P030

Defining the Co-occurrence of Bacterial Communities Within the Biofilms of Diabetic Wounds

Emma Wright[1,2], Katja Hill[1], David Thomas[1], Faizan Sadiq[1]

[1]Advanced Therapies Group, Cardiff University, Cardiff, United Kingdom. [2]QBiotics Group Limited, Yungaburra, Australia

Abstract

Diabetic foot ulcers (DFUs) are non-healing chronic wounds and a rising cause of morbidity and mortality. The polymicrobial wound microbiota plays a critical role in wound progression and treatment outcomes. This study systematically reviewed the literature to characterise the microbiota in DFUs and model bacterial association. Following PROSPERO registration (ID: CRD42024587324), we identified studies in which molecular or culture-based methods had been utilised. Ecological modelling of association was studied using 'Co-occur' in R and 'HiOrCo' (Python). Statistical significance was assessed using a probabilistic co-occurrence model and determined at a false discovery rate (FDR)-corrected *P*-value threshold of 0.05. Nine studies were selected including a total of 248 DFU samples with 89 bacterial genera identified. High bacterial diversity was observed for samples analysed by molecular methods, with *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Anaerococcus* and *Finegoldia* being the most abundant genera. Modelling revealed 117 positive and 37 negative pairwise associations. Among the significant positive associations, the most frequently co-occurring pairs were *Staphylococcus-Streptococcus* (46.7% of samples), *Corynebacterium-Staphylococcus* (34.3%), and *Anaerococcus-Finegoldia* (30.7%). Higher-order co-associations (>2 species) predominately included members of these genera, along with *Peptoniphilus* and *Prevotella*. Across all co-occurrence patterns, there was a high prevalence of facultative and obligate anaerobic Gram-positive bacteria. These findings define the microbial associations within DFU biofilm communities and highlight the importance of anaerobic Gram-positive bacteria in these communities and their involvement in metabolic networks. This analysis offers a framework for building rationally-designed model biofilm communities representing DFUs to study their ecological interactions and develop targeted antimicrobial therapies.

P031

Fluorescence imaging reveals organization of *Mycobacterium marinum* biofilms *in vitro* and *ex vivo*

Nelli Vahvelainen[1], Leena-Maija Vanha-aho[1], Milka Hammarén[1,2], Aleksandra Svorjova[1], Alina Sillanpää[1], Saara Lehmusvaara[1], Mataleena Parikka[1]

[1]Tampere University, Tampere, Finland. [2]European Molecular Biology Laboratory, Heidelberg, Germany

Abstract

Biofilm forming bacteria contribute to many chronic and recurring diseases. The respiratory illness tuberculosis is caused by *Mycobacterium tuberculosis* that forms biofilm *in vitro* and *in vivo*. The high antibiotic tolerance of biofilms reduces the efficacy of tuberculosis treatment. Therefore, novel treatment methods, such as antibiofilm compounds, are needed.

The zebrafish pathogen *Mycobacterium marinum* is a close relative to the human pathogen. Fluorescent *M. marinum* strains were created by introducing plasmids for fluorescent protein expression into the bacterium. We also created fluorescent deletion mutant strains and strains that overexpress certain *M. marinum* proteins. The selected proteins were hypothesized to affect biofilm formation. Mature biofilms were imaged with confocal microscopy to reveal their 3D-structures. The fluorescent bacteria were also used to infect adult zebrafish. Bacterial granulomas were collected from the fish 2–13 weeks after infection and used either for whole-mount staining or to prepare paraffin sections for staining. A variety of dyes that stain ECM components were used to visualize the structure and organization of bacteria in granulomas.

The 3D-structures of submerged-type biofilms revealed that *M. marinum* forms cord-like structures *in vitro*. Mutants forming thicker cords and overall thicker biofilm had increased tolerance. This suggests a strong link between biofilm structure and antibiotic tolerance.

Granulomas are composed of bacteria encapsulated by host immune cells. The imaged granulomas contained known ECM components indicating that *M. marinum* forms biofilm *in vivo*. Understanding the structure and organization of mycobacterial biofilm *in vivo* could help the development of more rapid and effective tuberculosis treatment.

P032

Testing Novel Antimicrobial Surface Coatings using Biocalorimetry to Prevent Biofilm Formation

Erica Li, Jontana Allkja, Rasmita Raval

Open Innovation Hub for Antimicrobial Surfaces, Surface Science Research Centre, Department of Chemistry, University of Liverpool, Liverpool, United Kingdom

Abstract

Bacterial biofilms are prolific in a variety of different environments and are difficult to control due to their complexity and intrinsic ability to resist the action of antimicrobials. The burden of biofilms is a global challenge that has huge economic costs and impact on human quality of life. In order to tackle the issue of biofilms, new innovative surfaces are being explored that are antimicrobial or anti-fouling. Biocalorimetry provides data on the metabolic activity of biofilms and the effect of antimicrobial compounds with a high degree of sensitivity. By measuring metabolism, we are able to acquire more comprehensive information about the activity of a bacterial population as a whole. Importantly, surfaces that are complex in nature can be assessed with biocalorimetry. Dynamic changes to bacterial cells that cannot be captured by normal culture techniques can be seen, which may indicate changes to their behaviour and gene expression. Biocalorimetry also allows the assessment of surfaces to be conducted in a way that requires no additional dyes, is non-destructive to a bacterial sample and allows for follow-up tests to be conducted on the same samples. In our work, we are using biocalorimetry to test the efficacy of novel surface coatings in preventing the growth and attachment of organisms such as *Staphylococcus aureus* on non-porous surfaces.

P034

Synthetic phage-inducible chromosomal islands efficiency depends on biofilm composition.

Nahiara Garmendia-Antoñana[1,2], Pedro Dorado-Morales[3], Carmen Gil[4], Begoña García[2], Maite Echeverz[1], Cristina Solano[1], Iñigo Lasa[1]

[1]Universidad Publica de Navarra, Pamplona, Spain. [2]Navarrabiomed, Pamplona, Spain. [3]Institut Pasteur, Paris, France. [4]CIMA, Pamplona, Spain

Abstract

Treatment of infections caused by *Staphylococcus aureus* is increasingly limited to a very narrow group of antibiotics because a significant percentage of strains are resistant to several families of antibiotics. This problem is compounded by the fact that *S. aureus* is also a relevant pathogen in farm animals, where the treatment of infections such as mastitis in the dairy industry involves prolonged antibiotic therapy. In this study, we constructed genetically engineered phage-inducible chrosomomal islands (ePICIs) carrying a CRISPR-Cas9 system targeting genes encoding small RNAs. Our results show that ePICIs have a broader spectrum of bactericidal activity compared to phages. ePICIs do not need to integrate into the chromosome of the recipient bacterium to exert their bactericidal effect, making it feasible to produce them without the integrase gene, as long as the producing strain provides the integrase in trans. The bactericidal efficacy of ePICIs against the same strain depends on the guide RNA (gRNA) activity, while their activity against different strains relies on the composition of the extracellular matrix. Specifically, a Bap-mediated extracellular matrix protects against ePICIs under planktonic and biofilm conditions, whereas a PIA/PNAG-mediated exopolysaccharide matrix does not. In a mouse model of mastitis, ePICIs demonstrated similar bactericidal activity to vancomycin in treating *S. aureus* infections. Based on these findings, we propose ePICIs as a promising candidate for controlling mastitis infections.

P035

Understanding the role of mycobacterial biofilm formation in antibiotic tolerance through genetic screening of *Mycobacterium marinum*

Emilia Lintilä[1], Nelli Vahvelainen[1], Jesse Anttila[1], Erika Niininen[1], Laura Kantanen[1], Milka Hammarén[1,2], Mataleena Parikka[1]

[1]Tampere University, Tampere, Finland. [2]European Molecular Biology Laboratory, Heidelberg, Germany

Abstract

Tuberculosis is an infectious disease that caused over 1 million deaths in 2023. The causative agent of the disease, *Mycobacterium tuberculosis* forms biofilms *in vitro* and *in vivo*, which likely contributes to antibiotic-tolerant phenotype of mycobacteria. Thus, we aim to find and characterize mutants with biofilm defects to understand the role of biofilms and ECM components during tuberculosis infection. The goal of this study is to discover regulatory mechanisms underlying biofilm formation and drug tolerance of mycobacteria.

The study is carried out with *Mycobacterium marinum* which is closely related to *M. tuberculosis*. We aim to screen 18,000 ϕMycoMar T7 transposon mutant clones on Congo red plates to identify mutants with different color or colony morphology from wild type *M. marinum*. The Congo red dye binds to ECM components such as amyloids and cellulose. Currently, 4200 mutants have been screened, and 158 potential hits with red and/or rough or smooth morphology have been identified. The biomass per cell of the hit clones were quantified by crystal violet staining. Mutants with biofilm defects were screened for antibiotic tolerance. Among the hits identified so far, 40% of mutants with red or smooth morphology were significantly less tolerant to a common first-line antibiotic, rifampicin, than the wild type *M. marinum.* Lastly, the most interesting mutants will be sequenced, further characterized using proteomics, and tested for virulence and tolerance *in vivo* in the adult zebrafish tuberculosis model.

P036

Multi-excitation Raman spectroscopy for direct bacterial identification and antimicrobial resistance characterisation

Callum Highmore, Niall Hanrahan, Yoshiki Cook, Sumeet Mahajan, Jeremy Webb

University of Southampton, Southampton, United Kingdom

Abstract

Time to diagnosis of bacterial infection is reliant on culture techniques, which leads to delays in effective treatment and the unnecessary prescription of broad-spectrum antibiotics. To expedite diagnosis and delivery of effective treatment, we have developed a multi-excitation Raman spectroscopy methodology (MX-Raman) that enhances Raman capacity for pathogen identification and characterisation, combined with machine learning techniques.

Twenty clinical isolates of *Pseudomonas aeruginosa* were identified by their Raman spectra using a latent Dirichlet allocation (LDA) to an accuracy of 93%. The MX-Raman approach outperformed equivalent single-wavelength analyses. Antimicrobial sensitivity profiles of the isolates to four antibiotics were predicted using their Raman spectral signatures and twelve classification models. Best performing models were partial least squares k-nearest neighbours (PLS-KNN) and partial least squares support vector machine (PLS-SVM), classifying AMR profiles of each strain with 90-99% success. Resistance to ciprofloxacin was predicted with 99% accuracy. Multispecies biofilms of *P.* aeruginosa, *Staphylococcus aureus,* and *Burkholderia cenocepacia* were subjected to Raman imaging and confocal microscopy to validate the identification of bacterial species by Raman spectroscopy in a spatially resolved context. Finally, bacterial signals have been identified directly in patient sputum samples using MX-Raman.

Together these data demonstrate the potential for MX-Raman as a rapid and direct alternative diagnostic tool for infection, that will reduce the burden on hospital bed space, improve clinical outcomes and slow the rise of antimicrobial resistance.

P037

AI-2 Production and Biofilm Formation in Meat-Spoiling *Pseudomonas* spp.

Yasemin Şefika Küçükata[1], Hasan Yetim[1], Banu Metin[2]

[1]İstanbul Sabahattin Zaim Üniversitesi, İstanbul, Turkey. [2]Yıldız Technical University, İstanbul, Turkey

Abstract

Pseudomonas spp. are major contributors to refrigerated meat spoilage, utilizing biofilm formation and quorum sensing (QS) mechanisms for survival. Autoinducer-2 (AI-2), a universal QS signal, plays a role in bacterial communication, yet its relationship with biofilm production in meat-spoiling *Pseudomonas* remains unclear. This study investigates AI-2 and biofilm production in *Pseudomonas* isolates to assess their potential role in spoilage. A total of 81 *Pseudomonas* isolates were obtained from refrigerated beef and minced meat, with molecular characterization identifying 15 as *P. fragi* and 57 as *P. bubulae*. All isolates showed active motility for swimming, swarming, and twitching. Biofilm formation was initially assessed using Congo red agar, where 37 isolates exhibited biofilm-positive phenotypes. Quantification via a microplate spectrophotometer confirmed significant biofilm production in these strains. AI-2 production was evaluated using a *Vibrio campbellii* (ATCC BAA-1117) biosensor, revealing luminescence in 25 isolates, with the highest activity observed in YK50 (69.87%), YK107 (63.72%), and YB91 (19.10%). Principal component analysis (PCA) was performed to explore potential correlations between AI-2 and biofilm formation, revealing no direct relationship. These findings suggest that meat spoiler *Pseudomonas* sp. can use different signal molecules or regulators to maintain its spoilage characteristics. Future research will focus on AI-2-related gene deletions to determine their functional role in biofilm development. This study provides insights into biofilm formation mechanisms in meat-spoiling *Pseudomonas*, contributing to a better understanding of spoilage-associated activities and potential strategies for mitigating bacterial persistence in food environments.

P038

Enhancing early-stage antimicrobial screening: The importance of physiologically relevant assays for predicting drug success

Adam Varney[1], Eden Mannix-Fisher[1], Vanina Garcia[2], Samantha McLean[1]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

Background: The rise of antimicrobial resistance has driven interest in developing novel drugs to combat resistant pathogens. Early drug development relies on cost-effective high-throughput screening, but expenses escalate as promising leads advance through preclinical and clinical trials. Failures at these stages result in significant financial losses for pharmaceutical companies. With biofilms causing up to 80% of infections, early assessment of antimicrobial efficacy that includes both planktonic growth and biofilm testing requires standardized assays and models that better reflect real-world conditions to provide better prediction of downstream success. In vitro bespoke assays and models, often developed in academic settings, lack the standardization, cost-effectiveness, and reproducibility needed for industrial application. Ensuring that screening methods are both practical and relevant is crucial for streamlining antimicrobial development and minimizing costly failures is crucial to antimicrobial drug discovery.

Methods: We assessed the impact of growth conditions on biofilm formation and antimicrobial activity for a variety of clinically relevant pathogens, utilising commercially available equipment and media components that better reflect the real-world environment under study.

Results and conclusions: We found that media composition significantly influenced growth, biofilm formation, and antimicrobial sensitivity. This highlights the importance of developing physiologically relevant assays and models early in drug development, as they offer a more accurate predictor of market success than traditional methods relying on nutrient-rich laboratory media. Implementing such approaches can ultimately reduce the cost of antimicrobial product development.

P041

Impact of surface properties on biofilm formation and antimicrobial production in *Phaeobacter piscinae*.

Xavier Ferrer-Florensa, Yuyan Liu, Claus Sternberg, Paul Kempen, Rafael Taboryski, Lone Gram

DTU, Kongens Lyngby, Denmark

Abstract

In recent decades, aquaculture fish production has increased significantly; however, it continues to face challenges from infectious diseases and the use of antibiotics. Sustainable alternatives, such as marine probiotics, are needed. *Phaeobacter piscinae*, a member of the *Roseobacter* group, is a promising probiotic due to its production of tropodithietic acid (TDA), which is enhanced in the biofilm state. The purpose of this study was to determine surfaces that would enhance biofilm formation and, consequently, increase TDA production.

P. piscinae biofilms were formed on PP, PMMA, COC, and PDMS surfaces in microtiter plates growing in 3% Instant Ocean, 0.3% CasAmino Acids, 0.3% HEPES, and 0.2% glucose. Syto 62 staining analysed bacterial coverage and biovolume, while GFP expression driven by the *tda*CDE promoter was used to monitor TDA biosynthesis gene expression. Z-stack images were captured using a confocal laser scanning microscope (CLSM), and coverage, biovolume, and *tda*CDE gene expression were analyzed using BiofilmQ with Otsu's thresholding method.

During the early attachment (6 hours incubation) PDMS supported the highest biofilm coverage at 60%, significantly surpassing the other surfaces, which had less than 10%. However, after 24 and 96 hours, biovolume and TDA biosynthesis gene expression were similar across all surfaces, indicating that biofilms eventually colonize each surface equally.

In conclusion, *P. piscinae* shows a preference for initial attachment on PDMS surfaces, but after prolonged growth, biofilm distribution becomes uniform across all tested surfaces.

P042

Deciphering the role of the c-di-GMP network in the control of the biofilm master regulator CsgD in *Salmonella*

Leire Azparren, Laura Imedio, Maite Echeverz, Begoña García, Iñigo Lasa, Cristina Solano

Laboratory of Microbial Pathogenesis, Navarrabiomed-Universidad Pública de Navarra (UPNA)-Hospital Universitario de Navarra (HUN), IdiSNA, Pamplona, Spain

Abstract

In *Escherichia coli* and *Salmonella enterica*, the transcription factor CsgD is considered the master regulator of biofilm formation because it activates the production of cellulose and curli fimbriae, principal components of the biofilm extracellular matrix. In *E. coli*, expression of *csgD* is tightly controlled by the levels of the second messenger cyclic diguanylate monophosphate (c-di-GMP), that is synthesized by diguanylate cyclases (DGCs) and degraded by specific phosphodiesterases (PDEs). The transcriptional regulator MlrA binds the DGC DgcM and the complex activates *csgD* transcription. When intracellular c-di-GMP levels are low, the PDE PdeR binds the complex and blocks *csgD* transcriptional activation. Conversely, when intracellular c-di-GMP levels rise due to the activity of the DGC DgcE and the PDE PdeH, PdeR releases both DgcM and MlrA, enabling expression of *csgD* and biofilm production. In this work, we aimed to decipher the c-di-GMP network controlling *csgD* expression, and thus, biofilm formation in *Salmonella*. For that, we constructed single DGC and PDE mutants, sequential DGC mutants and a multiple DGC mutant devoid of c-di-GMP. Analysis of their biofilm phenotype together with quantification of c-di-GMP, evaluation of *csgD* expression levels, and two-hybrid analysis showed notable differences with respect to the network in *E. coli*. First, MlrA does not need to bind a DGC to be fully active. Second, most DGCs and the PDE PdeC control c-di-GMP levels upstream PdeR and third, the PDE PdeK shows an unforeseen role in enhancement of diguanylate activity of certain DGCs.

P043

Proof-of-Concept study on innovative approaches for monitoring and controlling Listeria monocytogenes on food contact surfaces

Ana Victoria Gutierrez[1], Jatin Joshi[2], Kasper Nørskov[3], Matthew Gilmour[1], Maria Diaz[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Vitec Microgenix, London, United Kingdom. [3]Symcel, Stockholm, Sweden

Abstract

Biofilms containing pathogens like *Listeria monocytogenes* can persist on food contact surfaces, posing significant food safety challenges in processing environments (FPE). Conventional disinfectants often fail to completely eradicate these resilient biofilms. This proof-of-concept study explores novel strategies developed by industrial collaborators to enhance pathogen control by: 1) assessing the capability of microcalorimetry technology, using the calScreener instrument, to monitor biofilm viability and biocide efficacy under realistic FPE conditions, and 2) evaluating the potential of surface coatings to prevent biofilm formation.

L. monocytogenes biofilms will be cultivated at 10°C and 20°C on stainless steel and plastic surfaces. Biofilm development will be monitored by measuring metabolic activity through heat production as a proxy for cell viability. Leveraging the calScreener's ability to evaluate biofilm growth in a non-destructive manner, we will evaluate subsequent treatments, including biocide application, washing, and media replacement, with all findings validated against traditional plate count methods. Additionally, biofilm formation will be compared between VSC-1000-coated and uncoated food contact surfaces, with effectiveness evaluated based on metabolically activity and CFU reduction.

This study introduces a less labour-intensive approach for monitoring biofilm development and assessing biocide efficacy under conditions resembling those used in the food industry. Furthermore, it evaluates the potential of VSC-1000, previously successful in reducing hospital-acquired infections, as a preventive measure against biofilms containing foodborne pathogens in FPE. These findings aim to provide actionable solutions for improving food safety by addressing critical gaps in biofilm control.

P044

A Systematic Review of the Co-occurrence of Bacteria Within Chronic Venous Leg Ulcer Biofilm Communities

Jacob Evans[1,2], Emma Wright[1,2], David Thomas[1], Katja Hill[1], Faizan Sadiq[1]

[1]Advanced Therapies Group, Cardiff University, Cardiff, United Kingdom. [2]QBiotics Group Limited, Yungaburra, Queensland, Australia

Abstract

Chronic venous leg ulcers (CVLUs) in elderly patients affect their quality of life and are a source of increased morbidity. Within CVLUs, biofilm communities and their bacterial metabolites play pivotal roles in sustaining inflammation and contributing to the impaired wound healing that characterises the disease. The interactions between different bacterial populations within the wound biofilm is important in directing these responses but remains poorly defined.

Following PROSPERO registration (CRD42024587324), a systematic review of the literature was conducted to identify studies characterising the CVLU wound microbiota using molecular methods. Ecological modelling of pair-wise co-occurrence was conducted using the R-based tool Co-occur (Probabilistic Species Co-occurrence Analysis). Statistical significance was evaluated using the hyper-geometric distribution, with a significance threshold set at an FDR-adjusted p-value of < 0.05.

Six studies were included, defining the microbiota of 72 CVLU samples, comprising tissue biopsies, swabs, and debridement. These studies identified a mean of 3.97 bacterial genera per sample, documenting 53 genera and 24 species. The most prevalent genera were *Staphylococcus* (61.5%), *Bacteroides* (35.4%), *Pseudomonas* (30.8%), *Corynebacterium* (29.2%), and *Peptoniphilus* (29.2%). Facultative- and obligate anaerobes accounted for 52.1% and 42.3% of isolates, respectively, with obligate anaerobes present in 61.1% of all samples. Five discrete positive pair-wise associations were identified, namely: *Bacteroides-Streptococcus, Bacteroides-Prevotella, Anaerococcus-Peptoniphilus, Anaerococcus-Finegoldia*, and *Peptoniphilus-Finegoldia.*

This study reinforces the importance of anaerobic organisms in CVLUs, defining their microbial inter-relationships within the wound biofilm and their co-occurrence networks. This understanding is being used to inform the design and delivery of novel antimicrobial therapies for CVLUs.

P045

A continuous biofilm evolution model of *Listeria monocytogenes* identified mechanisms of persistence and niche adaptation.

Oleksii Omelchenko[1,2,3,4], Ana Victoria Gutierrez[1,4], Maria Diaz[1,4], Mark Webber[2,1,4], Matthew Gilmour[2,1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Food Standards Agency, London, United Kingdom. [4]Centre For Microbial Interactions, Norwich, United Kingdom

Abstract

Effective cleaning and disinfection are essential for controlling microbial pathogens in food production environments (FPE). Yet, *Listeria monocytogenes (Lm)* can persist despite these measures, posing a significant concern due to its ability to cause severe foodborne illness. Persistence mechanisms of *Lm* include biofilm formation and increased tolerance to biocides such as benzalkonium chloride (BC), for which several tolerance mechanisms are known. We hypothesised that *Lm* biofilms when under pressure of disinfectants and other FPE stressors, undergo adaptive evolution through SNPs or indels linked to biofilm formation and biocide tolerance. To test this, we developed a biofilm persistence model, subjecting *Lm* biofilms to 30 consecutive passages on stainless steel under prolonged subinhibitory BC exposure. Whole-genome sequencing of adapted populations at passages 11, 23 and 30 identified mechanisms of persistence and niche adaptation of *Lm*: non-synonymous mutations became fixed in genes/pathways related to metal homeostasis, stress response and pyrimidine synthesis. In addition, high-frequency mutations within *fepRA* operon, encoding the FepR transcriptional repressor and FepA MATE efflux pump, resulted in increased BC tolerance in both planktonic and biofilm lifestyles. Novel mutations linked to BC tolerance were also identified at lower frequencies in pathways related to amino acid biosynthesis and metal ion homeostasis. These findings provide insights into the genetic mechanisms underlying *Lm* biofilm persistence and biocide tolerance in the context of FPE, highlighting potential novel targets for improving sanitation strategies and mitigating the establishment of persistent *Lm.*

P047

Investigating biofilms of *Desulfovibrio desulfuricans*

Marnie Barham, Lizbeth Sayavedra, Melinda Mayer, Nathalie Juge, Arjan Narbad

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

Sulphate-reducing bacteria (SRB) are a diverse group of anaerobic microorganisms that can form biofilms. During anaerobic respiration, SRB produce hydrogen sulphide, a corrosive and genotoxic gas as a by-product of dissimilatory sulphate reduction. In the environment, SRB contribute to corrosion of metal structures, causing financial losses for oil and gas industries. In humans, SRB blooms in the gut microbiota have been linked to Parkinson's disease and inflammatory bowel diseases. However, little is known about the role of SRB biofilm formation in gut colonisation in humans.

To address this gap, 23 SRB strains were isolated from the gut microbiota of healthy people over 60 years old, and their genomes were sequenced. Of these strains, five were identified as *Desulfovibrio desulfuricans*. Phylogenomic analyses revealed that *D. desulfuricans* strains clustered into five distinct clades, suggesting a need for species reclassification. Biofilm formation, assessed using crystal violet staining on polystyrene, varied among strains and did not correlate with taxonomic classification. Transcriptomic analysis revealed 26 genes upregulated in a *D. desulfuricans* biofilm compared to planktonic state, including genes encoding glycosyltransferases, flagella proteins and protein transporters. Future work will focus on generating mutant strains to investigate the functional role of these genes in biofilm formation. Understanding the molecular mechanism underpinning biofilm formation and dispersal of SRB in the gut could inform strategies to control their populations and promote gut health.

P048

The pharmacokinetics of hexylresorcinol-containing lozenges and their antimicrobial efficacy against oral and respiratory microorganisms

Priyanka Sharma[1,2], Charlotte Richards[1], Kirsten Bentley[1], Nicholas Claydon[1], Thomas Hallett[3], Anuradha Kulasekaran[4], Graca Coutinho[4], Lydia Powell[5], Richard Stanton[1], Elaine Ferguson[1], David Thomas[1], Katja Hill[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [3]Reckitt Healthcare, Hull, United Kingdom. [4]Reckitt Healthcare, Slough, United Kingdom. [5]Swansea University, Swansea, United Kingdom

Abstract

The substituted dihydroxybenzene Hexylresorcinol (HR) are employed in lozenge formulations to provide symptomatic relief for oropharyngeal inflammation. Whilst evidence of bactericidal activity in these formulations is limited, it has recently been described in planktonic bacteria. We defined antimicrobial/antiviral activity in planktonic and biofilm models and characterised the pharmacokinetics of HR release from lozenges. Antimicrobial activity (purified or released from lozenges) was determined against oropharyngeal pathogens using minimum inhibitory concentration (MIC) and Log_{10} reduction assays. Antiviral activity was determined by suspension test (EN14476). Antibiofilm effects employed minimum biofilm eradication concentration assays and confocal laser scanning microscopy. HR release from lozenges was studied *in vitro* and *in vivo* using HPLC. HR exhibited MICs ≤16 µg/mL against 19/25 strains including: *Streptococcus, Staphylococcus* and *Candida* spp. Marked bactericidal activity (> 3_{log10}; >99.9% reduction) occurred within 10 minutes. Significant anti-biofilm activity was evident in streptococcal and candidal biofilms ($P < 0.05$) and reduction in virucidal infectivity of HR in lozenges ranged from 1-log_{10} to 3.5-log_{10}. *In vivo*, HR exhibited rapid release from lozenges into saliva [t_{max}, 5 minutes; C_{max}, 82.5 $\pm$24 mg/mL; t > MIC (16 µg/mL), 22.5 minutes]. Rapid release and antimicrobial activity of HR against oropharyngeal pathogens was evident, occurring at concentrations ≥ 2-fold lower than present in saliva, highlighting the potential application of HR in the treatment of oropharyngeal infections.

P049

Inhibiting Pseudomonas aeruginosa with Staphylococcus epidermidis encapsulated in a honey-based prebiotic-probiotic hydrogel

Sophie Hellé[1], Victor C. Igbokwe[1], Vincent Ball[1], Chelsea Fesselier[1], Simon Gree[2], Eric Mathieu[1], Corinne Nardin[3], Lydie Ploux[1]

[1]Inserm UMR_S 1121, CNRS EMR 7003, Université de Strasbourg, Biomaterials and Bioengineering, Strasbourg, France. [2]CNRS UMR 7361 IS2M, Université de Haute-Alsace, Mulhouse, France. [3]Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau, France

Abstract

Bacteriotherapy has emerged as an effective strategy to combat bacterial infections prevalent in skin wounds, particularly those associated with diabetic ulcers and burns. In this contest, we found that the supplementation of hydrogels with honey enhanced the potential of *Staphylococcus epidermidis*, a skin commensal bacterium, to inhibit the growth of *Pseudomonas aeruginosa*. In this study, we varied the concentration of *S. epidermidis* encapsulated in the hydrogels with and without honey. After gelation, we inoculated *P. aeruginosa* on the surface on the hydrogel and 24h later, we observed a remarkable difference in morphology and size of the colony of *P. aeruginosa* in presence of either honey or *S. epidermidis*. Importantly, a distinct absence of *P. aeruginosa* colony was noted in the agarose + honey hydrogel at the two highest concentrations of *S. epidermidis*. These observations suggest that *S. epidermidis* and honey, alone or together, create an unfavorable environment for *P. aeruginosa* growth. Also, combining honey with *S. epidermidis* inhibits the growth of *P. aeruginosa*, in a concentration-dependent manner. The results of our experiment on the hydrogels excluded a direct effect of the viscoelastic or hydration properties of the hydrogels on *P. aeruginosa* growth whereas the ATR-FTIR fingerprint of the solution extracted from this hydrogel suggests a crucial role of the sugar catabolism by *S. epidermidis* in honey-containing hydrogels, probably triggered by *P. aeruginosa*. These anti-pathogen effect of encapsulated *S. epidermidis* enhanced by honey could advance the use of bacteriotherapy in developing therapeutic dressings to prevent bacterial infections associated with skin wounds.

P051

Dry biofilms contribute to bacterial persistence on environmental surfaces

Kelli Buckingham-Meyer, Elizabeth Sandvik, Lindsey Miller, Chris Jones

Center for Biofilm Engineering, Bozeman, USA

Abstract

A comprehensive definition of dried biofilm has yet to be determined, however, it is recognized that dried biofilms survive longer than 12 months and are causative agents of healthcare-acquired infections (HAIs) and food contamination events. We hypothesize that microbial biofilms have different tolerance to drying, which affects their ability to persist in various environments.

A panel of HAI and food contamination microorganisms were selected for development of a microtiter screening assay. Biofilms were formed in microtiter plates, the media was removed at the end of the growth period and dried in a humidity-controlled environment for 1, 4, 28 days and 4 months. To determine viability of the biofilm, media was added after drying and bacterial growth was monitored for 24 hours by optical density.

Variability was observed among strains in biofilm desiccation tolerance over time and humidity conditions. Representative strains were chosen from tolerant, sensitive, and conditional groups for antimicrobial testing. We report varied responses to antimicrobial treatments based on the desiccation of the biofilm, suggesting that the degree of dryness alters the sensitivity of the microbes.

Dried biofilms are relevant across many industries where they can serve as a reservoir for microbes, affecting the ability to effectively clean and sanitize surfaces. Here, we have established methodologies for screening strains for biofilm desiccation tolerance and determining the effect on antimicrobial sensitivity. Future work will investigate the mechanism of antimicrobial tolerance by a variety of dry biofilms.

P053

Experimental evolution enhances biofilm formation in *Paenibacillus xylanexedens* for improved biocontrol against Hairy Root Disease

Xander Mols[1,2], Bram Lories[3], Hans Steenackers[3], Hans Rediers[1,2]

[1]Centre of Microbial and Plant Genetics, Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium. [2]Leuven Plant Institute (LPI), KU Leuven, Leuven, Belgium. [3]Centre for Microbial and Plant Genetics, Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium

Abstract

Hairy root disease (HRD), caused by rhizogenic *Agrobacterium*, results in a considerable reduction in the yield of several hydroponically grown crops, including tomato and bell pepper, leading to substantial economic losses. HRD is a persistent disease, partially because rhizogenic *Agrobacterium* forms biofilms in the irrigation pipeline, making it more resistant to chemical biocides. *Paenibacillus xylanexedens*, a biocontrol organism (BCO) with proven activity against HRD, offers potential for disease management. However, its limited biofilm-forming capacity restricts its ability to combat the *Agrobacterium* residing in biofilms.

To address this limitation, experimental evolution was used to enhance the biofilm-forming capacity of *P. xylanexedens*. The ancestral strain was cultured in 24-well plates under various conditions (25°C-30°C; optimal and suboptimal growth media; two BCO strains). To create a selection pressure favouring biofilm formation, only biofilm cells were harvested and transferred to fresh medium after 2 or 4 days of growth, initiating a new cycle. Biofilm formation and planktonic growth were assessed in each cycle.

After 11 cycles (~53 generations) of experimental evolution, biofilm formation increased up to 40-fold in two independent *P. xylanexedens* strains, consistently across all tested conditions, compared to the ancestral strain. A subset of clonal isolates retained their enhanced biofilm-forming capabilities and their anti-pathogenic properties, making them promising candidates for further characterization. Population sequencing is ongoing to identify the underlying mechanisms of these improvements.

In conclusion, this study highlights the potential of experimental evolution as a tool for optimizing traits important for the efficacy of biocontrol agents like *P. xylanexedens*.

P055

Understanding the interaction between lipid A modifications and type IX secretion

Beichang Zhang

King's College London, London, United Kingdom

Abstract

Porphyromonas gingivalis is a key pathogen for periodontitis. *P. gingivalis* utilizes a type IX secretion system to export virulence factors (e.g. gingipain proteases) to the bacterial surface and into outer membrane vesicles (OMVs). Lipopolysaccharides are composed of lipid A, a core oligosaccharide, and an extended O-antigen polysaccharide. Lipid A modifications such as the phosphatases LpxE, LpxF, and the deacylase LpxR, play a significant role in host immune responses. However, lipid A modification has been linked with OMV biogenesis, and this study investigates the interaction between lipid A modifications and type IX secretion.

Colonies of wild-type, Δ*lpxF1*, Δ*lpxF2*, and Δ*lpxR* mutants, as well as complemented strains, were cultivated under anaerobic conditions. All mutants showed smaller, light brown colonies on blood agar, indicative of type IX secretion defects, unlike the black-pigmented wild-type colonies. Despite similar growth curves across strains, mutants exhibited growth defects on blood agar. Gingipain activity was reduced in all mutants, with increased activity observed in the complemented strains. SDS-PAGE and Western-blot analyses revealed significantly reduced gingipain levels in the supernatant of Δ*lpxF1* and Δ*lpxR* mutants, while cell surface levels remained unchanged.

The data suggest that lipid A modifications by LpxF and LpxR are essential for gingipain export, potentially through their influence on OMV formation. Reduced OMV formation in Δ*lpxF1* mutants correlated with decreased biofilm production, further confirming the role of lipid A in type IX secretion and biofilm development. These findings underscore the importance of lipid A heterogeneity in pathogenicity and its potential as a therapeutic target for periodontitis.

P056

Development of different models for biofilm evaluation

María José González[1], Nicolás Navarro[1], Erlen Cruz[1], Luciana Robino[1,2], Paola Scavone[1]

[1]Laboratorio de Biofilms Microbianos, Depto. de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay. [2]Unidad Académica de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Abstract

Bacterial biofilms are complex microbial communities that adhere to surfaces and exhibit increased resistance to antimicrobial agents. Various models have been developed to study biofilm formation, structure, and behavior, each with distinct advantages. *In vitro* models, including microtiter plate assays, flow cells, and microfluidic devices, provide controlled environments for biofilm growth and analysis. Microtiter plate assays assess biofilm-forming capacity, while flow cells enable real-time visualization. In vivo models, such as murine, rabbit, and zebrafish systems, offer insights into host-biofilm interactions and immune responses, though ethical concerns and physiological differences remain challenges. The choice of model depends on research objectives, balancing complexity, reproducibility, and clinical relevance. Advances in microscopy, including high-resolution and *in vivo* imaging, allow the study of biofilm dynamics without disrupting their natural structure.

We have developed static and dynamic imaging models to elucidate biofilm formation, revealing their complex architecture, which has led to considering biofilms as ancient tissues. Our research has provided insights into biofilm development in pathogens such as *Escherichia coli, Proteus mirabilis, Staphylococcus aureus,* and *Acinetobacter baumannii* and has facilitated nanoparticle-based strategies to prevent biofilm formation. Notably, we have described intracellular biofilms in eukaryotic cells from urine samples of urinary tract infection patients. Understanding biofilm formation through advanced microscopy is crucial for biomedical applications, public health, and ecological studies.

P057

Development of a reproducible dual-species *in vitro* chronic wound biofilm model

Vânia Pinheiro[1,2], Anabela Borges[1,2,3], Nuno Cerca[4,5], Darla Goeres[6]

[1]LEPABE – Laboratory for Process Engineering Environment, Biotechnology and Energy, Faculty of Engineering of the University of Porto, Porto, Portugal. [2]ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering of the University of Porto, Porto, Portugal. [3]DEQB – Department of Chemical and Biological Engineering, Faculty of Engineering of the University of Porto, Porto, Portugal. [4]CEB – Centre of Biological Engineering, Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), University of Minho, Braga, Portugal. [5]LABBELS – Associate Laboratory, Braga, Portugal. [6]CBE – Center for Biofilm Engineering, Montana State University, Bozeman, USA

Abstract

Background: Chronic wounds, are challenging to heal due to the recurrence and persistence associated with biofilm infections. This study aimed to develop an innovative *in vitro* biofilm model that better replicates the conditions contributing to chronic wound persistence.

Methods: A novel in-house *in vitro* chronic wound model was developed by integrating three existing models described in the literature: the Lubbock chronic wound biofilm (LCWB) model, the layered chronic wound biofilm (CWB) model, and the collagen chronic wound model. The LCWB model was used to establish a dual-species biofilm with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This biofilm inoculated the layered CWB model, which was nourished with simulated wound fluid from the collagen chronic wound model, every 24 hours for three days. Colony forming units (CFU) were quantified, and fluorescence in situ hybridization (FISH) combined with confocal microscopy assessed spatial organization and species distribution.

Results: CFU analysis and FISH combined with confocal microscopy, confirmed the presence of *P. aeruginosa* and *S. aureus* over three days. *P. aeruginosa* dominated the dual-species biofilm, maintaining CFU counts similar to its single-species biofilm, while *S. aureus* showed reduced CFU levels in co-culture.

Conclusion: The presence of *P. aeruginosa* and *S. aureus* over three days confirms the model's ability to replicate their coexistence in chronic wounds. This model provides a valuable tool for studying biofilm spatial transcriptomics and developing targeted therapies for biofilm-associated infections.

P059

Phage bioengineering of complex communities for enhanced wastewater treatment

Matt Irwin[1], Jeremy S. Webb[1,2], Franklin Nobrega[1], Yongqiang Liu[1], John Williams[3], Juhani Kostiainen[4]

[1]University of Southampton, Southampton, United Kingdom. [2]NBIC, Southampton, United Kingdom. [3]University of Portsmouth, Portsmouth, United Kingdom. [4]Plantwork Systems, Alton, United Kingdom

Abstract

Wastewater treatment is a globally critical infrastructure for the reclamation of water after human utilisation. Ubiquitous problematic microorganisms within water treatment reactors negatively impact treatment efficiency and can ultimately cause reactor collapse. Problematic glycogen accumulating organisms (GAOs) make consistent phosphorus (P) removal unfeasible and flourish under elevated and rising global temperatures. To combat these current solutions are untargeted and expensive, requiring constant monitoring and modification of operation parameters, impacting long term reactor health. Here, we developed a bioengineering approach harnessing phages for targeted removal of problematic organisms within complex microbial wastewater communities. As a proof of concept, the problematic GAO, *Micropruina glycogenica Lg2*, was selected. We developed an industrial-scale high-throughput virome concentration procedure for collected seasonal viromes from the England's South Coast region. Phage cocktails were introduced to small scale reactors (Pioreactors) to bioengineer a sludge-GAO enriched community, recovering a consistent P removal. The collective potential of bioengineering wastewater could revolutionise treatment approaches, advancing treatment limits and assist in developing new closed-loop industrial-scale treatment for full nutrient recovery.

P060

ZnO Nanoparticles mediated potentiation of gentamicin to eradicate in vitro biofilms of drug- resistant Gram- negative bacteria.

Akshit Malhotra[1], Kwthar Debbarma[2], Ashwini Chauhan[1]

[1]Department of Microbiology, University of Delhi South Campus, New Delhi, India. [2]Department of Bio Engineering, National Institute of Technology, Agartala, India

Abstract

Background: Globally, antibiotics are becoming ineffective with the emergence of multi-drug resistant microbes posing a daunting threat to the well-being of people. Altogether, the virulence mechanisms of bacteria due to resistant genes and biofilm formation pose a tremendous clinical challenge. This has necessitated developing efficient alternatives to antibiotics to combat ESKAPE bacterial infections.

Methods: We report a simple, green and novel method to synthesize zinc oxide (ZnO) nanoparticles using ethanolic extracts of *Diplazium esculentum* by precipitation method. The physio-chemical characteristics were assessed using Field Emission- Scanning Electron Microscopy, Energy dispersive X-Ray spectroscopy, X-Ray Diffraction (XRD) and UV-Visible Spectroscopy. Clinical isolates of Gram- positive *S. aureus* and Gram- negative *E. coli, K. pneumoniae* were treated with the ZnO NPs to check antibacterial and antibiofilm activity. Besides, we assessed the ability of ZnO NPs to improve the anti-biofilm activity of gentamicin.

Results: ZnO NPs displayed more than 4-log reduction of *E. coli* and *K. pneumoniae* clinical isolates at 500 µg/ ml concentration in planktonic state. Moreover, ZnO NPs significantly reduced the biofilm bacterial cell viability of clinical isolates of Gram- negative bacteria. Interestingly, complete eradication of biofilm bacteria was achieved for *E. coli* clinical isolate using a combination of sub- MIC concentration of gentamicin and 500 µg/ ml ZnO NPs.

Conclusion: Our study focuses on sustainable synthesis of ZnO NPs for treatment of infections caused by pathogens belonging to the high priority ESKAPE group. Green synthesized ZnO NPs potentiate the efficacy of gentamicin to eradicate biofilms of drug resistant gram- negative bacteria.

P061

Exploring the Genomic Mechanisms of Biofilm Formation in *Enterococcus faecium* from Catheter-Associated Urinary Tract Infections

Ayu Rahayu[1,2], Sayyidah Auliany Aminy[1], Dian Asti Umaroh[2], Nila Rezha Diyestia Rakhma[2], Niar Gusnaniar[3,2], Puspita Lisdiyanti[3,2], Estelle Peraud[4,5], Christine Imbert[4], Titik Nuryastuti[1,2]

[1]Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. [2]Indonesian Biofilm Research Collaboration Center, Yogyakarta, Indonesia. [3]National Research and Innovation Agency, Bogor, Indonesia. [4]Laboratoire Ecologie et Biologie des interactions, Université de Poitiers, Poitiers, France. [5]Laboratoire de Parasitologie et Mycologie, CHU de Poitiers, Poitiers, France

Abstract

Enterococcus faecium is a major pathogen implicated in 60–80% of microbial infections, particularly due to its biofilm-forming ability on medical devices, which complicates infection management and enhances antimicrobial resistance. This study employed Whole Genome Sequencing (WGS) to investigate the genetic basis of biofilm formation in *E. faecium*. The genome analysis revealed a G + C content of 35.1% and a single circular chromosome spanning 3,322,669 base pairs, encoding 2,321 genes, 47 tRNAs, and 13 rRNAs, with no plasmids detected. Several biofilm-associated genes (*sgrA, acm, fss3, efbA,* and *scm*) were identified, potentially contributing to *E. faecium*'s persistence in clinical settings. Additionally, multiple antimicrobial resistance genes, including *AAC(6'), Msr(C), Tet(M), ANT(6)-Ia, EfmA,* and *Tet(L)*, were detected, highlighting the bacterium's resilience against antibiotic treatments. Computational predictions using AlphaFold identified key structural conformations in *fss3* and *efbA*, with over 90% of their regions in highly stable configurations, suggesting potential targets for antibiofilm therapy. These findings provide a foundation for molecular docking studies to explore novel therapeutic interventions aimed at disrupting biofilm formation. Understanding the genetic and structural factors underpinning *E. faecium* biofilm development is crucial for designing targeted strategies to mitigate biofilm-associated infections and improve clinical outcomes.

P062

Coordinated Regulation of Curli and Colanic Acid during Biofilm Formation

Qonita Afinanisa, Tim Overton

School of Chemical Engineering and Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

The ongoing effort to mitigate biofilms in industrial and medical fields has been mainly focused on surface adherent biofilms based on the five-step model of biofilm formation and maturation described in *Pseudomonas aeruginosa* [1]. Recent studies show that biofilm-like communities such as clusters or pellicles can form without attaching to a solid surface [2]; mechanisms include sloughing, planktonic cell growth, nucleation of cell surface components, polymer depletion, and polymer bridging. This raises the question of whether there are common mechanisms for formation of solid surface-associated and free-floating biofilms.

E. coli initially adheres to solid surfaces using curli, and biofilm maturation involves colanic acid production. Regulation of these biofilm components is linked through the envelope stress sensor RcsAB, suggesting tight coordination in the stages of biofilm formation. Colanic acid production has also been shown to physically prevent curli from mediating *E. coli'*s adherence to the surface [3]. This study aims to explore the mechanism of biofilm formation in *E. coli* from new perspectives, such as the intersection between curli and colanic acid regulation. We have exposed bacteria to various stressors and used reporters to observe the effect on curli and colanic acid regulation on population and single-cell levels. We have also explored the involvement of key second messenger c-di-GMP in this process.

References

1. Sauer et al. (2002) doi: 10.1128/jb.184.4.1140-1154.2002.

2. Sauer et al. (2022) doi: 10.1038/s41579-022-00767-0

3. Hanna et al. (2003) doi: 10.1128/AEM.69.8.4474-4481.2003.

P063

Shutting down Pseudomonas aeruginosa quorum sensing, virulence production, and disrupting biofilms using novel multifunctional peptide.

Theerthankar Das[1], Michael Radzieta[1], Greg Whiteley[2], Mark Lutherborrow[2], Trevor Glasbey[2], Slade Jensen[1]

[1]Microbiology and Infectious Diseases, School of Medicine, Ingham Institute, Western Sydney University, Sydney, Australia. [2]Whiteley Corporation, Sydney, Australia

Abstract

Background and aims: *Pseudomonas aeruginosa* is an opportunistic Gram-negative pathogen responsible for causing life-threatening infections in humans. Like many bacterial species, *P. aeruginosa* secretes small signaling molecules (e.g., N-acyl homoserine-lactone) to activate the Quorum sensing (QS) mechanism. Through QS *P. aeruginosa* regulates gene expression thus facilitating the expression of critical phenotypes, including virulence factor biosynthesis, biofilm formation, and antibacterial resistance.

This study aims to develop a novel peptide-based strategy to disrupt QS in bacteria, inhibit virulence factor biosynthesis, and facilitate biofilm dispersion.

Methods: QS inhibition in *P. aeruginosa* was investigated using strains containing QS response regulator-reporter gene fusions (for example, *lasR-gfp*) and measuring fluorescence. Virulence factor pyocyanin was quantified by chloroform-HCl. Biofilm was quantified using a crystal violet assay, Confocal microscopy imaging, and counting CFU/ml, and peptide cytotoxicity was examined using a human fibroblast cell line (HFF-1).

Results: The peptides tested, exhibited inhibition of the primary QS response regulators LasR, RhlA, and PqsR in *P. aeruginosa*. QS inhibition directly translated into decreased pyocyanin biosynthesis in *P. aeruginosa* reporter strains and clinical isolates. The peptide tested, also significantly inhibited biofilm formation and triggered the dispersion of pre-established biofilms. The peptide also directly bound and modulated pyocyanin structure, and inhibited its ability to intercalate with DNA. No cytotoxicity was observed when the peptides were tested using a human fibroblast cell line.

Conclusions: The peptide's multifunctional profile against *P. aeruginosa* proves a pathway to developing a new treatment strategy for chronic infections, such as those associated with wounds and diabetes-related foot ulcers.

P064

Regulatory effects on biofilm formation revealed by *sdiA* mutation in *Klebsiella pneumoniae*

Sergio Silva-Bea, Pablo Maseda, Ana Otero, Manuel Romero

Department of Microbiology and Parasitology, Faculty of Biology - Aquatic One Health Research Center (ARCUS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Abstract

The formation of biofilms is a significant virulence factor in *Klebsiella pneumoniae*, a bacterium recently classified by the WHO as a maximum-priority pathogen for developing new antimicrobial strategies due to its multi-resistance (MDR) and hypervirulence traits. SdiA, a quorum-sensing receptor that responds to acyl-homoserine lactones (AHLs), plays an important regulatory role in biofilm formation in Gram-negative bacteria. However, the role of this receptor in *K. pneumoniae* remains unclear. The present study utilised the Rolling Biofilm Bioreactor system to investigate the impact of AHL signalling in both wild-type and *sdiA* deficient (Δ*sdiA*) strains of *K. pneumoniae* KLEB-33, an MDR hyper-biofilm-forming strain harbouring hypervirulence genes. Quantification of biofilm biomass was conducted at 24-hour intervals (up to 72 hours) using a Crystal Violet staining method and Confocal microscopy examination. Furthermore, biofilms were examined for cell filamentation and matrix composition after eDNA and exopolysaccharide fluorescent dye staining. The results obtained demonstrated that, in comparison with the parental strain, the Δ*sdiA* mutant exhibited enhanced biofilm formation and elevated rates of cell filamentation. Additionally, it was observed that AHL supplementation promoted biofilm formation exclusively in the wild-type strain, with no such effect observed in the Δ*sdiA* mutant. This finding suggests that AHL biofilm promotion is SdiA-dependent. Furthermore, no significant alterations in matrix composition were detected after *sdiA* mutation or AHL supplementation. Consistent with observations made in other bacteria, our results indicate that SdiA is a biofilm-repression regulator in *K. pneumoniae*, and its biofilm-repression effect could be related to cell filamentation in sessile communities of this pathogen.

P065

The role of fouling layer composition on its mechanical properties: implications for CIP strategies in membrane filtration

Shifa Shaikh, Kees Theo Huisman, Peter Desmond, Khaled Mahmoud

Hamad Bin Khalifa University, Doha, Qatar

Abstract

Fundamental understanding of the chemical composition and mechanical properties of fouling layers on the surface of reverse osmosis membranes (RO) applied in seawater desalination plants is critical for optimized pretreatment and cleaning-in-place (CIP) procedures. However, the current state of art is built on mechanisms derived from pure biofilm systems cultivated using synthetic feedwaters, limiting insights into fouling behaviors encountered in real environments. The presented work overcomes this limitation by juxtaposing the behavior of synthetic fouling layers with those from spent membrane elements obtained from a full-scale seawater RO desalination plant in the State of Qatar. The fouling layer morphology and chemical composition were respectively determined by coupling SEM and EDX techniques. The mechanical characteristics of the fouling layer were established using shear rheology. SEM images with EDX spectra revealed a heterogenous fouling layer dominated by the presence of various inorganic magnesium aluminum silicates alongside iron oxide and various microbial cell clusters. Shear rheology analyses of the foulant layer exhibited high yield stress and viscoelastic gel-like behavior indicating high material resistance to deformation. Evidently, the abundance of interlocked clay particles can be correlated to the enhanced fouling layer mechanical properties compared to pure biofilms cultivated using raw synthetic feedwaters. Their presence may also promote synergistic interactions with organic foulants, microbial cells, and extracellular polymeric substances, reinforcing the cohesion and structural integrity of fouling layers that resist detachment. These outcomes present a fundamental understanding of the impact of fouling layer composition on its mechanical strength, with practical implications on optimizing CIP strategies.

P066

Identifying roles for hypothetical genes in *Vibrio cholerae* lifestyle switching and biofilm formation

James Haycocks[1], Micheal Alao[2], Georgia Williams[2], Huda Ahmad[2], Danesh Moradigaravand[3], David Grainger[2], Manuel Banzhaf[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom. [3]King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

Abstract

A major challenge in microbiology lies in assigning functions to hypothetical genes, particularly in pathogens where the full repertoire of virulence and biofilm-related factors remains poorly understood. *Vibrio cholerae* is the causative agent of cholera, and has a biphasic lifestyle with stages in the both marine environment and the human small intestine, with biofilm formation critical to both phases. Despite extensive research into *V. cholerae* biology, over 40% of genes lack functional annotation, many of which are likely important for transitioning between, and survival within, these distinct environments. To address this gap we took a chemical genomics approach, conducting a high-throughput phenotypic screen of a single-gene disruption library in *V. cholerae* C6706 (generated by Cameron *et al.*, 2008) under 86 unique conditions to identify functions for orphan genes.

We were able to a phenotype for 52% of the hypothetical genes in C6706, with 40% of these exhibiting a phenotype highly associated with that of an annotated gene, many of them involved in biofilm regulation and lifestyle switching. These findings provide new insights into factors linked to *V. cholerae* biofilm biology and its ability to persist in diverse ecological niches. In this presentation, we will showcase the most interesting findings from the screen.

P067

Wound In a Cup - Utilizing Isothermal Microcalorimetry for a *In Vivo-Like* Chronic Wound Biofilm Model

Kasper Nørskov Kragh[1], Emma Frisinger[2], Madle Sirel[2]

[1]Symcel AB, Copenhagen, Denmark. [2]Symcel AB, Stockholm, Sweden

Abstract

Background: Chronic wounds, including diabetic foot ulcers and pressure ulcers, pose a significant healthcare burden, exacerbated by biofilm-associated infections. Traditional biofilm models fail to adequately capture the complexity of chronic wound environments, which limits the translational relevance of studies on antimicrobial efficacy. These models often rely on removing microbes from the biofilms for CFU counts, risking the exclusion of non-growing or viable-but-nonculturable subpopulations within the model.

Methods: We employed chronic wound media (CWM2) to establish an *in vitro* biofilm model mimicking the biochemical conditions of chronic wounds. *Pseudomonas aeruginosa* biofilms were cultivated in the calScreener calorimetric vials, allowing real-time metabolic analysis via isothermal microcalorimetry (IMC). Biofilms were treated with tobramycin and ciprofloxacin at up to 10× MIC, and metabolic activity was continuously monitored *in situ*. A traditional LB agar-based biofilm model was included for comparison.

Results: Biofilms grown in CWM2 media exhibited distinct metabolic behaviors compared to those in LB agar, demonstrating prolonged lag phases and increased metabolic heterogeneity. IMC analysis revealed that subpopulations persisted in the chronic wound model despite high antimicrobial concentrations, whereas the LB agar model displayed greater uniform susceptibility.

Conclusions: The combination of IMC and a chemically defined chronic wound medium provides a physiologically relevant model for studying biofilm behavior and antimicrobial treatment efficacy. This approach enables non-destructive, real-time metabolic monitoring, capturing bacterial resilience often overlooked in conventional models. Our findings emphasize the need for advanced biofilm models to bridge the gap between laboratory studies and clinical outcomes, ultimately improving chronic wound infection management.

P069

Candida auris, an Emerging Threat: Can Essential Oils Be a Promising Antifungal Strategy?

Liliana Fernandes, Bernardo Portugal, Mariana Henriques, Maria Elisa Rodrigues

University of Minho, Braga, Portugal

Abstract

Background: *Candida auris*, a drug-resistant species, has recently emerged as a significant global health threat due to its high prevalence in hospitals and associated mortality rates. Its transmissibility and resistance to antifungal agents, coupled with its adaptability, are a challenge in the treatment of these infections. The intensified virulence, attributed to its multidrug resistance and biofilm formation, underscores the urgent need for an understanding of its biology. Such insights are essential for the development of effective strategies in the treatment, prevention, and management of *C. auris* infections.

Methods: This study screened 17 essential oils (EOs) for antifungal activity against *C. auris* NCPF8971 using the disc diffusion method. The impact of the vapor phase (VP-EOs) of 6 previously selected EOs on biofilms was evaluated through colony-forming unit enumeration. The mechanism of action of selected EOs was investigated via flow cytometry and their cytotoxic effects were evaluated through hemolysis testing.

Results: Several EOs significantly inhibited *C. auris* planktonic growth. *Cymbopogon citratus*, *Ocimum basilicum*, *Coriandrum sativum*, *Cinnamomum verum* and *Cymbopogon flexuosus* exhibited inhibition halos between 76.4 ± 11.0 mm and 85.0 ± 0.01 mm. Among them, *C. citratus* and *C. flexuosus* completely eradicated pre-formed biofilms, while *C. sativum* significantly reduced them. Flow cytometry indicated VP-EOs affect membrane integrity (PI internalization) and metabolic activity.

Conclusions: This study highlights the antifungal potential of EOs against *C. auris*, suggesting their role as complementary or alternative treatments. Flow cytometry confirmed that VP-EOs damage *C. auris* cell walls. These findings support EOs as promising anti-yeast agents exerting primary cellular damage.

P070

Biofilm formation of diverse *Klebsiella pneumoniae* strains isolated from hospital surfaces in South-East Asia

Maria Nieto-Rosado[1], Kathryn M. Thomson[1], Greta Zaborskytė[1], Refath Farzana[1], Brekhna Hassan[2], Amy Cooke[1], Julia Rzaska[1], Sarah Langhorne[1], Leonie Wilson[1], Rabaab Zahra[3], Eman Rashid[3], Sadia Riaz[3], Abdur Rehman[4], Asim Ali Shah[4], Amjad Mehboob[4], Muhammad Haroon Hamid[5], Rabina Temur[5], Maryam Babar[5], Shahanaz Pervin[6], Rahimatul Farzana[7], Nafia Tasnim[8], Timothy R. Walsh[1], BARNARDS group[1], Kirsty Sands[1]

[1]University of Oxford, Oxford, United Kingdom. [2]Division of Infection and Immunity, Cardiff University, Cardiff, United Kingdom. [3]Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan. [4]Bacha Khan Medical Complex, Swabi, Pakistan. [5]Mayo Hospital, Lahore, Pakistan. [6]Khulna Medical College Hospital, Khulna, Bangladesh. [7]Dhaka Medical College Hospital, Dhaka, Bangladesh. [8]Mymensingh Medical College Hospital, Mymensingh, Bangladesh

Abstract

Background: Sequence type 15 (ST15) *Klebsiella pneumoniae* isolates carrying bla_{NDM}/bla_{OXA-48}-like genes were recovered from neonatal ward surfaces in Pakistan during the BARNARDS study (Burden of antibiotic resistance in neonates from developing societies) 2015-2018, and were identical to strains causing neonatal sepsis. Multidrug-resistant bacteria embedded in biofilms on hospital surfaces may hinder cleaning/disinfection regimes, posing infection risks. This study aims to evaluate *in vitro* biofilm formation of *K. pneumoniae* on materials commonly found on hospital surfaces and biocide effectiveness.

Methods: Biofilm formation was evaluated by crystal violet staining and viable cell counts (CFU counts) over 48 h, using the FlexiPeg device with silicone-coated, 316L stainless steel- and nylon-printed pegs. Biocide effectiveness on pre-formed 24 h and 48 h biofilms was evaluated for recommended biocides including 70% isopropanol. To identify the genetic basis for the biofilm phenotype, whole genome sequencing was performed using Oxford Nanopore Technologies.

Results: On silicone, 6/47 isolates formed significantly more biofilm (63 to 293-fold) than the low biofilm forming control *K. pneumoniae* strain; and they formed robust biofilms on stainless steel and nylon too. Three of these six isolates potentially carried bla_{NDM}; one non-carbapenemase isolate was positive for six hypervirulence genes (*rmpA, rmpA2, iutA, magA, iucA, peg-344*). Initial assays showed significant reduction in CFU counts (70% isopropanol treatment vs no treatment) for the early 24h pre-formed biofilms on silicone.

Conclusions: This *in vitro* dataset will provide insights on how *K. pneumoniae* forms biofilms on diverse surfaces and information on to perform appropriated infection prevention and control practises.

P071

Why is Cell Segmentation Still a Challenge? A Comparative Study on 3D Biofilm Analysis

Arefeh Mazarei[1], Diogo Narciso[2], Albert Parker[3], Joana Vilas Boas[1], Jaime Cardoso[1], Darla Goeres[3,1], Nuno Azevedo[1]

[1]University of Porto, Porto, Portugal. [2]Instituto Superior Técnico, Lisbon, Portugal. [3]Montana State University, Bozeman, USA

Abstract

Background. Biofilms play essential roles in healthcare, environmental sustainability, and biotechnology. Quantitative analysis at the cellular level is critical to understanding their structure, growth, and behavior. However, the inherent complexity and variability of biofilms across imaging platforms create significant challenges for accurate quantification. Automatic cell segmentation, especially in 3D confocal microscopy datasets, remains indispensable for extracting meaningful insights but continues to face numerous obstacles.

Methods. A comparative evaluation of three state-of-the-art automatic cell segmentation methods designed for bacterial biofilms was completed. The methods included DeepSeeded, a cascaded deep learning architecture that estimates seed points for watershed-based segmentation; StarDist OPP, a deep learning framework optimized for single-cell segmentation in 3D biofilm images with post-processing enhancements; and BCM3D 2.0, an advanced pipeline leveraging deep convolutional neural networks for detecting and segmenting bacterial cells in 3D fluorescence time-lapse images.

Early-stage *Pseudomonas aeruginosa* biofilms were used for the evaluation, employing a partition distance metric to measure segmentation accuracy relative to the reference dataset.

Results. The analysis highlighted each method's strengths and limitations, providing crucial insights into their performance under biofilm-specific conditions. While all methods leverage deep learning, differences in architecture, preprocessing, and postprocessing approaches impacted segmentation performance. The findings suggest that the methods still struggle to segment under different biofilm conditions robustly and that combining deep learning with conventional image processing techniques can improve the reliability of segmentation.

Conclusions. By addressing the challenges in biofilm segmentation, this study contributes to developing reliable tools for biofilm research and quantitative image analysis.

P074

Drug conjugates for targeted delivery of antineoplastic drugs to biofilm infections

Magnus Schou Dybtved[1], Lærke Glud[1], Maiken Engelbrecht Petersen[2], Alif Firman Firdausy[2,3], Alexander Zelikin[1], Rikke Meyer[2]

[1]Department of Chemistry, Aarhus University, Aarhus, Denmark. [2]Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark. [3]Department of Pharmacy, Universitas Islam Negeri Maulana Malik Ibrahim, Malang, Indonesia

Abstract

Background
Biofilm infections persist due to antibiotic tolerance of non-growing persister cells. The antineoplastic mitomycin C is known to be effective against persister cells, but it is also cytotoxic and immunosuppressive. To increase therapeutic impact and minimize toxicity, we developed a vancomycin-mitomycin conjugate with a thiol-reactive linker, demonstrating targeted antimicrobial activity against Gram-positive pathogens.

Methods
Various strains of *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli*, and *P. aeruginosa,* were treated with free mitomycin-C or vancomycin-mitomycin conjugate for 15 minutes under non-growing conditions followed by 24 h in buffer with or without N-acetylcysteine (NAC) for drug release. Antimicrobial efficacy was quantified by CFU enumeration. Thiol-reactive groups naturally present on the bacteria were quantified using the DTNB titration assay. Fluorescently labeled vancomycin was used to document vancomycin's targeting capability.

Results
CLSM images confirmed that vancomycin could be used to target bacteria – also strains that are not susceptible to vancomycin as an antibiotic. Vancomycin-mitomycin conjugates were more effective than free mitomycin C, both against vancomycin-susceptible and -resistant strains. Some strains triggered drug-release from the conjugate by interacting with reactive thiols on the cell surface, while other strains required NAC for drug release and antimicrobial activity.

Conclusion
We demonstrate targeted delivery of antineoplastic drug as a prodrug conjugated to a glycopeptide antibiotic as the targeting agent. The efficacy of this therapeutic against non-growing bacteria, resistant strains, and biofilms suggests that this type of drug conjugate offers a new strategy for treating chronic biofilm infections.

Keywords:

Drug conjugates, antineoplastic, biofilm

P075

Exploring the Potential of Silver Nanoparticles Against Haemophilus Influenzae: Impact of Surface Coatings on Bacterial Proliferation and Biofilm Formation

Giorgia Dal Pan[1], Lavinia Rita Doveri[1], Vittorio Maceratesi[1], Noemi Ghelardini[1], Junkal Garmendia[2,3], Carlos Ortiz-de Solórzano[4,5,6], Yuri Antonio Diaz Fernandez[1,7,8]

[1]Department of Chemistry, University of Pavia, Pavia, Italy. [2]Instituto de Agrobiotecnología, Consejo Superior de Investigaciones Cientificas (IdAB-CSIC) - Gobierno de Navarra, Mutilva, Spain. [3]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [4]University Clinic of Navarra, Center for Applied Medical Research (CIMA), Pamplona, Spain. [5]Centro de Investigación Biomédica en Red de Enfermedades Oncológicas (CIBERONC), Madrid, Spain. [6]University of Navarra Clinic Cancer Center, Pamplona, Spain. [7]Interdepartmental Research Center in Advanced Material and Devices, Pavia, Italy. [8]Open Innovation Hub for Antimicrobial Surfaces, University of Liverpool, Liverpool, United Kingdom

Abstract

The increasing number of multi-drug-resistant strains significantly limited treatment options with conventional antibiotics. Nanomaterials, particularly silver nanoparticles (AgNPs) have been considered as a promising alternative. Ionic silver released from AgNPs is well known for its antibacterial properties, inducing oxidative stress leading to cell membrane structural changes and cell death. The colloidal form enhances stability and enables controlled ion release.

While the susceptibility of many bacterial strains to silver is well-documented, there is still much to explore regarding *Haemophilus influenzae* (H. influenzae). This Gram-negative bacterium, capable of forming biofilms, is responsible for airway infections and is a major pathogen in early-stage cystic fibrosis.

In this study, 40 nm diameter silver nanoparticles were synthetized and functionalized on the surface with a library of biocompatible functional molecules, including mercapto-polyethylenglycol-carboxylate (SH-PEG2000-COOH); Human Serum Albumin (HSA) and two mucines derived from porcine stomach (Type II and Type III mucines). Colloidal stability of the nanoconjugates in simulated physiological conditions was demonstrated up to one month by UV-visible spectroscopy, dynamic light scattering and Z-Potential measurements. Antimicrobial activity against H. influenzae was tested in vitro, showing a total inhibition of planktonic bacteria at concentrations ranging from 12ppm to 25ppm of total silver, depending on particle surface coating. All nanoconjugates were able to reduce by 5 orders of magnitude the population of H. influenzae biofilms at the highest concentration tested (200ppm).

This preliminary study proves that AgNPs could be used to treat H. influenzae infections and lays the foundations for further studies to improve efficacy and specificity.

P077

Complexities in determining live-dead state of bacteria at surfaces

Jiaqi Luo, Rasmita Raval

University of Liverpool, Liverpool, United Kingdom

Abstract

Determination of the physiological state of bacterial cells attached to a surface is crucial for antibacterial evaluation in biofilms research. The commercial counter-staining SYTO 9 – propidium iodine kit is a popular choice for viability studies, enabling bacterial cells with compromised membranes to be distinguished from intact ones. Scanning electron microscopy is another useful tool, enabling visualisation of single bacterial cells at much higher resolution. Here we combined these two methods to conduct correlative studies at the single-cell level of early-stage *Staphylococcus aureus* biofilms. This works shows that there is not a straightforward correlation between electron microscopy images and live-dead states indicated by fluorescence. For a number of cells, a double-staining phenomenon was also observed and further investigated. This revealed unexpected complexities and distributions of dyes, which significantly impacts evaluation of live-dead populations.

P078

Pathogen Characteristic of Biofilm Related CA-UTI in Intensive Care Unit

Titik Nuryastuti[1,2], Sayyidah Auliany Aminy[1], Ema Damayanti[2,3], Ayu Rahayu[1,2], Estelle Perraud[4,5], Christine Imbert[4]

[1]Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, Yogyakarta, Indonesia. [2]Indonesian Biofilm Research Collaboration Center, UGM-BRIN, Yogyakarta, Indonesia, Yogyakarta, Indonesia. [3]Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia. [4]Laboratoire Ecologie et Biologie des Interactions, Université de Poitiers, UMR CNRS 7267, F-86073 Poitiers, France, Poitiers, France. [5]Laboratoire de Parasitologie et Mycologie, CHU de Poitiers, Poitiers, France, Poitiers, France

Abstract

Background: Catheter-associated urinary tract infection (CA-UTI) in ICU is one of the HAIs with increased length of stay, healthcare costs, morbidity, and mortality. This study aimed to analyze the pathogens, antimicrobial sensitivity and biofilm forming of microorganisms causing CAUTI in ICU patients of UGM Academic Hospital Yogyakarta, Indonesia.

Method: This study was conducted on 71 latex-urinary catheterized patients from October 2022 to March 2023. Isolate identification and antimicrobial susceptibility testing by using Vitek2 compact system. Biofilm-producing bacteria were identified by microtiter plate assay. Biofilm visualization test on several urinary catheter tips was conducted using SEM.

Results: This investigation collected a total of 71 urine specimens obtained from patients with indwelling latex-urinary catheters, resulted in 49 bacterial isolates and 28 yeast identified. Polymicrobial infection was detected in 8.45%. The most abundant microorganisms are *E. coli* (15.6%), *A. baumannii* (13.0%), *C. albicans* (11.7%), *C. tropicalis* (10.4%), *E. faecium* (10.4%), *E. faecalis* (9.1%), *C. krusei* (6.5%), and *P. aeruginosa* (6.5%). As many as 62.5% of Enterobacterales isolates are ESBL producers, 90% of *A. baumannii* and 20% of *P. aeruginosa* are CRO, respectively. *C. albicans* and *C. tropicalis* showed good level of sensitivity to antifungals. About 91.8% of bacterial, and 100% of Candida isolates were able to form biofilms. SEM of urinary catheter showed the biofilm structure and confirmed with bacterial morphology from urine culture.

Conclusion: *E. coli, A. baumannii, and C. albicans* are the predominant pathogen causing biofilm infections in CAUTI. Uropathogens are mostly able to form biofilms and regarded as MDR strains.

P079

Effect of local surface topography on cell division of Staphylococcus spp.

Yuri Antonio Diaz Fernandez[1,2], Ioritz Sorzabal Bellido[1], Luca Barbieri[1], Arturo Susarrey-Arce[3], Roald M Tiggelaar[3], Rasmita Raval[1]

[1]University Of Liverpool, Liverpool, United Kingdom. [2]University of Pavia, Pavia, Italy. [3]MESA+ Institute, University of Twente, Enschede, Netherlands

Abstract

Abstract: Surface engineering is a promising strategy to limit or prevent the formation of biofilms. The use of topographic cues to influence early stages of biofilm formation has been explored, yet many fundamental questions remain unanswered. In this work [1], we develop a topological model supported by direct experimental evidence, which is able to explain the effect of local surface topography on early-stage micro-colonies of *Staphylococcus spp*. We demonstrate how the topological memory at the single cell level, characteristic of this genus of Gram-positive bacteria, can be exploited to influence both the architecture and surface interaction of micro-colonies of *Staphylococcus spp*. over nano-patterned surfaces. The surfaces are not intrinsically antimicrobial, yet they delivered a topography-based effect and a significant disruption of the local morphology and surface anchoring points of micro-colonies at the surface. The insights from this work could open new avenues towards designed technologies for biofilm engineering and prevention, based on surface topography.

Reference:

1. Sorzabal Bellido *et al*, Nanomaterials 2022, 12, 683.

P081

Comparative Analysis of Gene and Cytokine Expression in *Streptococcus mutans*: Biofilms vs. Planktonic Forms

Shatha Ahmasani

Cardiff University, Cardiff, United Kingdom. King Khalid University, Abha, Saudi Arabia

Abstract

Background and Aim: *Streptococcus mutans* is a Gram-positive facultative anaerobe and a key contributor to dental caries due to its biofilm-forming ability. The extracellular polymeric substances (EPS) in *S. mutans* biofilms may influence immune modulation. This study investigated immune responses to planktonic and biofilm forms of *S. mutans,* with and without EPS, using THP-1 and PBMC derived macrophages to identify potential immunomodulatory targets.

Methodology: *S. mutans* was cultured under planktonic and biofilm conditions, with and without EPS. Heat-inactivated bacterial preparations were co-cultured with THP-1 and PBMC-derived macrophages. Gene expression was analysed using nCounter Nanostring, with RT-qPCR and ELISA validation.

Results: Nanostring analysis showed significant immune gene upregulation in response to *S. mutans* biofilms compared to planktonic cells. Glucan-rich biofilms induced higher *IDO1, CXCL10,* and *MX1* expression, suggesting immune modulation. Biofilms without EPS triggered a different and reduced response, indicating an EPS-mediated effect. ELISA revealed increased TNF-α in macrophages exposed to glucan-rich biofilms, while planktonic cells induced higher IL-10, supporting differential cytokine production. Enzymatic EPS disruption partially restored immune responses, confirming its role.

Conclusion: *S. mutans* biofilms elicited distinct immune responses, with EPS playing a key role. Targeting EPS could enhance immune recognition and clearance, offering potential therapeutic strategies.

P082

PROBIOTIC THERAPY: A NATURAL SOLUTION FOR ANTIBIOTIC RESISTANCE IN WOUND HEALING PROCESS

Prathima Morbagilu Nagabhushana, Iza Radecka

University of Wolverhampton, Wolverhampton, United Kingdom

Abstract

Antimicrobial resistance and microbial infection is a major threat to global public health. Infection of wounds leads to poor healing, sepsis, and often death. Some bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* have multi-drug resistance ability due to their virulence factors. This study explores bacterial cellulose (BC) as a wound dressing material, incorporated with lactic acid bacteria (LAB) for antimicrobial properties.

BC was produced using *Glucanoacetobacter xylinus* ATCC 23770, under static condition at 30°C (15 days) and characterised using moisture content, transparency test, SEM and FTIR. FTIR results of BC were shown similar peaks (0-H, C-H stretching) to commercial grade cellulose. SEM images indicates pore size of BC varied from 3-8 µm, which helps in incorporation of antimicrobial substances, as BC itself lacks antimicrobial property of its own. BC was incorporated with LAB using adsorption incubation method. LAB strains used were *Lactobacillus acidophilus* UOW 3-34 and *Lactobacillus casei* NCIMB 8835.

Antimicrobial activity of LAB was evaluated using a disk diffusion assay against *P. aeruginosa* NCIMB 6571 and *S. aureus* NCIMB 8295. *L. acidophilus*-incorporated BC showed significant ($p<0.05$) inhibition of *S. aureus*. LAB incorporated cellulose disks showed highly significant ($p<0.001$) inhibition compared to ampicillin against *P. aeruginosa* and *S. aureus*, which shows potential antimicrobial activity of LAB.

This study highlights LAB-incorporated BC as a promising alternative for next-generation wound dressings. Its antimicrobial properties make it particularly effective against infections caused by resistant bacteria, offering a potential solution to combat antimicrobial resistance and improve wound healing outcomes.

P083

Inhibition of biofilm growth in microchannels via alternating electric fields with embedded 3D microelectrodes

Srinivasu Puttaswamy[1], Saikat Jana[2], James McLaughlin[2], Ian Johnston[1]

[1]University of Hertfordshire, Hatfield, United Kingdom. [2]Ulster University, Belfast, United Kingdom

Abstract

Biofilms in microchannels are a key area of research in microbiology, biomedical engineering, and microfluidics. Microchannels provide an ideal environment for biofilm formation due to their high surface-area-to-volume ratio and controlled fluid dynamics. Understanding biofilm behaviour in these systems is crucial for applications such as lab-on-a-chip devices, biofouling prevention, and microreactors.

In this study, we fabricated a microfluidic device using photolithography. The cured PDMS layer was bonded to a glass slide via oxygen plasma treatment, which also made the channel walls hydrophilic. The device included a primary channel for biological samples and two side electrode channels forming a 3D integrated electrode system to apply an alternating electric field.

A Pseudomonas fluorescens bacterial suspension was introduced at 0.1 µL/min, allowing biofilm formation over 48 hours. The biofilm developed on both sides of the main channel. To investigate electric field effects, voltages from 5 Vpp to 20 Vpp were applied at 1 MHz for different durations. The frequency was also varied from 1 MHz to 20 MHz at a constant voltage of 10 Vpp. The electrode channels, separated from the main channel by a 10 µm PDMS structure, enabled close-field exposure. Disrupted biofilms were removed using flow rates from 1 to 10 µL/min.

This study optimizes electric field parameters for biofilm disruption in microchannels, providing a non-invasive approach for biofilm management with potential biomedical, industrial, and environmental applications.

P084

Do polymicrobial communities support the persistence of *Ornithobacterium hominis* in the human nasopharynx?

Susannah Salter, Julian Parkhill

University of Cambridge, Cambridge, United Kingdom

Abstract

Background: *Ornithobacterium hominis* is a recently described Gram negative bacterium that can colonise the upper respiratory tract for prolonged periods in childhood. Despite its tenacity in the human host environment, *O. hominis* is a fastidious species in isolated culture and its growth requirements are not fully understood. We hypothesise that *O. hominis* receives nutritional benefits from other members of the microbiome.

Methods: We screened >200,000 publicly available respiratory tract microbiome samples from 83 countries, to identify studies where *O. hominis* was present. Datasets that contained *O. hominis* were analysed using Mothur and FastSpar to identify shared bacteria that correlate with *O. hominis* carriage.

Results: Several bacterial taxa are significantly correlated with *O. hominis* in nasopharyngeal samples from across the world. These include *Moraxella lincolnii, Helcococcus* sp, *Bergeyella* sp, *Suttonella/Rappaport* sp, and an unclassified Gracilibacteria. Furthermore, this combination of bacterial species is rare among samples that lack *O. hominis*.

Conclusions and further work: Due to the frequent co-occurrence of these bacteria in different settings, we hypothesise that they constitute a functional and persistent polymicrobial community. We propose to culture them in combination to examine triggers of biofilm formation, biofilm structure, and nutritional interactions between members that may support *O. hominis* colonisation.

P086

Bacteriological and Clinical Analyses of Biofilm-Forming Pathogen from Diabetic Foot Ulcers

Salsabilla Hasna Mutiara Rizki[1], Siti Nurhayati Kholidah[2], Rizka Humardewayanti Asdie[3], Ema Damayanti[4], Titik Nuryastuti[1,2]

[1]Indonesian Biofilm Research Collaboration Center, UGM-BRIN, Yogyakarta, Indonesia. [2]Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. [3]Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. [4]Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia

Abstract

Background: Biofilm infections complicate diabetic foot ulcers (DFUs) in 15-20% of diabetes mellitus (DM) patients annually. These led to amputation in 30% of patients, with a 14.8% mortality rate within a year post-amputation.

Methods: This study explores the relationship between biofilm formation and the antimicrobial sensitivity patterns of diabetic foot ulcer, considering clinical features. Patients (n = 65) from two Yogyakarta hospitals were recruited from October 2022 to May 2023. Foot ulcer swabs were cultured, isolated, identified, and subjected to antimicrobial susceptibility tests (VITEK2® system). Biofilm formation was assessed using a microtiter plate assay and scanned electronic microscope.

Results: Most patients were male (61.54%) with polymicrobial infections (56.92%), Wagner grade of 2-5 (98.47%), and 86.15% had no amputation history. Of the 115 bacterial isolates, 86.09% were Gram-negative, with *P. aeruginosa* (22.61%) and *E. coli* (20.87%) being most prevalent. Gram-positive bacteria, primarily *S. aureus* (10.43%), accounted for 13.91%. Almost all isolates (n = 111; 97.39%) were biofilm formers, with 61% being strong producers. Non-MDR and MDR bacteria were similarly common (47.83% vs 46.09%), and 6.09% were possibly XDR. The WHO 2024 Priority Pathogen List included CRA (100%), MRSA (58.33%), ESBL Enterobacterales (42.18%), CRPA (26.92%), and CRE (4.68%). Strong biofilm-forming carbapenem-resistant bacteria had higher biofilm-formation capacity than carbapenem-sensitive strains ($p \leq 0.05$). However, no significant correlation was found between biofilm formation and bacteria type, multidrug resistance, amputation history, wound length, or Wagner grade.

Conclusion: Most of biofilm-forming bacteria from diabetic foot ulcers have low antibiotic sensitivity. Rational antibiotic therapy is essential for treatment.

P087

From quantification of dry biofilms to the development of bio-adhesives for dry environments

Eleri Holmes[1,2], Daniya Babu[1], Liam Haglington[1,3], Geertje van Keulen[1], Christopher Phillips[2]

[1]Institute of Life Sciences, Swansea University, Swansea, United Kingdom. [2]Welsh Centre for Printing and Coating, Faculty of Science and Engineering, Swansea University, Swansea, United Kingdom. [3]Materials Engineering, Faculty of Science and Engineering, Swansea University, Swansea, United Kingdom

Abstract

Dry biofilms present significant challenges in sectors such as healthcare and marine environments, yet there is potential to exploit the inherent adhesive properties of dry biofilms in developing new bio-adhesives for bonding challenging materials under dry conditions. A limitation in screening of dry biofilm-forming microbes is the lack of a suitable quantitative high-throughput assay. This research therefore evaluates Crystal Violet Assay for quantification of dry biofilm formation on various surfaces, under various media and bacterial strains. The ability of microbes (*Escherichia coli* K12, *Staphylococcus epidermidis* 1457, and *Staphylococcus aureus* 16A) to form dry biofilms on glass coupons was assessed through wet and dry cycling in rich and defined media. Wet biofilm formation was assessed first, followed by drying of the biofilms through multiple drying methods. Biofilm mass was then quantified using crystal violet staining and absorbance measurements. Weak biofilm formers in the wet state were also weak in the dried state, with significant differences between weak and strong biofilm formers. Biofilm formation data was then used to screen for microbes capable of adhering to various materials from wettable to low-surface energy. This research highlights that understanding of dry biofilm formation, and the development of reliable quantification methods, will benefit sectors that require either prevention of biofilm formation or utilisation of their adhesive properties. The screening of the adhesive properties and mechanisms of the dry biofilm formation on the substates of interest, will determine which microbes to move forward with for further adhesive testing and for the development of novel bio-adhesives.

P089

Evaluation of the bead Assay for Biofilm to investigate biofilm form by different Candida species and their biofilm resistance to known yeasticidal disinfectants

Britta Brands[1], Nicole van Leuven[2], Dirk Bockmuehl[2]

[1]Dr. Brill + Prof. Bockmühl GmbH Institute for Applied Hygiene, 47533, Germany. [2]Rhine-Waal University of Applied Sciences, Kleve, Germany

Abstract

The Bead Assay for biofilms has been developed as a method to test the susceptibility of bacterial biofilms against different disinfectants [1].

In this model, biofilms are grown in glass or PTFE beads in 24-well multiwell plates under continuous agitation. The biofilms are then used to test the resistance to disinfectants or the influence of other substances.

We have investigated the suitability of this model for biofilms formed by different *Candida* species that are relevant in health as well as domestic settings. These included the well-known test strain *C. albicans,* but also *C. parapsilosis* and *C. auris* which gained attention in different health-care settings over the last years. [2–7]

The main focus was to evaluate whether the method in general was fit to grow biofilms of the yeasts and then in a further step to evaluate whether the grown biofilms show a resistance against known yeasticides that differs from the corresponding values achieved in normative tests in which planktonic cells are used.

P091

Cold Plasma Enhances Antibiotic Susceptibility and Immune Activation in Staphylococcus aureus Biofilms

Thomas Thompson[1], Ross Duncan[1], Carly Smith[2], Jordanne-Amee Maybin[1], Paula Bourke[3], Tom schaer[4], Noreen Hickock[2], Theresa Freeman[2], Brendan Gilmore[1]

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

Abstract

Biofilm infections remain a major challenge in antimicrobial resistance, limiting antibiotic efficacy and driving persistent infections in orthopaedic implants. Cold atmospheric plasma (CAP) is emerging as a novel biofilm-targeting therapy, capable of disrupting biofilm resilience, altering microbial metabolism, and increasing antibiotic susceptibility.

This study evaluates CAP as a pre-treatment for enhancing antibiotic penetration and biofilm eradication, focusing on *Staphylococcus aureus* orthopaedic implant infections in both in vitro biofilm models and a rat infection model. CAP exposure reduces biofilm biomass (confocal imaging), increases oxidative stress (REIMS analysis), and significantly lowers MBICs for intracellular-targeting antibiotics such as ciprofloxacin and tetracycline. Importantly, biofilm structure and MBIC reductions varied with CAP exposure time, underscoring the need for optimised treatment conditions.

Mechanistically, transcriptomic profiling of *Pseudomonas aeruginosa* biofilms (Maybin et al., 2023) identified upregulation of membrane stress response pathways (oprG, lptF, mreB), supporting plasma-induced permeability shifts. Building on this, ongoing proteomic analysis in *S. aureus* suggests similar metabolic disruption, reinforcing CAP's cross-species antibiotic-enhancing effects.

In vivo, CAP-treated infections exhibited increased B cell activation (transcriptomics from rat tissue), supporting an immune-stimulatory effect. This was further reinforced by macrophage activation observed in vitro. These findings suggest that CAP's impact extends beyond direct biofilm disruption to potentially enhancing host immune responses.

These findings establish CAP as a dual-action therapy—directly weakening biofilms thereby sensitising bacteria to antibiotics while stimulating host immune activity. With further refinement, CAP could serve as a scalable, non-antibiotic intervention for implant-associated infections, overcoming biofilm-mediated antibiotic tolerance.

P094

Quantitative Analysis of Dynamic Biofilm Structures via Time-Resolved Droplet Microfluidics and Artificial Intelligence

Daniela Pérez Guerrero

Gothenburg University, Gothenburg, Sweden. Jesús Manuel Antúnez Domínguez, Gothenburg, Sweden. Giovanni Volpe, Gothenburg, Sweden. Daniel Midtvedt, Gothenburg, Sweden. Wylie Ahmed, Toulouse, France. Lisa Muiznieks, Paris, France. Aurelie Vigne, Paris, France. Caroline Beck Adiels, Gothenburg, Sweden

Abstract

Biofilms are structured communities of microorganisms that play a crucial role in medicine, biotechnology, and ecology, contributing to microbial adaptation to any environment. Despite their significance, understanding their formation, development, and behavior remains a challenge for the community.

We utilize high-throughput droplet microfluidics to enable biofilm growth in miniaturized environments, generating extensive time-lapse bright-field microscopy images. To overcome experimental constraints, including dense structural heterogeneity and skewed illumination, we developed a deep learning-based segmentation approach capable of identifying biofilm structures in complex imaging conditions. Our method operates in an unsupervised manner, reducing the need for ground truth annotations and mitigating the introduced bias of manual segmentation approaches.
Our unsupervised model effectively detects and quantifies biofilm structures, even in late-stage growth, where traditional segmentation techniques fail. The neural network demonstrates robust performance across the development cycle, distinguishing biofilm boundaries and bacteria aggregates separated from the main biofilm structure despite imaging inconsistencies. Additionally, our approach reduces manual intervention, streamlining the analysis of high-throughput biofilm imaging data.
This AI-powered segmentation technique provides a reliable and scalable tool for biofilm analysis, addressing key limitations of conventional methods. By bridging the gap between microbiology research and automated image analysis, our approach facilitates more efficient and reproducible biofilm studies.

P095

Battlestar™: A Biodegradable Delivery System for Addressing Biofilm-Associated Infections Using In Situ Generated Oxidative Biocides

Harsha Siani[1], Adrian Fellows[1], Leah Conway[1], Guy Braverman[1], Allen Hanouka[1], Richard Day[2], Carey Lambert[3], Dimitra Panagiotopoulou[3], Kim R. Hardie[3]

[1]AGA Nanotech, Hemel Hempstead, United Kingdom. [2]University College London, London, United Kingdom. [3]University of Nottingham, Nottingham, United Kingdom

Abstract

Biofilm-associated infections remain a major challenge in antimicrobial resistance (AMR), reducing the efficacy of conventional treatments and increasing the risk of persistent infections. Battlestar™ is a novel, biodegradable antimicrobial platform that generates peracetic acid (PAA) and hydrogen peroxide (H_2O_2) in situ at the infection site.

Battlestar™ encapsulates biocide precursors within biodegradable poly(lactic-co-glycolic acid) (PLGA) microspheres, providing a sustained and controlled antimicrobial response. Upon exposure to biological fluids, the precursors react to generate PAA and H_2O_2, which penetrate biofilm matrices, disrupt extracellular polymeric substances (EPS), and induce oxidative damage to embedded microbial cells. Unlike conventional liquid PAA formulations, Battlestar™ enables controlled, on-demand PAA generation at optimised concentrations, reducing off-target cytotoxicity while preserving host tissue integrity.

Preliminary in vitro data in a monospecies biofilm model demonstrates biofilm eradication within twenty-four hours. Antimicrobial efficacy is sustained for up to 72 hours. In vivo assessments indicate minimal cytotoxicity, with no observable erythema or edema at the implantation site. Additionally, Battlestar™ generates PAA locally at a near-neutral pH, improving safety, stability, and biofilm disruption efficacy.

This novel platform represents a next-generation approach for biofilm-associated infections, with potential applications in chronic wounds, osteomyelitis, and medical device-related biofilms. By combining precision biocide activation with a biodegradable delivery system, Battlestar™ provides a scalable, resistance-mitigating solution to AMR.

P096

Development of NAM-FISH for the study of biofilms formed in advanced 3D substrates: insights into cystic fibrosis biofilm complexity

Isadora Maia[1,2], Sofia R. Cruz[1,2], Daniela Pacheco[3], Nuno F. Azevedo[1,2], Andreia S. Azevedo[1,2]

[1]LEPABE, Porto, Portugal. [2]ALiCE, Porto, Portugal. [3]Bac3Gel, Porto Salvo, Portugal

Abstract

Background. Biofilms play a key role in chronic infections, including cystic fibrosis (CF), where persistent polymicrobial biofilms in the lungs contribute to disease progression and premature death. Physiological models and improved methods for biofilm analysis promote efficient strategies for treating biofilm-associated infections. The goal of this work was to implement and validate a Locked Nucleic Acid/2'-OMethyl-Fluorescence *in situ* Hybridization (LNA/2'OMe-FISH) method for biofilms cultured in a novel 3D substrate - Universal Bac^{3}Gel - that reproduces key properties of human mucus.

Methods. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were selected for the case-study for their relevance in CF. Complementarily, confocal laser scanning microscopy (CLSM) was used to determine the spatial distribution and interactions of each pathogen in the biofilm.

Results. Validation experiments showed LNA/2'OMe FISH detected ~95% of the cells detected by DAPI, with a correlation coefficient of 0.99. In single-species biofilms, LNA/2'OMe-FISH detected log 8.8 and log 8.9 cells/mL of *P. aeruginosa* and *K. pneumoniae*, respectively. For dual-species biofilms, cell counts reached similar values (detected by LNA/2'OMe FISH), log 9.2 and log 8.9 cells/mL, for *P. aeruginosa* and *K. pneumoniae*, respectively.

Conclusion. This study validates LNA/2'-OMe-FISH as a reliable method for biofilm detection in Universal Bac^{3}Gel, enabling high-resolution visualisation of microbial communities. The observed co-aggregation of *P. aeruginosa* and *K. pneumoniae* suggests potential synergistic interactions in CF-related biofilms. Combining LNA/2'-OMe-FISH with CLSM offers a robust approach for studying biofilm architecture and pathogen behavior, facilitating improved infection models and therapeutic development.

P097

The combined effects of biofilm dispersal agents and ultrasound: a novel therapeutic strategy for biofilm-associated infections

Aaron Crowther[1,2], Gareth LuTheryn[2], Maryam Parhizkar[1], Ramón Garcia Maset[1], Sara Keller[2], Mark Sutton[3], Charlotte Hind[3], Dario Carugo[2]

[1]University College London, London, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]UKHSA, Porton Down, United Kingdom

Abstract

Biofilm-associated infections (BAIs) account for up to 80% of human infections, often contributing to chronicity and antibiotic resistance. Biofilms, commonly found in chronic wounds or in the urinary tract, create a diffusional barrier that limits antimicrobial penetration and provide a niche for dormant, antibiotic-tolerant bacteria. Novel antibiofilm agents, including signalling molecule nitric oxide (NO) and chelator tetrasodium ethylenediaminetetraacetic acid (T-EDTA), show promise due to their multifaceted effects, such as bactericidal activity, biofilm dispersal, and wound healing. Additionally, ultrasound (US)-mediated cavitation has emerged as a potential adjuvant therapy, enhancing antimicrobial penetration by physically disrupting the biofilm architecture. This study investigates the combination of chemical antibiofilm agents with US-mediated cavitation. NO donors propylamine propylamine NONOate (PA-NO) and spermine NONOate (SP-NO) and T-EDTA were evaluated against early-stage (24-hour) and mature (48-hour) *Pseudomonas aeruginosa* PAO1 biofilms. T-EDTA demonstrated consistent antibiofilm efficacy, reducing biofilm biomass by 57% (early-stage) and 64% (mature) after a 2-hour exposure period. After the same treatment period, PA-NO was effective against early-stage biofilms (21% reduction) but had no effect on mature biofilms, whilst SP-NO showed no efficacy against either model. When phospholipid-shell, perfluorobutane-core microbubbles ($PFBMB^{+}$s) were stimulated with US in the presence of ciprofloxacin and PA-NO, a 91% reduction in biofilm coverage was observed, while T-EDTA showed reductions of 73% (without ciprofloxacin) and 88% (with ciprofloxacin). Findings from this study suggest that the combination of biofilm dispersal compounds with cavitation-mediated approaches represent a promising strategy for addressing the challenges of BAIs, particularly through enhancement of antimicrobial delivery and efficacy.

P099

Imaging the Internal Architecture of *Pseudomonas aeruginosa* Biofilms in Single- and Dual-Species Communities with *E. coli*

Connor MacDonald, Liam Rooney, Gail McConnell

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom

Abstract

Pseudomonas aeruginosa and *Escherichia coli* have been extensively studied in monoculture and are commonly found in polymicrobial biofilms in clinical settings, such as catheters and the airways of cystic fibrosis patients. Biofilm growth is influenced by dynamic biological and physical factors and in natural environments often consist of multiple species, exhibiting collective behaviours that cannot be easily predicted from individual species' biofilms. Previously, intra-colony channels have been identified in *E. coli* macrocolony biofilms and shown to facilitate nutrient transport from the surrounding environment. This study used conventional and novel imaging techniques, such as confocal microscopy and widefield mesoscopy with the Mesolens and discovered undocumented channel structures within *P. aeruginosa* macrocolony biofilms. Specimen preparation methods enabled visualisation of internal structures of *P. aeruginosa* biofilms, revealing, for the first time, that *P. aeruginosa* forms radial structures capable of transporting fluorescent microspheres into the biomass. Co-cultured *E. coli/P. aeruginosa* biofilms developed sinuous channel structures with a different morphology to its monoculture, indicating that *E. coli* may alter the internal architecture of *P. aeruginosa* biofilms. Demonstrating that these structures form and can uptake exogenously in two phylogenetically distinct bacteria indicates the need to determine how widespread these channel structures are among other bacterial genera. Along with these novel findings of internal architecture in *P. aeruginosa,* the current work is focused on expanding this research to clinically relevant isolates to investigate whether these channel structures are conserved across diverse strains and to better understand their potential role in polymicrobial biofilm development.

P100

"Photo-responsive surfaces based on Prussian Blue: towards antibiofilm technologies based on FDA approved chemistries"

Lavinia Rita Doveri[1], Emanuela Peluso[2,3,4,5], Laura Ghidoni[6,1], Livia Visai[2,4,3,5], Piersandro Pallavicini[1,3], Angelo Taglietti[1], Yuri Antonio Diaz Fernandez[1,7,8], Giacomo Dacarro[1,3]

[1]Department of Chemistry, University of Pavia, Pavia, Italy. [2]Department of Molecular Medicine, University of Pavia, Pavia, Italy. [3]Centre for Health Technologies (CHT), Pavia, Italy. [4]Consorzio Interuniversitario Nazionale per la Scienza e la Tecnologia dei Materiali (INSTM), Pavia, Italy. [5]Research Unit (UdR) Pavia, University of Pavia, Pavia, Italy. [6]PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy. [7]Interdepartmental Research Center in Advanced Materials and Device (MADE), University of Pavia, Pavia, Italy. [8]Open Innovation Hub for Antimicrobial Surfaces, University of Liverpool, Liverpool, United Kingdom

Abstract

Antibiotic resistance represents a major challenge for managing bacterial infections related to biofilms. This study explores the use of FDA approved material Prussian Blue, formulated as nanoparticles (PBnps), that exhibit light-triggered antibacterial activity directly at surfaces.

PBnps, fabricated with different coatings, were stable under physiological conditions, as confirmed by UV-Vis spectroscopy and dynamic light scattering. Cryo-TEM analysis revealed the formation of a protein corona at the surface of the particles, providing additional chemical stability to PBNPs. Cytotoxicity tests on EAhy926, NCI-H1299, and A549human cell lines showed that PBnps do not affect cell viability in a wide range of concentrations, ensuring safety for biomedical applications.

PBnps were anchored onto poly-L-lysine (PLL) functionalised glass, forming multilayers of uniformly arranged nanoparticles, as verified by complementary characterization techniques such as UV-Vis spectroscopy and scanning electron microscopy (SEM). These substrates can deliver a surface-localized increase in temperature upon exposure to a near-infrared laser (808nm), using regulatory compliant light intensities while providing the advantage of low phototoxicity and enhanced transparency of tissues at this wavelength. The photoactivated response achieved can be exploited for the photothermal eradication of biofilms. In fact, exposure of *Staphylococcus aureus* ATCC25923 biofilms to 1h irradiation at 808nm with laser power 330mW/cm^{2}led to a reduction of over 97% of the bacteria population at the surface, probed *in-vitro* with MTT assay and confocal fluorescence microscopy.

This innovative approach paves the way to emerging technologies that can combine already approved materials to produce light-triggered antimicrobial surfaces, able to prevent or eradicate biofilms on medical devices.

P102

Mycobacterial carbonic anhydrases as targets for tuberculosis research

Jenny Parkkinen, Ashok Aspatwar, Seppo Parkkila

Tampere University, Tampere, Finland

Abstract

Tuberculosis is amongst the deadliest infectious diseases worldwide with approximately 10 million annual cases and notable amount of drug-resistant infections. Carbonic anhydrases are believed to have important roles in mycobacterial biofilm formation and thus, these enzymes could act as novel targets in tuberculosis drug development.

The study uses *Mycobacterium marinum*, a natural pathogen of zebrafish, to model tuberculosis. Carbonic anhydrase inhibitors were screened to find bactericidal compounds safe to administer to zebrafish. Carbonic anhydrase-depleted strains are created by knocking out the enzyme-coding genes. Knockout phenotypes are assessed via studies on growth pace, colony morphology, antibiotic sensitivity and especially, any visual changes in biofilm formation.

Our inhibitor screening has revealed compounds bactericidal towards mycobacteria, with a preferential activity in biofilm environment. To further aid the design of effective inhibitors, we seek to reveal the biological mechanisms and interactions of the mycobacterial carbonic anhydrases by creating knockout strains. The produced MMAR_5088-knockout lacks target gene expression, whilst complemented strain has rescued expression. The knockout grows slower than its control strains and growth assays have indicated an increased sensitivity for rifampicin and isoniazid. Encouraged by the *in vitro* results, we are currently studying changes in virulence of the knockout with an adult zebrafish infection model.

MMAR_5088 encodes a carbonic anhydrase involved in biofilm formation of *Mycobacterium marinum*. We aim to unravel its mechanisms of action to help optimize the design of carbonic anhydrase inhibitors to be used as new anti-tubercular agents.

P103

Using predatory bacteria as alternative treatments of complex biofilms

Carey Lambert, Kim Hardie

University of Nottingham, Nottingham, United Kingdom

Abstract

Bdellovibrio bacteriovorus is a small predatory bacterium which preys upon other Gram-negative bacteria by burrowing inside the periplasm and consuming them from within. They prey upon a wide range of pathogens, including multi-drug resistant "ESKAPE" organisms and so have great potential for use as a novel therapeutic intervention. As well as efficient predation on planktonic bacteria, *Bdellovibrio* have been shown to eradicate biofilms of Gram-negative bacteria and even to disrupt biofilms of non-prey Gram-positive bacteria.

We hypothesise that *Bdellovibrio* could be an effective agent for combating problematic biofilms, potentially effective against a wide range of Gram-negative organisms and dispersing polymicrobial biofilms. Here, we describe novel adhesins involved in prey handling; a diverse group of mosaic adhesive trimeric proteins expressed on the predator surface and MIDAS domain proteins. Better understanding of prey attachment and handling could give insights into the nature of prey range and allow us to understand which pathogens in biofilms can be targeted by *Bdellovibrio.* Testing the prey ranges of predatory bacteria and adapting them to prey upon specific pathogens in biofilms could optimise the predators for such usage. Training *Bdellovibrio* by repeated passage on specific pathogens, or selection of specific adhesin mutants could result in more efficient predation on these pathogenic biofilms.

Insights into *B. bacteriovorus* attachment to, and predation on diverse biofilms could bring us closer to applying this organism as a novel intervention approach to address problematic biofilms in different environmental settings.

P104

Polymer-directed inhibition of reversible to irreversible attachment prevents Pseudomonas aeruginosa biofilm formation

Jean-Frédéric Dubern[1], Alessandro Carabelli[2], Maria Papangeli[3], Andrew Hook[2], Morgan Alexander[2], Paul Williams[1]

[1]Biodiscovery Institute, National Biofilms Innovation Centre and School of Life Sciences, University of Nottingham, Nottingham, United Kingdom. [2]School of Pharmacy, University of Nottingham, Nottingham, United Kingdom. [3]Department of Infectious Disease, Imperial College London, London, United Kingdom

Abstract

Bacteria readily attach to surfaces forming biofilms. These are commonly associated with medical device-associated infections and highly refractory to antibiotics. Biocompatible, weakly amphiphilic acrylate polymers with rigid hydrocarbon pendant groups resist bacterial biofilm formation *in vitro* and *in vivo* but the biological mechanism involved is not known. By comparing the biofilm resistant acrylate, polyethylene glycol dicyclopentenyl ether acrylate (pEGdPEA) with a biofilm supporting polymer, neopentyl glycol propoxylate diacrylate (pNGPDA) and a glass surface, we demonstrate that pEGdPEA inhibited the transition from reversible to irreversible attachment. Confocal laser scanning microscopy showed that fewer *Pseudomonas aeruginosa* cells accumulated on pEGdPEA. Single-cell tracking and controlled flow microscopy indicated that bacteria reaching the polymer surface exhibited shorter residence times and greater asymmetric division with more cells departing from the surface post-cell division, characteristic of reversible attachment. On the resistant polymer, the lack of accumulation of the second messenger cyclic diguanylate or expression of *sadB* (coding for Surface Attachment Deficiency Protein B) were consistent with the failure to transit from reversible to irreversible attachment. Additionally, we showed that negative regulation of rhamnolipid production by SadB inhibits biofilm formation on pEGdPEA. Rhamnolipids are a class of glycolipids produced by *P. aeruginosa* and have the properties of biosurfactants. Their functions cover modification of surface properties, stimulation of bacterial motility, formation and disruption of biofilms. We hypothesize that pEGdPEA inhibits *sadB* expression resulting in rhamnolipid overproduction causing bacterial cells to depart from the surface.

P105

Advancing Sustainable Biotransformation: Utilizing µMBR for Biofilm-Based Simultaneous CO_2 Fixation and Production of Platform Chemicals

Carmen Mandel, Johannes Gescher, Miriam Edel

Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany

Abstract

Background

Leveraging renewable carbon sources or atmospheric CO_2 for a more sustainable chemical production is vital. We propose using a genetically engineered strain of *Cupriavidus necator* H16 thriving as biofilm on gas diffusion membranes, utilizing CO_2 fixation with H_2 and converting organic acids, e.g., from biogenic waste streams, into valuable platform chemicals.

Methods

Microfluidic flow cells with an integrated gas diffusion membrane (µMBR) were developed as screening platform for biofilm-based production of acetoin, allowing flexible substrate input configurations. Biofilm growth and product formation was investigated under auto-, hetero- and mixotrophic conditions, monitored non-invasively using fully automated optical coherence tomography (OCT). Microprofiling was applied to elucidate substrate availability under different process conditions and gas flow rates.

Results

With the µMBR, we established a versatile screening platform for continuous biofilm-based production of platform chemicals, exemplified with acetoin. The impact of process conditions on the biofilm could be closely monitored. By fine-tuning the gas flow rate and composition, growth was limited to the membrane surface and the biofilm height controlled. Acetoin production was correlated to the biofilm volume during different stages of the fermentation. Cultivation as a membrane-bound biofilm allowed a substantially higher cell density compared to planktonic systems.

Conclusion

The application of genetically engineered *C. necator* on gas diffusion membranes holds significant promise for producing valuable chemicals with high carbon efficiency and cell retention. The µMBR serves as an effective platform for optimizing conditions for larger-scale applications, showcasing the potential of biofilm-mediated production from CO_2 in a sustainable biotechnology.

P106

FT-NIR and FTIR spectroscopic analysis of *Aureobasidium pullulans* biofilm

Anja Černoša[1,2], Jose Manuel Amigo[3,4], Anna Sandak[1,2,5]

[1]InnoRenew CoE, Izola, Slovenia. [2]Andrej Marušič Institute, Koper, Slovenia. [3]Department of Analytical Chemistry, University of the Basque Country UPV/EHU, Bilbao, Spain. [4]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain. [5]Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Koper, Slovenia

Abstract

Aureobasidium pullulans is ubiquitous, non-pathogenic, dimorphic fungus with biotechnological significance due to its production of extracellular polysaccharide pullulan, key component of its biofilm's EPS. However, the structure of its biofilm remains poorly understood.

This study analyzed biofilm structure of three morphologically different *A. pullulans* strains on four solid media – Yeast Nitrogen Base (YNB), Potato Dextrose Agar (PDA), Sabouraud Agar (SA) and Synthetic Nutrient Deficient Agar (SNA). Depending on their morphology, strains were inoculated onto media and incubated at 25°C. After two weeks, biofilms were analyzed using Fourier-transform near-infrared spectrometer (FT-NIR) and Fourier-transform infrared spectrometer (FTIR). Each sample underwent five measurements. Spectral pre-processing and data mining were performed using Opus 6.5 and PLS_Toolbox, an extension of Matlab package.

Principal Component Analysis (PCA) of all spectra indicates no major differences between biofilms of different strains, but showed distinct spectral differences on nutrient-poor SNA medium compared to other media. This trend appears in data from both instruments, confirming that chemical variations in the fingerprint region of IR spectra are also reflected in NIR spectra, which capture overtones and combination bands. Loadings analysis highlights bands related to -OH groups (around 5000 cm^{-1} and 7000 cm^{-1}) and -CH groups (around 1000 cm^{-1}) as the most relevant for the model. Previous findings indicate all strains grow in yeast form on SNA medium, possibly explaining variations in biofilm structure, however more in-depth investigation is needed.

These findings enhance understanding of *A. pullulans* biofilm formation, crucial for optimizing its use as a living coating in ARCHI-SKIN project.

P108

Real-time integrated diagnosis and antimicrobial resistance infection profiling by Raman spectroscopy and machine learning

Grace McCurdy[1,2], Callum Highmore[1,2], Niall Hanrahan[3,4], Jeremy Webb[1,2], Sumeet Mahajan[3,4]

[1]School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, United Kingdom. [2]National Biofilms Innovation Centre (NBIC), Southampton, United Kingdom. [3]School of Chemistry, Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, United Kingdom. [4]Institute for Life Sciences, University of Southampton, Southampton, UK, SO17 1BJ4, Southampton, United Kingdom

Abstract

Ventilator Associated Pneumonia (VAP) is typically acquired in hospital settings following 48 hours of endotracheal intubation with mechanical ventilation. VAP infections represent one in four infections in critically ill patients, posing a worldwide health risk. Biofilm formation in endotracheal tubes is associated with the development of VAP by acting as a reservoir for pathogens, resulting in relapses of VAP following antibiotic treatments.

Delayed diagnosis is a key challenge to effective treatment, often resulting in overuse of broad-spectrum antibiotics and incorrect diagnosis. The absence of real-time diagnostics for VAP and antibiotic susceptibility testing hinders efforts aimed at preventing antibiotic resistance proliferation. Current methodologies are time-consuming and often diagnostics follow the administration of broad-spectrum antibiotics. Bacterial species and strains can be identified using their unique Raman spectroscopic fingerprint, which reflects differing genotypic and phenotypic characteristics of different species and their growth conditions.

Raman spectroscopy has clear advantages over traditional diagnostics as it is non-destructive and requires minimal sample preparation. A Raman spectral database of VAP-related pathogens and their associated biofilms has been developed using Raman mapping which will be used for real-time identification and differentiation of VAP pathogens in the future. The continued development and growth of this Raman spectral database in combination with machine learning is a key step in enabling real-time integrated diagnosis of infections, helping restrict future antibiotic resistance proliferation.

P109

Impact of Environmental Stress on Biofilm Formation and Antibiotic Tolerance of *E. coli* Strains Isolated from Ghanaian Hospital

Tosin Senbadejo[1], Samuel Ntiamoah[1], Elizabeth Bugase[1], Christina Bourne[2], Abiola Isawumi[1]

[1]University of Ghana, Accra, Ghana. [2]The University of Oklahoma, Norman, USA

Abstract

Biofilm formation is a critical survival strategy for *Escherichia coli*, enhancing resistance to phenotypic stress and antibiotics. However, the extent to which stress conditions influence biofilm formation and subsequent antibiotic tolerance remains unclear. This study investigates the impact of temperature (4°C, 45°C) and pH (3, 9) on biofilm formation and antibiotic tolerance in four *E. coli* strains.

Biofilm formation was assessed using crystal-violet staining assay before and after stress. Optical density (OD) cut was used to categorize strains as strong, moderate, or weak formers. Using cell counts assay, antibiotic tolerance of biofilm and planktonic cells was evaluated at (2× MIC) of meropenem and polymyxin-B. Whole-genome sequencing (WGS) of strains was conducted to identify biofilm-associated genes.

The strains were moderate to strong biofilm-formers and showed 100% resistance to standard concentrations of the antibiotics. Environmental stress modulated biofilm formation, with pH altering biofilm classification (moderate ↔ strong), while multiple stressors maintained biofilm status. Biofilm-forming and planktonic cells exhibited complete tolerance to polymyxin-B, showing initial growth in 3 hours ($4x10^{-3}$ – $8.36x10^{-1}$; $2x10^{-2}$ – $1.574x10^{0}$ CFU/ml), followed by partial survival at 6 hours ($1x\ 10^{-3}$ – $2x10^{-3}$; $3x10^{-3}$-$9x10^{-3}$ CFU/ml) respectively. In contrast, meropenem exposure led to cell death. WGS identified key biofilm-associated genes (*csgG, afa operon, papF, fimH, papI*) in all strains, suggesting an inherent genetic potential for biofilm formation in complement to environmental conditions.

The findings highlight genetic basis of biofilm formation and its modulation by environmental stress, contributing to a deeper understanding of *E. coli* adaptation and informing antimicrobial treatment strategies.

P112

The skin and soft tissue microbiome in lymphoedema: A target for reducing cellulitis-associated morbidity?

Hannah Rossiter[1], Mitra Kanagaraj[2], Faizan Sadiq[3], Zita Jessop[3], David Thomas[3]

[1]Cardiff and Vale University Health Board, Cardiff, United Kingdom. [2]Cwm Taf Morgannwg University Health Board, Bridgend, United Kingdom. [3]Cardiff University, Cardiff, United Kingdom

Abstract

Lymphoedema is a chronic condition characterised by the accumulation of protein-rich lymphatic fluid, resulting in inflammation, fibrosis and often repeated episodes of cellulitis. Cellulitis episodes have significant impacts on quality of life, necessitate frequent antibiotic courses and worsen disease progression. It is hypothesised that microbiome alterations of affected regions may contribute towards infections. Knowledge of this would facilitate tailored prophylactic and empiric antimicrobial use.

A systematic review was conducted using Medline, Embase and Cochrane databases. Studies which sampled skin and soft tissue of patients with limb lymphoedema and described the genera of microorganisms present using molecular or culture based methods, or which reported microorganisms isolated from blood cultures of patients with lymphoedema and active cellulitis were included. Papers were screened and data extracted by two independent authors.

This review highlighted a lack of high quality, large volume primary research and the lack of standardised sampling and microbial detection methods in this patient cohort. Just two papers had collected primary data via skin or wound sampling. The majority of included papers were single patient case reports utilising blood culture. Nonetheless, the most commonly identified organisms across the included data were *Staphylococcus epidermis, Staphylococcus aureus, Streptococcus agalactiae and Streptococcus dysgalactiae*.

Further large scale research utilising standardised sampling and analysis methods are required to allow detailed data analysis and an understanding of the patterns of microbial colonisation in lymphoedema. This is an unmet need which has potential to facilitate better antimicrobial use and reduced morbidity for this patient group.

P113

Mutanofactin affects interactions of mucin-coated surfaces and *Streptococcus mutans*

Konstantin Nikolaus Beitl[1], Leon Thies[2], Lukas Schrangl[3], Guruprakash Subbiahdoss[1], Erik Reimhult[1], Christina Schäffer[2]

[1]Institute of Colloid and Biointerface Science, Department of Natural Sciences and Sustainable Resources, BOKU University, Vienna, Austria. [2]Institute of Biochemistry, NanoGlycobiology Research Group, Department of Natural Sciences and Sustainable Resources, BOKU University, Vienna, Austria. [3]Institute of Biophysics, Department of Natural Sciences and Sustainable Resources, BOKU University, Vienna, Austria

Abstract

Streptococcus mutans (*S. mutans*) is an early colonizer of the human oral cavity and key player in the establishment of dysbiotic biofilms associated with dental caries. Mutanofactins were recently identified as small-molecule secondary metabolites of *S. mutans*. Only one has been investigated and demonstrated to promote biofilm formation. However, the molecular mechanisms of mutanofactin action remain unclear.

Contact angle (CA) and microbial adhesion to hydrocarbons (MATH) assays were used to assess Muf-697's impact on *S. mutans* cell surface hydrophobicity (CSH). Quartz crystal microbalance with dissipation monitoring (QCM-D) and atomic force microscopy (AFM) were employed to characterize the interaction of mutanofactins with the glycoprotein mucin, a major component of saliva-coated surfaces in the oral cavity. Additionally, the effect of Muf-697 on bacterial adhesion to mucin layers was investigated using a microfluidics setup.

Muf-697 exposure did not affect *S. mutans* CSH, contradicting prior findings that proposed Muf-697-induced changes in CSH as the driving force in promoting *S. mutans* biofilm formation. Instead, QCM-D measurements showed that Muf-697 uniquely interacts with mucin molecules, roughly tripling mucin layer thickness. We interpret our data as an irreversible change in mucin structure and hydration. AFM force-distance mapping revealed increased mucin layer heterogeneity and localized substrate exposure after Muf-697 treatment. Flow adhesion assays demonstrated that Muf-697 increases *S. mutans* adsorption to mucin-coated surfaces tenfold, showing that mucin restructuring facilitates bacterial attachment.

Our findings suggest that Muf-697 promotes *S. mutans* biofilm formation through a previously unrecognized mechanism that may provide opportunities for new therapeutic approaches to oral biofilm-associated diseases.

P114

The effect of stagnation on biofilm structure

Francisca Ferreira[1,2], Luis F. Melo[1,2], Ana Pereira[1,2]

[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal. [2]ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

Abstract

Biofilm structure plays a crucial role in protecting bacteria, including pathogens, and resisting detachment under external stresses. Hydrodynamic conditions influence biofilm microbiome composition and structural characteristics. Particularly, water stagnation (no flow), a common issue in engineered water systems, is associated to water quality deterioration, increased microbial growth, and microbial population shifts. For example, stagnation fosters *Legionella* colonization of existing biofilms, raising concerns for Legionnaire's disease prevention.

In stagnant pipelines, biofilms form in varying orientations, such as vertical and horizontal surfaces. This study explores whether these orientations impact biofilm structure differently under stagnant conditions.*Pseudomonas fluorescens* biofilms were developed under flow conditions (Re: ~4440) for eleven days in a laboratory flow cell equipped with coupons and Surface Sensor Technology for real-time biofilm monitoring. Coupons were periodically sampled, biofilm structure was analysed through Optical Coherence Tomography and culturability was assessed.

Preliminary findings suggest that stagnation effects vary with biofilm positioning. When biofilms remain in a vertical position, thickness and porosity decrease, whereas horizontal position results in increased thickness and porosity. Those differences may be due to gravity effect on biofilms or suspended solids in the bulk phase. Additionally, stagnation may impact biofilm detachment patterns, leading to heterogeneous structural adaptations depending on flow history and biofilm maturity. The real-time monitoring was very relevant to assess the biofilm attachment/detachment.

Further research is needed to clarify these mechanisms and their implications in biofilm persistence. Understanding how stagnation influences biofilm behaviour is important to develop effective water management strategies and improve biofilm control in water systems.

P115

EPS production, disruption, and antimicrobial susceptibility in oral biofilms

Abdullah Albarrak

Cardiff University, Cardiff, United Kingdom

Abstract

Background:

Biofilms form when microorganisms adhere to surfaces and produce extracellular polymeric substances (EPS), which provide structural integrity and protection. Understanding EPS composition and its impact on antimicrobial susceptibility is crucial for biofilm control. This study aimed to characterise EPS production and assess enzymatic disruption to enhance antimicrobial efficacy.

Methodology:
Single- and mixed-species biofilms (*Candida albicans, Streptococcus mutans, Streptococcus Salivarius, Streptococcus gordonii, Streptococcus sanguinis* and whole saliva) were grown under batch and flow-through conditions, in differing culture media, incubation times, and periods of drying. EPS was assessed using fluorescent probes and Confocal Laser Scanning Microscopy (CLSM). Confocal Raman Scattering (CRS) microscopy, CLSM, and carbohydrate quantification evaluated enzymatic EPS disruption. Antimicrobial susceptibility of biofilms with and without EPS to chlorhexidine digluconate (CHX) and fluconazole was measured.

Results:
CLSM showed that EPS production was dependent on growth conditions, with highest levels occurring in biofilms supplemented with 1% sucrose. CLSM, carbohydrate quantification, and CRS confirmed significant EPS reduction post-dextranase treatment.

While sucrose supplementation did not affect planktonic susceptibility, biofilm growth was found to be more resistant. Minimum biofilm eradication concentrations were lower for EPS-inhibited biofilms but not for EPS-disrupted and EPS-intact biofilms. CLSM did however show an increased biovolume of dead cells. Fluconazole treatment of dual species biofilms significantly lowered *C. albicans* CFU counts post-EPS disruption.

Conclusion:
This study highlighted the role of culture conditions on EPS formation and the potential of enzymatic disruption to enhance antimicrobial efficacy.

P117

Investigating the impact of biofilm formation on multiphase flow and wettability in porous media using X-ray micro-CT.

Cian "CJ" Jones[1], Andreas Busch[1], Julia De Rezende[2], Kamaljit Singh[1]

[1]Heriot-Watt University, Edinburgh, United Kingdom. [2]-, Edinburgh, United Kingdom

Abstract

Underground hydrogen storage (UHS) in porous media proposes the large-scale storage of hydrogen in depleted hydrocarbon reservoirs.

Hydrogenotrophic microbes are able to consume hydrogen injected into the subsurface through metabolic pathways such as methanogenesis, sulphate reduction and acetogenesis (Thaysen et al., 2021). Microbes inhabiting the deep biosphere can also form biofilms that can influence the flow of fluids through the porespace of the reservoir rock (Boon et al., 2024). These factors combined pose a significant threat to UHS in porous media.

Biofilm formation can significantly change the petrophysical properties of porous media (Jin & Sengupta, 2024). These include changes in pore geometry, tortuosity, porosity, wettability, capillary pressure and phase saturations across the system which, in the context of underground hydrogen storage, can lead to hydrogen trapping and reduced recovery over several cycles of hydrogen injection and production (Pasca et al., 2015; Jangda et al., 2022; Raza et al., 2022).

This interdisciplinary project employs anaerobic and selective culturing techniques to effectively grow and cultivate relevant biofilm forming isolates. Batch experiments run under representative reservoir conditions with relevant strains and enrichment cultures will provide a time-resolved look at gas consumption and microbial activity over time in bulk fluid and in rock samples. X-ray micro-CT imaging will be utilised to look inside inoculated cores without destroying them. Digital image analysis of the scanned cores will allow for the observation of changes in multiphase flow in these rock-hydrogen-brine-biofilm systems and determine what impact biofilms could have on hydrogen recovery over several injection cycles.

P118

A novel, antibiotic-free and prophylactic topical in-wound applied formulation for combating biofilms and improving chronic wound healing.

Alice Plane, Dr Lian Liu, Professor Roberto La Ragione

University of Surrey, Guildford, United Kingdom

Abstract

Chronic wounds will affect the quality of life of an estimated 1-2% of developed populations. It is seen that biofilms are present in 90% of chronic wounds, a problem compounded by the increasing development of resistance to antibiotic drugs. Hence, attention is turned to formulating new non-antibiotic prophylactic and therapeutic strategies that can reduce the burden on existing antibiotic treatments. A novel, non-antibiotic treatment utilising polyhexamethylene biguanide (PHMB) and the vitamin A family member retinol in a D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) nano-micellar suspension has been developed in response. PHMB is a broad-spectrum antimicrobial, and in a wound setting retinol stimulates the neogenesis and re-structuring of epidermal cells.

The formulation is deployed against biofilms produced *in vitro* of *P. aeruginosa* and *S. aureus* with optimisation integrated. To date, we have shown our formulation to have significant antimicrobial efficacy against our isolates as planktonic cells and in preventing biofilm formation, with subsequent biofilm clearance assays in progress. Future work is looking to include the gold-standard organotypic, mammalian cell-seeded *ex vivo* tissue models in infection and wounding experiments. We will use these models to expand understanding of biofilm-host tissue interaction, alongside further demonstrating our formulation's efficacy in anti-biofilm activity and improvement of wound closure in an exemplary model.

The outcome of this research has the potential to offer an alternative therapeutic strategy for the prevention and clearance of bacterial biofilm infection, and the reversal of consequent wound chronicity, without the addition of burden to already overwhelmed usage of antibiotics.

P120

A novel method for monitoring biofilm build-up in industrial laundry equipment

Jake Dunlop, Raymond Allen, Katie Laird

De Montfort University, Infectious Disease Research Group, Leicester, United Kingdom

Abstract

Biofilm formation is a potential issue in industrial laundries. Microorganisms present in biofilms can induce degradation of the surfaces to which they are attached, impacting upon the tunnel washer's performance and stability. No methods are currently available to monitor biofilm formation in difficult to access areas of industrial tunnel-washers.

Aim: To develop a new approach to detect and quantify biofilm formation within healthcare industrial laundry machines.

Methods: Specially engineered frames were created for the suspension of stainless-steel coupons within two areas within an industrial laundry machine; a tunnel-washer module (used for disinfection of laundry) and a recycled wastewater tank. After one month of exposure, coupons were assessed by bacterial enumeration, scanning electron microscopy (SEM) to detect the presence of biofilms, and 16S metagenomic analysis. A Bactiscope was used as a rapid, simple method for identification of biofilms within the machines.

Results: Frames were successfully engineered for detection of biofilms within tunnel washers. Biofilm formation was detected in the wastewater tanks but not in the module exposed to high temperature (71°C) and chemical disinfection processes. Lint appears to play a role in biofilm formation within the wastewater areas of the laundry machines, with all SEM images from this area evidencing biofilms in association with lint.

Conclusion: Healthcare laundry equipment with difficult to access areas that are used continuously require specific engineering solutions for the detection of biofilms. This new approach presents an effective method to monitor biofilm formation and therefore improve industrial laundry machine design and disinfection processes.

P121

Comparative Analysis of Oral Biofilm Growth on Dental Surfaces: Static vs. Dynamic Models

Srinivas Kore[1,2], Marta Roldo[1], Rob Howlin[2]

[1]University of Portsmouth, Portsmouth, United Kingdom. [2]Haleon, Weybridge, United Kingdom

Abstract

Background: The research aims to identify the best model for studying the formation of biofilm on different materials, thereby supporting efforts to significantly reduce biofilm growth and enhance oral hygiene.

Methods: Oral biofilm from pooled saliva was cultivated for 5 days using a CDC biofilm reactor or on a static well plate model on different substrates - hydroxyapatite (HA), polymethyl methacrylate (PMMA), and stainless steel (SS) - which are commonly used in dental appliances. Surface characterization was conducted using profilometry, contact angle measurement, SEM, and imaging of biofilm via confocal microscopy.

Results: The results showed that the static model had greater biofilm growth in both volume and surface area compared to the dynamic model across all surfaces (PMMA 1.2 times, HA 2.6 times, and SS 3.4 times).

PMMA exhibited the highest biofilm growth in the CDC model (volume 235 μm^3, surface area 14874 μm^2), with statistically significant growth compared to HA and SS. There was no significant difference in biofilm growth between HA and SS.

The static model results showed the highest biofilm growth on stainless steel (contact angle θ 79°, volume 365 μm^3, surface area 26936 μm^2). Stainless steel differed significantly in volume compared to HA and PMMA, but only differed from HA in surface area.

Conclusion: This study shows static models lead to higher biofilm growth than dynamic models on all surfaces, emphasizing the significance of choice of material and model in biofilm research aimed at designing dental appliances to reduce biofilm issues.

P122

Control of *Listeria monocytogenes* biofilms in the fresh food supply chain

Lucy Sutton[1,2], Callum Highmore[1], Innogen Carter-Hall[2], Sandra Wilks[1], Charles Keevil[1]

[1]University of Southampton, Southampton, United Kingdom. [2]Vitacress, Chichester, United Kingdom

Abstract

Listeria monocytogenes is an opportunistic food-borne pathogen that can survive under harsh conditions such as refrigeration temperatures, low oxygen levels and low nutrient levels – therefore is a problem in the fresh food supply chain. Pathogenesis involves intracellular infection after which infection with *L. monocytogenes* can result in listeriosis which can be fatal in immunocompromised patients. This study aims to evaluate the efficacy of common sanitisation methods used in the fresh food supply chain, using appropriate laboratory models of *Listeria* biofilms. *L. monocytogenes* Scott A, *L. monocytogenes* CECT 936 and *L. innocua* biofilms were grown at 20°C or 4°C, on 1 cm^2 steel coupons for 7 days, and treated with 4 different concentrations of Cl_2 (0 ppm, 25 ppm, 50ppm, 100 ppm) on days 1, 3, 5, and 7. Coupons were then processed for culturable cell counts, EDIC microscopy, and Raman spectroscopy. The results of this study show that temperature effects biofilm growth; biofilms became established faster at 20°C but could still form at 4°C. Temperature also influences chlorine efficacy, as 4°C biofilms were more susceptible to chlorine sanitisation. Overall, chlorine was not effective at treating *Listeria* biofilms at the concentrations used, however Raman spectroscopy did indicate a physiological response to treatment with chlorine. Future work would include increasing the concentrations of chlorine, and to investigate the stress response at the molecular level using transcriptomics. This work provides important information on sanitisation efforts in the fresh food supply chain, concerning factory temperature and age of biofilm.

P123

Illuminating the Invisible: Investigating the Structural and Temporal Spatial Development of Anthropogenic Marine Biofilms and Their Role in Hydrozoan Larval Settlement and Metamorphosis

Isabelle Firth

University of Essex, Colchester, United Kingdom

Abstract

Biofouling hydrozoans (Cnidaria, Medusozoa) exhibit complex life histories involving planktonic planula larvae settling and metamorphosing into benthic polyps. This transition is crucial for species survival and is influenced by bacterial biofilms already inhabiting oceanic surfaces. Despite the direct physiological and economic damage colonial hydrozoans induce within bivalve and finfish aquaculture industries, the direct interactions between hydrozoan planula larvae and marine biofilms are relatively unexplored. Bioassay-guided analysis provides an initial understanding of how monospecies biofilms influence planulae settlement and metamorphosis, however, current research fails to explore the architectural role of biofilm development.

To reduce this disparity, bacteria isolated from fouled boats in Helsingor Harbour (Copenhagen, Denmark) were assembled into monospecies biofilms. Using confocal scanning laser microscopy and the application of target fluorophores, key biofilm biomolecules were visualised. Image acquisition shows species-specific architectural phenotypes observed in each monospecies biofilm, with distinct structures and spatial pattern development over time. These findings suggest each bacterial isolate may present unique influences on planula settlement and metamorphosis.

To complement these findings, future studies will incorporate bioassay-guided analysis with the model hydrozoan *Clytia hemisphaerica,* to build a holistic understanding of hydrozoan planula-biofilm interactions, through a novel approach.

P124

mRNA PNA-FISH for Gene Expression Analysis to Study the Spatial Ecology in Legionella pneumophila Biofilms

Ana Barbosa[1,2,3], Carina Almeida[1,2,4], Albert Parker[3], Christopher J. Jones[3], Heidi J. Smith[3], Nuno F. Azevedo[1,2], Darla M. Goeres[1,2,3], Laura Cerqueira[1,2]

[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal. [2]ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal. [3]Center for Biofilm Engineering, Montana State University, Bozeman, USA. [4]INIAV, IP- National Institute for Agrarian and Veterinary Research, Vila do Conde, Portugal

Abstract

Fluorescence *in situ* hybridization using nucleic acid mimics (NAM-FISH), such as peptide nucleic acids (PNA), enables targeted detection of microorganisms and their spatial localization in biofilms without disturbing their structure. Using PNA-FISH to target mRNA allows for insights into gene expression at the single-cell level, combining spatial and functional analysis in both free-living cells and biofilms. The main goal of this work is to explore the use of mRNA PNA-FISH to study the regulatory network involved in the biphasic life cycle of *L. pneumophila* clarifying the organization and functional development of biofilms. Bioinformatics tools were used to design PNA probes targeting genes involved in this biphasic life cycle of *L. pneumophila*. Probe optimization was conducted in planktonic cells, and for this, the *L. pneumophila* serogroup 1, ATCC™ 33152 was grown on a buffered yeast extract (BYE) medium. Optimal hybridization conditions were established at 60°C for the *secE* (replicative gene), *rpoS*, *mip,* and *fliA* (transmissive genes), and 16S (housekeeping gene). These probes are now being tested in a flow cell to assess gene expression and localization in monospecies *L. pneumophila* biofilms. Additionally, image acquisition in a confocal laser scanning microscope (CLSM) is being optimized to enhance the accuracy of the probe signals.

The study results highlight the potential of a model that uses mRNA PNA-FISH to study the regulatory network of *L. pneumophila* biofilms. This model can provide essential gene expression data in individual cells in planktonic and sessile states with a single hybridization step, capturing functionality and spatial information.

P126

In vitro modelling of polymicrobial infections in the catheterized urinary tract: Polymicrobial biofilm community offers protection against ciprofloxacin treatment

Isobel Garratt[1], Maryam Yazdizadeh Ravari[1], Matthew Wand[2], Mark Sutton[2], Brian Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]UKHSA, Salisbury, United Kingdom

Abstract

Catheter associated urinary tract infections (CAUTI) are highly prevalent hospital acquired infections, causing significant burden on healthcare with many uropathogens being World Health organisation priority pathogens. Up to 75 - 85 % of patients with indwelling catheters have polymicrobial infections (Stickler *et al.,* 2008). There is a gap in knowledge within the current literature with there being few studies on polymicrobial catheter infections, therefore the development of a reproduceable method to model such communities is salient.

To address this, we have developed an in vitro model of polymicrobial CAUTIs with common uropathogens (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis* and *Staphylococcus aureus*). Development and validation of selective medias to facilitate enumeration of specific community members, as well as sampling strategies to investigate community dynamics in both planktonic and biofilm populations are described. We show polymicrobial communities can be reproducibly generated using this model and stable for at least 14 days. To demonstrate the utility of this model we used it to investigate the impact of antibiotic treatment on community dynamics and the potential for biofilms to protect community members. The application of ciprofloxacin significantly reduced E. coli and P. aeruginosa in planktonic populations, whilst they remained retained in biofilms.

This model provides a valuable tool for both basic and applied research in this area, facilitating more robust pre-clinical evaluation of approaches to control CAUTI and helping to address more fundamental questions of biofilm associated infections, the evolution of antimicrobial resistance, and other important traits in these pathogens.

P127

A Novel ST1 Lactonase Reduces Virulence and Inhibits Biofilm Formation and Extends the Lifespan of *Caenorhabditis elegans* Infected with *Pseudomonas aeruginosa* MMA83

Jovana Curcic[1], Ivano Merendino[2], Danka Matijasevic[1], Branko Jovcic[3], Andrea Cochis[4], Silvia Spriano[2], Milka Malesevic[1]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia. [2]Department of Applied Science and Technology, Politecnico di Torino, Turin, Italy. [3]Faculty of Biology, University of Belgrade, Belgrade, Serbia. [4]Biomaterials Lab, Department of Health Sciences, Università degli Studi del Piemonte Orientale, Novara, Italy

Abstract

Background: Bacterial biofilms represent a major challenge in clinical settings due to their resistance to antibiotics and immune responses. *Pseudomonas aeruginosa* is a significant opportunistic pathogen responsible for chronic infections, particularly in immunocompromised patients. Quorum quenching (QQ) enzymes, such as lactonases, show promise in disrupting biofilm formation and reducing bacterial virulence. This study examines the effects of ST1-YtnP lactonase from thermophilic *Bacillus licheniformis* on biofilm formation and virulence of *P. aeruginosa* MMA83.

Methods: The antibiofilm effect of recombinant ST1-YtnP lactonase on multi-drug resistant *P. aeruginosa* MMA83 was analyzed using fluorescence microscopy. *Caenorhabditis elegans* infection model was used to evaluate the enzyme's effect on virulence and host survival. The liquid killing assay was employed using the *C. elegans* AU37 mutant strain to quantitatively evaluate host survival in response to ST1-YtnP lactonase treatment.

Results: Fluorescence microscopy showed a significant reduction in *P. aeruginosa* MMA83 biofilm formation with ST1-YtnP lactonase, leading to a looser, less dense biofilm. Planktonic cell growth remained unaffected. In *C. elegans*, treatment resulted in an almost 100% survival rate, while untreated infected worms showed 0% survival within 24 hours. ST1-YtnP lactonase exhibited no toxicity, consistent with previous studies on QQ enzyme safety in eukaryotic models.

Conclusion: ST1-YtnP lactonase inhibits *P. aeruginosa* MMA83 biofilm formation and reduces virulence in *C. elegans*, indicating its potential as a safe and effective antivirulence agent for biomedical applications.

P128

Natural existing arbuscular mycorrhizal-bacterial biofilm associations and their functional behavior

Aditi Pandit[1,2,3], Leena Johny[1], David Cahill[2], Lambert Brau[2], Mandira Kochar[1]

[1]TERI Deakin Nanobiotechnology Centre, Sustainable Agriculture Division, The Energy and Resources Institute, TERI Gram, Gwal Pahari, Gurugram, Haryana 122103, India, Gurugram, India. [2]School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Geelong, Victoria 3216, Australia, Geelong, Australia. [3]Department of Genetics, University of Georgia, Athens, Georgia, USA, Athens, USA

Abstract

In symbiosis with plant, arbuscular mycorrhizal fungi (AMF) access the carbon stored in roots in exchange for increased uptake of nutrients and water from the soil. In mycorrhizal-plant symbiosis, AMF–associated bacteria (AAB) serve as a third partner and are tightly linked to AMF. AAB are involved in mycorrhizal activity and nutrient uptake enhancement and have impact on plant development. In order to create biofertilizer for sustainable crop production, it is important to understand the function and process of this inter-kingdom natural coexistence. In our research, we used in vitro and in situ co-cultures to screen 33 different AMF species, and we characterized 231 AAB using 16S rDNA analysis. 109 selected AABs were examined for ten functional qualities that promote plant growth, and it was found that different bacterial strains had a variety of advantageous traits. The association of AAB was seen as biofilm and endobacteria using microscopic methods. Further, by using an in vitro assay system, an association recreation of 12 AAB-*Rhizophagus irregularis* was investigated to look at the impact on mycorrhization and functional capabilities. It was observed that AABs moved along the developing *R. irregularis* hyphae and spores. Different AAB had an impact on AMF development as well as its capacity to solubilize phosphate and potassium, and fix nitrogen. We discovered both the synergistic interactions and partnerships between the two cross-kingdom microbial partners. Understanding the molecular elements of these fungal-bacterial connections, which will enable their later use and modification for sustainable agriculture practices, is another area of focus.

P129

Listeria monocytogenes is able to colonize and hide in a multi-species biofilm.

Eva Voglauer[1], Lauren Alteio[1], Nadja Pracser[1], Sarah Thalguter[1], Martin Wagner[2,1], Kathrin Rychli[2]

[1]FFoQSI GmbH – Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Austria, Tulln, Austria. [2]University of Veterinary Medicine Vienna, Vienna, Austria

Abstract

Background: The foodborne pathogen *Listeria* (*L.*) *monocytogenes* can survive for extended periods in the food producing environment, where they provide a niche for long-term survival.

Methods: This study examined the behaviour of a *L. monocytogenes* ST121 isolate in a multispecies biofilm composed of *Pseudomonas* (*P.*) *fragi*, *Brochothrix* (*B.*) *thermosphacta*, and *Carnobacterium* (*C.*) *maltaromaticum*, previously isolated from a meat processing facility. The composition of the biofilm community and matrix, and transcriptional activity were analysed.

Results: *L. monocytogenes* colonised the multispecies biofilm, accounting for 6.4% of all total biofilm cells after six hours. Transcriptomic analysis revealed 127 significantly up-regulated *L. monocytogenes* genes compared to the inoculum, including motility, chemotaxis, iron, and protein transport related genes. When comparing the differentially expressed transcripts within the multispecies biofilm with and without *L. monocytogenes*, only a cadmium/zinc exporting ATPase gene in *C. maltaromaticum* was significantly upregulated, while the other 9,313 genes in the biofilm community showed no significant differential expression. We further monitored biofilm development over time (6, 24 hours and 7 days). *P. fragi* remained the dominant species, while *L. monocytogenes* was able to survive in the multispecies biofilm accounting for 2.4 % of total biofilm cells after 7 days, without any significant changes in its abundance. The presence of *L. monocytogenes* did neither alter the biofilm community nor its matrix composition (amount of extracellular DNA, carbohydrates, and protein).

Conclusion: Our data indicate that *L. monocytogenes* resides in multispecies biofilms, potentially increasing survival against cleaning and disinfection in food processing environments, supporting persistence.

P130

Influence of growth conditions on structural and mechanical properties of *Microbacterium lacticum* biofilms

Caroline Bachlechner, Philipp Fuhrmann, Henry Jäger

BOKU University, Vienna, Austria

Abstract

Biofilms represent significant challenges to the food industry, where hygiene is critical. The aim of this study was to compare structural and mechanical properties of *Microbacterium lacticum* biofilms from the milk industry, grown under static and dynamic conditions, to provide a better understanding for the optimisation of cleaning strategies.

Microbacterium lacticum D84 biofilms were grown on stainless steel coupons with defined roughness values, either in a stationary environment or under flow conditions, for 12 days at 30°C. The mechanical properties were analysed with a MCR302 Rheometer (Anton Paar, Graz, Austria) with a parallel plate setup (25 mm diameter). In an amplitude sweep, G' and G'' were measured from 0.01 % to 100 % and at an angular frequency of 1 rad/s. Additionally, the chemical composition of the EPS matrices was analysed, as a function of the growth conditions, and microbiological parameters (i.e., CFU/mL) were assessed.

Depending on the growth conditions, the results reveal significant differences in the rheological behaviour of the biofilms. The differences in mechanical properties (increase in G') are further supported by effects of growth conditions on EPS composition. We propose that these effects, in turn, explain differences in cleaning behaviour.

This study provides a deeper understanding of how growth conditions influence *Microbacterium lacticum* properties. By gaining deeper insight into these mechanisms, we can optimise operational parameters for more targeted, and thus, sustainable cleaning strategies.

P131

Biofilm development on marine microplastics: insights from in-situ experiments in Viet Nam

Jennifer Scott[1], Hieu Vo Hoai[2], Max Webb[1], Ryan Pereira[1], Truong Van Thuong[3], Huong Thi Thuy Ngo[2], Michel Kaiser[1], Tony Gutierrez[1]

[1]Heriot Watt University, Edinburgh, United Kingdom. [2]Phenikaa University, Hanoi, Vietnam. [3]Fisheries and Technical Economic College Veitnam, Hanoi, Vietnam

Abstract

Viet Nam has emerged as one of the global hotspots of marine plastic pollution, with the marine environment heavily affected by plastic floating litter and submerged debris. There is therefore a vital need to understand the microbial colonisation and development of biofilm on these plastics to better understand their fate and potential to cause harm. This study is one of few long-term, in-situ experiments investigating the microbial colonisation of microplastics in the marine environment.

Experiment cages containing microplastic size (<5mm) polystyrene, high density polyethylene and glass (as a control surface) were deployed for 1 year in two different Vietnamese coastal sites: a floating fish farm facility and a popular tourist island archipelago. Using barcoded-amplicon Illumina sequencing and Scanning Electron Microscopy (SEM), we examined how biofilm developed and changed on the different surfaces over time.

Our findings reveal that biofilm formation on microplastics is detectable within 48 hours and progressively becomes thicker and changes over time. Biofilms were most predominant on polystyrene particles incubated within a fish farm facility. Microbial community analysis of the biofilms revealed distinct taxa and abundance patterns across the different particle surfaces, including the detection of pathogenic organisms. This work provides greater understanding on the evolution of microplastic biofilms in the tropics, including the potential risks to aquaculture produce and human health through exposure to marine-derived microplastics.

P132

Synergy Between Antibiotics and Crude Marine Extracts Against a Range of Biofilm-forming Pathogens

Monica Murphy

Munster Technological University, Cork, Ireland

Abstract

Introduction: Biofilm-associated bacteria are protected by an extracellular polymeric substance and undergo metabolic and genetic adaptations that reduce the efficacy of conventional antibiotics. This study investigated the synergistic effects of antibiotics, seaweed extracts, and bacterial cell-free supernatants (CFS) on antimicrobial and antibiofilm properties.

Methods: Seaweed extracts from *Fucus vesiculosus* and *Codium fragile* were prepared using water and methanol extractions. *Bacillus subtilis* and *Bacillus velezensis* CFS was obtained via centrifugation and filtration. Extract stability was tested under varying pH (4-10), temperature (4-90 °C), and salt concentrations (3-10%). Antimicrobial efficacy was determined using MIC assays, while biofilm inhibition (MBIC) was measured at 99, 90, and 50% inhibition using crystal violet assays. Synergy was evaluated using a checkerboard assay.

Results: Antimicrobial activity remained stable across the range of pH 4–7, salt 5–10%, and temperatures up to 60°C but decreased by 15–30% at 90°C. *C. fragile* exhibited broad-spectrum inhibition, achieving 50% across multiple Gram-positive and Gram-negative strains and 90% against *S. aureus*. *F. vesiculosus* was more effective against Gram-positive bacteria, inhibiting biofilms at lower extract concentrations. *B. subtili*s and *B. velezensis* CFS showed strong antimicrobial and antibiofilm activity, with *B. subtilis* most effective against *S. aureus* and *A. baumannii* and *B. velezensis* against MRSA and *S. aureus* clinical isolates. A combination of *B. velezensis* and *F. vesiculosus* reduced MRSA growth by 81%.

Conclusion: Findings showed that crude marine extracts were capable of increasing the susceptibility of strain-specific antibiotics against a range of pathogens.

P134

Characterisation of biofilms produced by *Listeria monocytogenes* isolates of two different lineages

Jorge Baixauli[1], Tonciulescu Mihai-Alexandru[2], Juan José Quereda[3], Alfredo Palop[1], Alberto Garre[1], Enriqueta Garcia-Gutierrez[1]

[1]Universidad Politécnica de Cartagena, Cartagena, Spain. [2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania. [3]Universidad CEU Cardenal Herrera, Valencia, Spain

Abstract

Listeria monocytogenes is the bacterium responsible for listeriosis, a severe foodborne illness. In 2023, a total of 2,952 cases were reported in Europe, 133 of which were foodborne, resulting in 11 fatalities (EFSA, 2024). This species is highly diverse, with isolates classified into groups of closely related genetic strains. Although four distinct lineages exist, the majority of clinically significant strains belong to lineages I and II. Currently, regulatory agencies treat all *L. monocytogenes* isolates as equally virulent. However, evidence suggests otherwise—lineage I is more commonly associated with clinical infections, whereas lineage II is predominantly found in industrial environment. This discrepancy could impact microbial risk assessments, as these lineages may exhibit different behaviours. To investigate this, we selected multiple strains from both lineages and assessed their growth characteristics in static conditions. Biofilm formation experiments were conducted using crystal violet and Congo red assays. Our findings revealed significant differences between the strains of the two lineages. A deeper understanding of the behaviour of these lineages will contribute to the development of improved predictive models, ultimately enhancing food safety measures.

P135

Synergistic evaluation of curcumin and meropenem on Biofilm forming genes in Staphylococcus aureus and Pseudomonas aeruginosa

Blessing Oyedemi, Mercy Ndukwe

Middlesex University, London, United Kingdom

Abstract

Biofilm-associated infections pose a significant challenge due to their resistance to antibiotics. This study evaluated the effects of curcumin, vancomycin, and meropenem—alone and in combination—on biofilm formation, gene expression, and metabolic activity of biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Mono-species biofilms were treated with antibiotics at different concentrations, and biomass reduction was assessed via crystal violet staining. While the first three concentrations effectively reduced biofilms in both species, curcumin showed limited efficacy against *P. aeruginosa*, reducing biofilm only at MIC×2 and MIC. Dual-species biofilms were treated with curcumin/meropenem and curcumin/vancomycin combinations, which showed no significant inhibitory effects.

Gene expression analysis via quantitative real-time PCR targeted *P. aeruginosa* biofilm-associated genes AlgR and PelF. The 2-ΔΔCt values (0.308 and 0.321 for *AlgR*; 0.223 and 0.154 for *PelF*) indicated that antibiotic treatment did not significantly alter gene expression. Metabolic profiling via UHPLC-MS are currently under evaluation.

The study provides valuable insights into antibiotic efficacy in biofilms , highlighting the challenges in biofilm treatment and the potential role of combinatory approaches.

P136

Investigation of mechanical and morphological properties of oral biofilm

Mrunmayee Joshi[1], Guannan Wang[2], Spring Wang[2], Xu Huang[2], Ayesha Rahman[3], Peter J Fryer[1], Zhenyu J Zhang[1]

[1]School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom. [2]Procter & Gamble Beijing Innovation Center, Beijing, China. [3]School of Dentistry, University of Birmingham, Birmingham, United Kingdom

Abstract

Biofilms such as dental plaque are structurally and functionally organised, stable and grow on different substrates, increasing the risk of disease due to calcification over time. Extensive microbiological investigations have been carried out in evaluating biofilms, whilst the knowledge and capability to characterize the mechanical properties and hence cleaning techniques are limited. This work develops a new strategy to develop biofilms of *S. mutans* and investigating their physical properties.

A customised high-throughput setup was established, using a modified container to hold substrates vertically in an oxygen-deficient environment. The container was placed in an orbital shaker at 37°C for 6 or 9 days with media replaced every 3 days. Biofilms were grown in media containing either 1% (HS) or 0.1% (LS) sucrose solution. The developed biofilms were stained with SYTO 9 and Propidium Iodide and visualised using Confocal Laser Scanning Microscopy (CLSM). Young's moduli were calculated using a home-built microindentation device with 2 mN of force and a spherical indenter.

Results show a range of Young's moduli (0.64 - 0.71 kPa) across different growth conditions of *S. mutans* biofilm likely due to the nutrient availability for the growth of bacteria. Differences in morphological differences were also observed in the two biofilms when CLSM images were compared for different number of growth days. This work contributes towards development of model biofilms that can be tested on different substrates with different techniques while establishing a better understanding of their mechanical strength.

P138

Studying the Factors Affecting Polymeric Pipes and Their Influence on Potable Water Quality

Mohammad Tariq

Hamad Bin Khalifa University, Doha, Qatar

Abstract

A drinking water distribution network aims to deliver biologically stable water to the customer through a network of pipelines, wherein the pipe materials are selected based on several factors aiming for cost effectiveness, durability, oxidation resistance, and resistance to leaching of organic carbon that may lead to biofilm growth. Although polymeric pipes are generally considered due to their cost efficiency, they are reported to release organic matter (total organic carbon (TOC)) when in contact with water. Since ClO2 is the primary disinfectant used in potable water networks within Doha, its interaction with polymeric materials and other oxidizing agents can lead to polymer degradation and result in premature pipe failure. The study investigates the interaction of chlorinated water with high-density polyethylene (HDPE) pipe materials used in Qatar's drinking water distribution systems (DWDS). Chlorination is the primary disinfectant for potable water in the region; understanding its effects on pipe materials is crucial for maintaining water quality and preventing biofilm formation. This study aims to evaluate the impact of chlorinated water on (i) carbon leaching from HDPE and (ii) material degradation.

The research will employ experimental techniques to assess organic carbon release and biofilm formation potential. TOC leaching will be measured by incubating the samples at 25°C for a period of 7 days, with migration potential measured each day. The biofilm formation potential will be measured by bacteria cell concentration after a two-week incubation. The study will also evaluate material degradation by assessing the carbonyl index and mechanical properties.

P139

Epoxytigliane induced migration of dHL-60 cells in *Escherichia coli* biofilms is related to the structural reorganisation of the extracellular polymeric matrix

Joana Stokniene[1,2], Jason Cullen[1,3], Glen Boyle[3], Katja Hill[2], Paul Reddell[1], Lydia Powell[4], David Thomas[2]

[1]QBiotics Group Limited, Yungaburra, Australia. [2]Cardiff University School of Dentistry, Cardiff, United Kingdom. [3]QIMR Berghofer Medical Research Institute, Brisbane, Australia. [4]Swansea University Medical School, Swansea, United Kingdom

Abstract

A novel, non-antibiotic therapeutic strategy has been described for chronic skin wound infections, employing a semi-synthetic epoxytigliane ester (EBC-1013) derived from the Australian blushwood tree (*Fontainea picrosperma*). Here, we investigate the direct and indirect mechanisms by which EBC-1013 may induce leukocyte invasion and biofilm disruption *in vivo.*

The effect of EBC-1013 treatment on established 96 h methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* biofilms was investigated alone, and in a three-dimensional co-culture model using neutrophil-like differentiated HL-60 (dHL-60) cells. Confocal laser scanning microscopy, COMSTAT and ImageJ analysis were used to quantify cellular and biofilm polymeric matrix changes and dHL-60 cell migration into these bacterial structures. The ability of EBC-1013 to induce chemotaxis and the generation of reactive oxygen species (ROS) was studied using fluorescent probes (CellROX and dihydroethidium).

EBC-1013 (at 256 μg/mL) induced significant biofilm disruption (through reorganisation and increased porosity of the biofilm matrix) and resulted in increased neutrophil-like cell migration ($p < 0.05$). Although EBC-1013 stimulated the generation of the ROS in dHL-60 cells and co-culture model system ($p < 0.05$), it did not induce chemotaxis *in vitro*. Exogenous ROS, used at a concentration similar to that produced by activated neutrophils (≤ 50 μM), failed to disrupt the established 96 h MRSA and *E. coli* biofilms ($p > 0.05$).

EBC-1013 induced dHL-60 cell migration into gram-negative biofilms, via the structural rearrangement of the cellular and extracellular components of the biofilm polymeric matrix. While in gram-positive biofilms, the predominant effects being upon the inflammatory cells.

The National Biofilms Innovation Centre (NBIC) exists to create a fusion of world-class interdisciplinary research and industry partnerships to deliver breakthrough science and technologies to control and exploit biofilms. The centre was established in 2017 by four lead universities (Edinburgh, Liverpool, Nottingham and Southampton), with funding from BBSRC and Innovate UK. NBIC's mission is to drive global leadership in biofilm research, training, and innovation by tackling key challenges crucial to the UK's future prosperity.

National Biofilms Innovation Centre | ukbiofilms | @ukbiofilms.bsky.social | @ukbiofilms | @ukbiofilms

biofilms.ac.uk **nbic@biofilms.ac.uk**

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

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

We are now on Bluesky @microbiologysociety.org

Registered as a Charity in England and Wales 264017. A Charity registered in Scotland SC039250.
Company Limited by Guarantee. Registered in England 1039582.