#Biofilms11

BIOFILMS 11

13–15 May 2025

Mercure Cardiff Holland House
Hotel & Spa, Cardiff, UK

INVITED AND OFFERED TALKS

National Biofilms Innovation Centre

MICROBIOLOGY SOCIETY

Invited talk: The development of novel supramolecular antimicrobial and antibiofilm agents

Jennifer Hiscock

University of Kent, Canterbury, United Kingdom

Abstract

Our novel patented (European Patent Application No. 18743767.8, U.S. Patent Application No. 16/632,194), Supramolecular Self-associating Amphiphiles (SSAs) technology incorporates a library of ~ 190 amphiphilic salts and associated compounds. The anionic component of this class of compounds have been shown to self-associate through the formation of intermolecular hydrogen bonds producing anionic dimers in the polar organic solvent DMSO. Moving into aqueous conditions, SSAs self-associate to form spherical aggregates between 100 nm and 550 nm in hydrodynamic diameter. However, the presence of inorganic salt can cause these spherical aggregates to morph from sphere to fibre, producing a series of hydrogel materials.

Within a biological context we have shown these SSAs to:

- act as antimicrobial (1) and antibiofilm agents; (2)
- increase the efficacy of other antibiotic/antiseptic agents and anticancer agents against bacteria (3) and ovarian cancer cells respectively; (4)
- selectively interact with phospholipid membranes of different compositions; (5)
- have the potential to act as drug delivery vehicles; (6)
- exhibit a druggable profile when delivered via i.v. in vivo to mice. (7)

References

1. *ChemMedChem*, **2020**, *15*, 2193.
2. *ACS Omega*, **2024**, *9*, 1770.
3. *Adv. Therap.*, **2022**, *5*, 2200024.
4. *RSC Advances*, **2021**, *11*, 14213.
5. *Chem. Commun*., **2020**, *56*, 4015.
6. *Molecules*, **2020**, *25*, 4126.
7. *Chem. Sci.*, **2022**,*13*, 9761-9773.

Invited talk: Effects of Antimicrobial Compounds in a Complex Biofilm Sink Drain Model

Dirk Bockmühl

Rhine-Waal University of Applied Sciences, Kleve, Germany

Abstract

Environmental biofilms harbour numerous microbial pathogens, which can be linked to antimicrobial resistance. Understanding the composition of biofilms in one-health related settings, such as hospitals, farms, and domestic environments, is crucial for controlling infection and preventing horizontal gene transfer. Since the microbial communities in biofilms are typically very complex, laboratory approaches to studying these systems are intricate and often focus on simpler setups, involving only one or a few species.

To overcome this drawback, the European project "COMBAT" developed a comprehensive multispecies biofilm model. A total of 86 sink drain biofilms from hospitals, farms and households across various European countries were sampled and transferred to a small scale complex biofilm model, originally described by Ledwoch *et al.* [1], which allows for the investigation of biofilms in conditions that closely mimic real-life scenarios.

We demonstrated the general feasibility of our model for studying complex biofilms in a standardized manner [2], and used it to show disinfectants can affect the composition and resistance profiles of these biofilms. Apart from providing a platform for better understanding antimicrobial resistance in biofilms and for designing more effective control measures, this model might also pave the way for a standardised test protocol to evaluate product efficacy against complex biofilms.

[1] Ledwoch *et al*., 2020, Journal of Hospital Infection, Vol.106 (4)

[2] van Leuven *et al.,* 2023 Journal of Microbiological Methods 2023; Vol. 212:106806

Invited talk: Bioprotection of stone heritage by biofilms

Francesca Cappitelli

University of Milan, Milan, Italy

Abstract

Recently, deep insight into microbial interactions with outdoor stone heritage has seen more than microbial ability for destruction or biodeterioration. At the core of the modified perspective lies the evidence that, in an astonishing number of cases, biofilm can be protective towards the heritage substrate and therefore microorganisms can be incredibly powerful allies for stone conservation. To understand the actual role of microorganisms is still an open challenge because the balance of biofilms' deteriorative or protective role is difficult to determine due to the complexity of the stone-biofilm-atmosphere ecosystem. In addition, the same biofilm can adapt over time to the changing environmental conditions, switching between a predominance of bioprotection over biodeterioration and vice versa.

Furthermore, microbial cells harbor huge metabolic potential. Consequently, novel opportunities in the use of living microbial cells have allowed restorers/conservators to perform green removal of unwanted substances (biocleaning) on stone as well as bioconsolidation of fragile inorganic surfaces.

The built heritage can be read as a symbol of the cultural history of the society and, at the same time, as an evolving ecosystem. Nurturing a culture of microbial value and appreciation opens up novel and sustainable perspectives, approaches and applications that were before unthinkable in stone heritage conservation.

Invited talk: The interplay between phage, phage resistance and microbial community dynamics

Edze Westra

University of Exeter, Penryn, United Kingdom

Abstract

Where there are bacteria, there will be bacteriophages. These viruses are thought to be important players in shaping the wider microbial community in which they are embedded.

However, bacteria possess a wide range of distinct immune mechanisms that provide protection against bacteriophages.

A key gap in our knowledge is how the evolution of these immune systems is shaped by the wider microbial community context, and how in turn immune systems shape the structure of microbial communities.

I will discuss how we may be able to identify general principles that govern bacteria-phage interactions in natural microbial communities using a combination of mathematical models and experiments with malleable synthetic communities.

Invited talk: Cyclic-di-GMP signaling as a target for biofilm control

Tim Tolker-Nielsen[1], Jens Bo Andersen[1], Louise Dahl Hultqvist[1], Charlotte Uldahl Jansen[2], Tim Holm Jakobsen[1], Martin Nilsson[1], Morten Rybtke[1], Christina Manner[3], Roland Seifert[4], Klaus Qvortrup[5], Claus Moser[1], Urs Jenal[3], Katrine Qvortrup[2], Michael Givskov[1]

[1]Costerton Biofilm Center, University of Copenhagen, Copenhagen, Denmark. [2]Department of Chemistry, Technical University of Denmark, Lyngby, Denmark. [3]Biozentrum, University of Basel, Basel, Switzerland. [4]Institute of Pharmacology, Hannover Medical School, Hannover, Germany. [5]Core Facility for Integrated Microscopy University of Copenhagen, Copenhagen, Denmark

Abstract

The ability to control and eradicate biofilms is important in both medical and industrial settings. For medically relevant biofilms various approaches are being studied with the ultimate goal of developing new medicine for the treatment of problematic infections. These approaches include the use of antibiotic combinations, bacteriophage therapy, enzymatic matrix disruption, modification of implant surfaces, and interference with bacterial signaling. We have done work to explore the possibility of controlling biofilms through interference with c-di-GMP signaling pathways. We employed a high throughput screening approach to identify chemical compounds that reduce the intracellular c-di-GMP content in *Pseudomonas aeruginosa*. This led to the identification of a small molecule that efficiently depletes *P. aeruginosa* for c-di-GMP, inhibits biofilm formation and disperses established biofilm. The combination of the anti-biofilm compound with standard of care antibiotics resulted in efficient eradication of biofilms *in vitro*, as well as in murine biofilm infection models. Genetic and biochemical analyses provided evidence that the anti-biofilm compound specifically stimulates the activity of the c-di-GMP phosphodiesterase BifA in *P. aeruginosa*. Our work constitutes proof of concept for c-di-GMP phosphodiesterase-activating drugs administered in combination with antibiotics as a viable treatment strategy for otherwise recalcitrant infections.

Invited talk: The structure and function of extracellular nucleic acids in biofilms

Rikke Louise Meyer

Aarhus University, Aarhus, Denmark

Abstract

The significance of extracellular DNA for biofilm formation has been recognized for more than two decades, and DNA seems to be a matrix component that most biofilms have in common. However, emergent properties of extracellular nucleic acids are changing our understanding of how this matrix component impacts the biology of bacteria in biofilms. In this talk, I will give an overview of our understanding of how eDNA and eRNA contribute to biofilm formation, and which new insights and questions that have arisen from recent research.

Some of these new insights relate to the structure of DNA (and RNA). In addition to the canonical B-DNA double helix, eDNA in biofilms also forms the left-handed Z-DNA helix, and several Gs coordinate to form G-quadruplexes involving 1, 2 or 4 DNA strands. These non-canonical DNA structures provide new functionality to eDNA. Z-DNA is mechanically strong and resistant to degradation by DNases. G-quadruplexes provide elasticity and may connect multiple strands to form a net-like superstructure.

G-quadruplexes also provide enzymatic activity to the biofilm matrix. G-quadruplexes bind iron porphyrins (heme/hemin) to form a DNAzyme with peroxidase-like activity. This complex can also transfer electrons from the cell to alternative electron acceptors via extracellular electron transfer, and it opens for the possibility of using catalytic DNA structures to protect biofilms from hydrogen peroxide and to use alternative electron acceptors to fuel the bacteria's metabolism.

Invited talk: Probing biofilm resilience with microfluidics: How do they withstand mechanical and chemical insults?

Eleonora Secchi

ETH Zurich, Zurich, Switzerland

Abstract

Biofilms are aggregates of microorganisms embedded in a self-secreted matrix of polymeric substances, which protect the microbial community from chemical and mechanical insults, thereby enhancing their survival and evolutionary success. Due to their resilience, biofilms significantly impact environmental, industrial, and medical settings. Typically, biofilms develop in moist environments where fluids are in motion— a ubiquitous factor that governs chemical transport and applies mechanical forces to them. However, a mechanistic understanding of how fluid flow modulates biofilm response to physicochemical stimuli is still lacking.

In our research, we employ microfluidic platforms to investigate biofilm assembly and the emergence of their properties. Using a newly developed microfluidic system, we explore the role of flow in forming biofilm streamers—suspended filamentous structures driving clogging. We analyze how varying flow conditions influence their mechanical response and demonstrate that biofilm streamers exhibit stress-hardening behavior, explaining their adaptability over both short and long timescales under dynamic flow conditions.

Beyond mechanics, we also investigate the permeability of biofilms to chemicals. To this end, we designed a microfluidic platform that enables the formation of surface-attached biofilms with controlled geometry while characterizing their hydraulic permeability over time. By correlating permeability with the biofilm developmental stage, matrix composition, and environmental conditions, we gain insights into their structural evolution. We then assess antibiotic penetration and treatment efficacy, providing a framework to better understand biofilm resistance to chemical challenges.

Together, these findings advance our understanding of biofilm resilience and inform strategies for their control and eradication in diverse settings.

Invited talk: Targeting the ECM to gain control of microbial communities

Agneta Richter-Dahlfors

Karolinska Institutet, Stockholm, Sweden

Abstract

Bacterial biofilms are fundamental to natural and industrial processes. Numerous mechanical and physicochemical properties confer important features to the structure the biofilm. As these properties influence bacterial growth and antimicrobial susceptibility, better understanding of these properties opens new opportunities to harness control of the biofilms. The extracellular matrix (ECM) is essential for the biofilm structure, thus we focus our research on the interplay between the carbohydrate and protein components of the ECM. Using conductive polymers, a uniqe class of material with combined electrical and ionic activity, we grew biofilms on electronically addressed conductive polymer (PEDOT:PSS), and showed how to control the amount of biofilm by electronic modulation of surface properties. A combination of the bioelectric effect and chemical surface modification nearly eradicated *Staphylococcus aureus* surface biofilm. We also use electronic addressing of electroactive surfaces to modulate ECM expression, i.e. increasing the ECM production while maintaining the cell component unaffected. Curli and cellulose are major protein and polysaccharide components in the ECM of *Salmonella* and *E. coli*. Mutants unable to express cellulose and/or curli revealed an essential role for cellulose to maintain viscoelastic properties of the ECM matrix. Real-time analysis of biofilm growth also showed the importance of curli and cellulose for biofilm maturation and a nanostructural arrangement of the ECM which is essential for the wettability of the biofilm structure. As we learn how to tune the organizational structure and physicochemical properties of biofilms, we also generate numerous new opportunities to gain control of microbial communities.

Invited talk: Individual leaf microbiota tunes a genetic regulatory network to promote leaf growth

Valéria Custódio[1], Isai Salas-González[2], David Gopaulchan[1], Paulina Flis[1], Régla Amoros[3], Yi-Qun Gao[1], Xianqing Jia[4], Ângela Moreno[3], Esther Carrera[5], Caroline Marcon[6], Frank Hochholdinger[6], M. Margarida Oliveira[7], David E. Salt[1], Gabriel Castrillo[8]

[1]University of Nottingham, Sutton Bonington, United Kingdom. [2]Universidad Nacional Autónoma de México, Campus Morelos, Mexico. [3]Instituto Nacional de Investigação e Desenvolvimento Agrário, Cidade da Praia, Cape Verde. [4]Northwest University, Shaanxi, China. [5]Instituto de Biologia Molecular y Celular de Plantas, Valencia, Spain. [6]University of Bonn, Bonn, Germany. [7]Instituto de Tecnologia Química e Biológica António Xavier, Lisbon, Portugal. [8]University of Nottingham, Sutton-Bonington campus, United Kingdom

Abstract

In natural ecosystems, microbes have the ability to stably colonise plant leaves, overcoming the fluctuating environmental conditions that the leaves represent. However, how the phyllosphere microbiota influences the growth of individual leaves is still poorly understood. Here, we investigated the growth of *Zea mays* leaves in plants grown in three soils with differing amounts of nutrients and water and identified a leaf-growth-promoting effect driven by the leaf microbiota. We built a bacterial strain collection that we used in recolonisation experiments to study the microbiota mechanisms involved in leaf growth-promoting effect. We established that prevalent bacteria inhabiting young leaves promote individual leaf growth. Using transcriptomic analyses, we reveal a defence-related genetic network that integrates the beneficial effect of the phyllosphere microbiota into the leaf development program. We demonstrated that the individual leaf microbiota differentially represses this genetic network to modulate the growth-defence trade-off at single-leaf resolution.

Invited talk: Death, taxes, biofilms and corrosion

Ian M. Head[1], Adrien Vigneron[1,2], Eric B. Alsop[2,3], Brian Chambers[4], Bartholomeus P. Lomans[5], Nicolas Tsesmetzis[2], Sven Lahme[6], Dennis Enning[6], Cameron M. Callbeck[7], Thomas P. Curtis[8], Demelza Menendez Vega[1], Neil D. Gray[1], Thomas P. Curtis[8], Casey R. J. Hubert[9], Mohammed E. Sindi[10], Xiangyang Zhu[9], Angela Sherry[11], Beate Christgen[1]

[1]School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom. [2]Shell International Exploration and Production, Inc.,, Houston, Texas, USA. [3]cDOE Joint Genome Institute, Walnut Creek, California, USA. [4]Shell Global Solutions (US), Inc.,, Houston, Texas, USA. [5]Shell Global Solutions International B.V.,, Rijswijk, Netherlands. [6]ExxonMobil Upstream Research Company, Spring, Texas, USA. [7]Max Planck Institute for Marine Microbiology,, Bremen, Germany. [8]School of Engineering, Newcastle University, Newcastle upon Tyne, United Kingdom. [9]Department of Biological Sciences, University of Calgary,, Calgary, Alberta, Canada. [10]Materials & Corrosion Services Division, Applied Microbiology Group, Saudi Aramco, Saudi Arabia. [11]Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom

Abstract

Microbially influenced corrosion (MIC) is one of the major contributors to the deterioration of metal infrastructure, affecting multiple industries and exerting a significant economic impact, estimated at $2,720 bn globally.[1] 20-40% of serious corrosion cases in the oil and gas industry and 70-95% of pipeline internal leaks have been attributed to MIC, with 20% of corrosion on metal surfaces attributed to biofilms.[1]

Even though MIC has been recognized for almost a century, control of MIC remains a major challenge. This stems from multiple mechanisms contributing to MIC, the diversity of organisms that have been implicated in the phenomenon and challenges in reproducing field-observed corrosion rates in the laboratory.

The mechanisms of MIC, the organisms that drive MIC and approaches to the control of MIC-associated biofilms will be explored and the potential for unintended consequences when managing MIC will be highlighted.

[1] ‘Quantification of Market Sectors Engaging With Biofilm Technologies’ PHS Consulting Ltd, NBIC commissioned report, 2021

Offered talk: Offered talk: Offered talk: Combined Nutrient Limitation and Biocide Strategy for Biofilm Prevention in a Water System in Space

Elizabeth Sandvik, Ian Novak, Darla Goeres, Paul Sturman, Phil Stewart

Montana State University, Bozeman, USA

Abstract

Background. Water is a precious resource in space and the capacity to recycle water is an important component of current and future life support systems for crewed space exploration. The longest operational microgravity water recycling system is on the International Space Station, where humidity condensate and urine distillate streams are treated to purify the water and dispense it as potable water. This wastewater recycle system works well, but has experienced biofouling events that threatened to compromise the recycle flow and have necessitated hardware replacements. In work sponsored by NASA, two strategies for reducing biofilm formation and fouling in the water recycling system were investigated.

Methods. A laboratory biofilm model system incorporating the essential microbiology (four bacteria and a fungus, all ISS isolates) a synthetic wastewater (based on decades of detailed chemical analysis of ISS wastewater) was developed. Flask assays identified two elements that drove biomass accumulation (P and Mg), whereas others (C, N, S) had little influence when altered. Twenty-three biocides were screened for biofilm inhibitory activity and three were selected for further investigation. Biofilm control approaches were tested using continuous flow CDC biofilm reactors operated for one month with Inconel coupons.

Results. Reducing or removing P and Mg from the synthetic wastewater significantly diminished biofilm accumulation. Dosing of 20 mg/l AgF or iodine daily also effectively suppressed biofilm formation. Combining these two strategies was synergistic.

Conclusions. Combined nutrient limitation and biocide dosing offers a high-performance approach for managing microbial stability in critical life support water systems.

Creating pathways to drive biofilm innovation: Building consensus on biofilm regulatory decision making

Darla Goeres[1], Paulina Rakowska[2], James Redfern[3], Maria Salta[4], Kasper Kragh[5], Martijn Riool[6], Martina Modic[7], Kazimierz Murzyn[8], Andreia Azevedo[9], Célia Fortuna[10]

[1]Center for Biofilm Engineering; Montana State University, Bozeman, USA. [2]National Biofilms Innovation Centre, Southampton, United Kingdom. [3]Manchester Metropolitan University, Manchester, United Kingdom. [4]Endures, Den Helder, Netherlands. [5]Symcel AB, Solna, Sweden. [6]Hospital Regensburg, Regensburg, Germany. [7]Jozef Stefan Institute, Ljubljana, Slovenia. [8]Klaster LifeScience, Krakow, Poland. [9]Faculty of Engineering U of Porto, Porto, Portugal. [10]Instituto Universitário de Ciências da Saúde, Gandra, Portugal

Abstract

Background. Regulatory science encompasses the development and application of scientific methods, research equipment, reference materials and model systems that enable informed decision making on antimicrobials, processes and devices that require regulatory approval. While every country has its own defined pathway for regulatory approval, all regulators rely on regulatory science tools to make decisions as to whether a product may enter the commercial market and what label claim the company who sells the product may make. As part of their decision making, regulators must consider how the product improves public health and its impact on the environment.

Challenge. Our challenge is that regulatory science has not stayed current, or even maintained, with what is considered "standard practice" in research laboratories, while the critical need to demonstrate the data collected on products tested *in vitro* translate to its end use is escalating. This has resulted in a large gap between fundamental science and current regulatory guidelines. This gap in regulatory tools is particularly immense for products formulated to kill, control, prevent or remove biofilm.

Approach. COST Action CA23152 will bring together teams of researchers to update the equipment, models, and methods used by regulatory, industrial, and clinical stakeholders for improved decision making with the intent of providing laboratory data that translates into regulatory decisions that protect public health and the environment.

From Architecture to Complex Infections: How Multi-Modal Cross-Scale Imaging Changes How We View Biofilms

Liam Rooney[1,2], Rebecca McHugh[2], Alexis Ramsey[3], Marina Haldopoulos[3], Khedidja Mosbahi[1], Daniel Walker[1], Marvin Whiteley[3,4], Suzie Humphrey[1], Andrew Roe[2], Gail McConnell[1]

[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom. [2]School of Infection and Immunity, University of Glasgow, Glasgow, United Kingdom. [3]School of Biological Sciences and Center for Microbial Dynamics and Infection, Georgia Institute of Technology, Atlanta, USA. [4]Emory-Children's Cystic Fibrosis Centre, Atlanta, USA

Abstract

Visualising infection dynamics across spatial scales is key to understanding the interplay between pathogens, the host, and the efficacy of clinical interventions. The difficulty in capturing the infection dynamics on a global scale lies in the lack of accessible imaging technologies to assess large tissue specimens with the high spatial resolution required to resolve individual pathogens. We present three separate case studies, where we have developed and applied innovative specimen preparation, imaging, and analysis methods to study infection biology across multiple spatial scales; (1) a high-efficiency optical clearing method for imaging whole *Galleria mellonella* larvae for the study of host-pathogen interactions, (2) quantitative multi-scale imaging to understand how anti-virulence therapies against enteric pathogens confer a protective role in renal remodelling, and (3) unprecedented visualisations of entire wound beds infected by polymicrobial biofilms and quantifying the spatial dynamics of wound infections in three dimensions. The three cases we present exemplify how cross-scale and multi-modal microscopy approaches offer new insights into colonisation, infection, and intervention studies. We propose that the open methods we have developed have great potential for translation to other infection biology scenarios, thereby opening the doors for microbiologists and immunologists to address key questions that span multiple length scales.

Reduced efficacy of efflux pump inhibitors as adjuvant therapy in polymicrobial biofilms

Brogan Richards[1], Luisa Martinez-Pomares[1], Shaun Robertson[1,2], Miguel Cámara[1,3]

[1]University of Nottingham, Nottingham, United Kingdom. [2]MiDx, Nottingham, United Kingdom. [3]National Biofilms Innovation Centre, Nottingham, United Kingdom

Abstract

Current *in vitro* biofilm models assessing the effectiveness of novel antimicrobials do not consider the impact of cohabiting microbes and their environment, often resulting in treatment failure. This results in recurrent and chronic lung infections in cystic fibrosis (CF) patients. To address this issue, we have developed accessible polymicrobial biofilm models highly adaptable to CF conditions and assessed the impact of polymicrobial communities on the effectiveness of antibiotics against *Pseudomonas aeruginosa*. Using these models, we assessed the role of RND efflux pumps on the antimicrobial tolerance of *P. aeruginosa* biofilms grown with *Staphylococcus aureus* and *Candida albicans*. Knockout *P. aeruginosa* mutants of four multidrug efflux pumps associated with increased tolerance to antibiotics were tested for sensitivity to meropenem and tobramycin, commonly used to treat CF-related infections. Minimal biofilm inhibitory concentrations were determined as colony-forming units and metabolic activity. From these mutants, only the MexAB-OprM showed significant effects on *P. aeruginosa* planktonic and biofilm tolerance to meropenem and tobramycin combination therapy. In contrast, in polymicrobial biofilms, the MexAB-OprM mutant did not show increased antimicrobial sensitivity. In agreement with these findings, MexAB-OprM specific and non-specific efflux pump inhibitors increased tolerance of *P aeruginosa* to antibiotics in single species biofilms but were significantly less effective in a polymicrobial context. This work indicates there is redundancy in efflux pump-mediated antibiotic tolerance and clear restrictions in using efflux pumps as adjuvant therapy in polymicrobial biofilms.

Impact of Residual Chlorine Concentration on Drinking Water Biofilms Including Coliform Incorporation and Mobilisation

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

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

Abstract

Drinking water distribution systems (DWDS) are designed to protect water quality during delivery from treatment works to consumers' taps. Biofilms form on the internal surfaces of DWDS, impacting water quality by their activity and/or mobilisation into the bulk-water. The impact of disinfection residuals upon biofilms, and the subsequent unintended risk they may present to water quality, is unclear.

An internationally-unique DWDS test facility, fed with water from the local DWDS, was used to grow biofilms under Low-, Medium- and High-chlorine regimes for 5 months. Each regime was spiked with an environmental contaminant and monitored for a further week to assess coliform incorporation. Biofilms were subsequently exposed to increasing shear stresses (flushing) to evaluate mobilisation and water quality impact. Biofilm and bulk-water cell counts/viability (flow cytometry), and coliform and *Escherichia coli* presence (colilert assay) were monitored throughout.

Residual-chlorine concentration impacted microbial water quality with a dose response observed during the growth-phase (Low-chlorine 500,000 TCC/mL; High-chlorine 600 TCC/ml on average). Biofilm cell concentrations and viability did not follow the same trend, although biofilm composition at 5-months was distinct between regimes. Coliforms were quickly inactivated in the bulk-water of High- and Medium-chlorine regimes, persisting in the Low-chlorine regime for 2 days post-spike. Active coliforms were subsequently detected again in the Low-chlorine regime bulk-water in response to flushing, demonstrating coliform incorporation, survival and mobilisation from the biofilm.

The derived understanding could impact the long-term management of DWDS water quality and biofilms, whilst challenging the mind-set of continuous residual-disinfection control strategies.

Silent Reservoirs: Understanding the Persistence of Vancomycin-Resistant Enterococcus in Dry Surface Biofilms

Ruby Harsent[1], Isabella Centeleghe[1], Michael Pascoe[1], Vincent Cattoir[2], Jean-Yves Maillard[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]Univeristy of Rennes, Rennes, France

Abstract

Dry surface biofilms (DSB) are undetectable by swabbing and often harbor antibiotic resistant bacteria such as vancomycin resistant *Enterococcus* (VRE) that persist for an unknown amount of time on surfaces.

Artificial DSB were produced on stainless steel coupons over 12 days. Three epidemic strains of *Enterococcus faecium*, including two VRE were selected. Other strains of *Enterococcus*, and *Staphylococcus aureus* were included for interspecies comparison. DSB were maintained at 20°C/55% relative humidity for up to 84 weeks, with viability determined at various time points. Scanning electron microscopy imaged DSB on surfaces. For comparison, bacterial suspensions were also deposited onto coupons which were maintained under identical conditions.

Enterococcus can form DSB on stainless steel in thin, expansive layers of extracellular polymeric substance. A mature Enterococcus DSB averaged 6.94±0.68 Log_{10} CFU/mL Between 0-84 weeks, a Two-Way ANOVA showed no statistical change in culturability for the two VRE and the standard *E. faecium* strain ($P<0.05$). The vancomycin susceptible *Enterococcus* and the *E. hirae* standard strain exhibited a significant reduction in culturability from 6.94±0.05 and 7.92±0.12 to 4.70±0.71 and 6.25±0.98 Log_{10} CFU/mL respectively ($P<0.05$). *S. aureus* became undetectable at 16 weeks. The viability of suspensions on steel decreased rapidly compared to DSB.

VRE persists on surfaces as DSB for long periods and are more resistant to disinfection and desiccation. The standard disinfectant efficacy testing strain does not persist as long as the E. faecium strain, prompting consideration of utilizing this strain for testing.

Close encounters on a micro scale: Interactions of microplastic biofilms in aquatic ecosystems and their effects on associated biofilm communities.

Jessica Song[1], Brittan Scales[2], Minh Nguyen[3], Emelie Westberg[4], Bartozs Witalis[5], Barbara Urban-Malinga[5], Sonja Oberbeckmann[1]

[1]Bundesanstalt für Materialforschung und -prüfung, Berlin, Germany. [2]Montana State University, Montana, USA. [3]IVL Swedish Environmental Research Institute, Stockholm, Sweden. [4]IVL Swedish Environmental Research Institute, Stockholm, Germany. [5]National Marine Fisheries Research Institute, Gdynia, Poland

Abstract

Microplastics circulate freely throughout aquatic ecosystems and, due to their interactive nature, accumulate complex polymeric matrices consisting of rich organic compounds and inorganic pollutants. Simultaneously, these hardy substrates offer nutrition and protection to diverse microbial communities and their theatre of activity, representing a new ecological niche. In our work, we investigate the interactions of microplastics in aquatic systems and characterise the effects of these interactions on associated microbial communities to better understand how these substrates might impact surrounding ecosystems. Demonstrating no specificity to polymer type, microplastic biofilms are shaped more by the strong influence of spatial and temporal factors. Microplastic sorption of polycyclic aromatic hydrocarbons (PAHs), in contrast, appear to be more strongly dictated by substrate type, with different polymers observed to sorb varying levels of different PAHs. These interactions between the different emerging contaminants were found in our study to have a significant effect on associated substrate biofilms. Elevated levels of specific 3- and 4-ring PAHs on polyethylene and polystyrene were found to coincide with a notable shift in community composition and structure, as well as a reduced diversity among biofilm communities. The findings in our study illustrate the importance of investigating the collective effect of pollutants in combination and their complex interactions in assessing their environmental impact. To fully understand how microplastics interact and alter surrounding ecosystems, the entire substrate must be considered, including all chemicals integrated into the polymeric matrix.

A new mechanism for outer membrane vesicle production in *Porphyromonas gingivalis*

Yichao Liu, Beichang Zhang, Sunjun Wang, Joseph Aduse-Opoku, Michael Curtis, James Garnett

King's College London, London, United Kingdom

Abstract

Periodontal (gum) disease is a destructive, inflammatory condition affecting the gingiva and hard and soft tissues of the periodontium and is ranked sixth most prevalent disease worldwide. *Porphyromonas gingivalis* (*Pg*) is a Gram-negative bacterium that plays a central role in this disease through the formation of complex biofilms. In *Pg*, a type-IX secretion system (T9SS) exports a range of essential virulence factors to the bacterial surface where they are attached to lipopolysaccharide (LPS) and packaged into outer membrane vesicles (OMVs). LPS is the major component of the outer membrane of Gram-negative bacteria, and is composed of lipid A, a core oligosaccharide, and a distal O-antigen of repetitive glycan polymers. *Pg* produces at least four lipid A modifying enzymes: LpxE (lipid A 1-phosphatase), LpxF1 and LpxF2 (lipid A 4-phosphatases), and LpxR (3-O-deacylase). We have demonstrated that a functional T9SS is required for LpxE activity, and in *T9SS* and Δ*lpxE* mutants, OMVs become enlarged/deformed. Using cryo-EM we have revealed that *Pg* LpxE contains a unique 25-kDa C-terminal extension, which mediates trimerisation and maintains the N-terminal type 2 phosphatidic acid phosphatase (PAP2) domain in an inactive state. We have also shown that Δ*lpxE*, Δ*lpxF1*, and Δ*lpxR* mutants (but not Δ*lpxF2*) are defective in OMV production and cargo sorting; and we have identified homologs in other Bacteroides species that colonise the gut and oral cavity and possess a T9SS and produce OMVs. We present a new mechanism where cargo sorting and vesicle blebbing in *Pg* is a coordinated process regulated by the T9SS.

Biofilm inhibitors, an evolutionarily robust anti-virulence strategy?

Sybren Van Ginneken[1], Mathieu Joos[1], Xabier Villanueva[1], Marie Dijkmans[1], Guglielmo Coppola[1], Camilo Andres Pérez-Romero[2], Thijs Vackier[1], Erik Van der Eycken[1,3], Kathleen Marchal[2], Bram Lories[1], Hans Steenackers[1]

[1]KU Leuven, Leuven, Belgium. [2]UGent, Gent, Belgium. [3]RUDN University, Moscow, Russian Federation

Abstract

Microbial biofilms present a significant challenge in both medical and industrial settings. In these structured communities, cells are embedded within a self-produced matrix of exopolymeric substances (EPS), which protects them from the immune system and antimicrobial treatments, making biofilm-associated infections persistent and difficult to eradicate.

Building on our previous work (*Dieltjens et al., 2020; Nat. Commun.*), we further developed 2-amino-imidazole-based biofilm inhibitors (2-AIs), which repress EPS production in a broad range of micro-organisms. Since EPS acts as an exploitable public good—costly to produce but benefiting the entire community—resistant mutants restoring EPS production would incur a private cost, while the benefit is shared within the population. This makes resistance evolution to an EPS inhibitor theoretically unfavorable. To test this, we exposed *Salmonella* biofilms to a 2-AI-based inhibitor for 40 days. As predicted, evolved biofilms remained susceptible to EPS inhibition. However, they consistently acquired mutations in the AcrAB efflux pump, which upregulated efflux and conferred cross-resistance to antibiotics. Selection for efflux was due to an off-target effect of the EPS inhibitor, which inadvertently delayed cell growth in addition to inhibiting biofilm formation. Promisingly, chemically modifying the inhibitor was able to mitigate the off-target effect, which reduced selection pressure for efflux.

In conclusion, targeting exploitable matrix components seems a promising and evolutionarily robust strategy for biofilm control, but minimizing off-target effects is essential. Our new findings are currently under review in *NPJ Biofilms & Microbiomes* and highlight the flexibility of 2-AI-based compounds as a platform for developing evolutionarily robust biofilm inhibitors.

Metallic Defence: Tackling the Biofilm Challenge

Rupika Gulati[1], Freya Harrison[1], Nicole Robb[1], Christopher Mills[2], Stuart Coles[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Tata Steel, Swansea, United Kingdom

Abstract

Metals exhibit antibacterial properties, with increasing interest in their antibiofilm potential, particularly in coatings for wound care and healthcare associated surfaces. This study employed a combination of in vitro and ex vivo methods, including minimum inhibitory concentration (MIC) assays, biofilm inhibition assays, ISO 22196, and a porcine wound model; to evaluate over 30 metal-based compounds against *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* USA300.

Selected metal compounds were incorporated into coatings and hydrogels for applications in surface coatings and wound dressings, respectively. Initial findings indicate that certain copper and silver compounds and formulations demonstrate potent antibacterial activity, with MIC values ≤2 mg/mL. These compounds were further evaluated in industrial coatings using a 96-well plate assay to assess MIC variability before additional testing.

For wound dressings, MIC comparisons between cation-adjusted Mueller-Hinton broth (MHB) and synthetic wound fluid (SWF) revealed medium-dependent variations. For instance, silver sulfadiazine exhibited MICs ranging from 32–256 µg/mL in MHB but 4–128 µg/mL in SWF, whereas other silver compounds/formulations displayed increased MICs in SWF. Additionally, optimization of an ex vivo wound model demonstrated that inoculation methods significantly influence biofilm formation, potentially affecting wound dressing efficacy.

These findings highlight that metal-based antibiofilm activity is highly dependent on assay conditions, including media composition and application method. Such variability underscores the need for standardized testing to consider the environment that metal-containing antimicrobial coatings will encounter in end-use scenarios to accurately assess their efficacy.

Transfer and persistence of plasmids in biofilm

Henriette L. Røder[1], Eleni Cristidi[2], Cristina Amador[1], Urvish Trivedi[1], Jakob Russel[1], Kasper N. Kragh[1], Tim Tolker-Nielsen[1], Thomas Bjarnsholt[1], Samra Music[1], Asmus K. Olesen[1], Birte Svensson[3], Jonas S. Madsen[1], Jakob Herschend[1], Jan-Ulrich Kreft[2], Mette Burmølle[1]

[1]University of Copenhagen, Copenhagen, Denmark. [2]University of Birmingham, Birmingham, United Kingdom. [3]Technical University of Denmark, Lyngby, Denmark

Abstract

Background: Bacteria can use horizontal gene transfer (HGT) to rapidly adapt to environmental changes by directly transferring mobile elements like, e.g., plasmids. This can ensure their survival even under otherwise detrimental conditions such as exposure to antimicrobial agents. Biofilms are the primary lifestyle of bacteria, and they have been proposed to be a hotspot for HGT because of their high cell density, proximity, and structural stability. Here, we set out to study the transfer and persistence of plasmids in biofilm communities.

Methods: Two different dual-labelled systems were used for detection of plasmid transfer or stability, respectively. Flow cytometry and confocal laser scanning microscopy was used for detection. Proteomics and knock-out mutants were used to confirm observations of increased plasmid uptake as well as the use of individual-based modelling to confirm or eliminate possible underpinning mechanisms.

Results: The results showed how biofilms can indeed act as spatiotemporal reserves for plasmids, allowing them to persist even under non-selective conditions. We further observed how the dynamic structure of biofilms directly impacts the number of plasmids that are retained. We also identified a new factor impacting the transfer of plasmids – flagella, which are required for motility by many bacteria. We demonstrate that their absence or altered activity can lead to enhanced plasmid dissemination. Furthermore, we demonstrate the utility of mathematical modeling to test hypothetical explanations.

Conclusions: Our findings identify biofilms as conducive towards maintaining genetic diversity as well as shedding light on the complex effects of flagella on bacterial conjugation in biofilms.

Metabolic crosstalk drives physiological differentiation in *Pseudomonas* aggregates and biofilms

Lindsey Florek, Lauren Unterreiner, Lars Dietrich

Columbia University, New York, USA

Abstract

Background: Bacteria in multicellular structures form subpopulations with distinct metabolisms and transcriptomes. This heterogeneity contributes to the resilience of biofilms, making infections more challenging to treat. In *Pseudomonas aeruginosa*, we have found that overlapping mechanisms in the sensing, transport, and metabolism of lactate and glycolate manifest as crosstalk between such subpopulations. While lactate is a key carbon source in cystic fibrosis lung infections and chronic wounds, little is known about glycolate metabolism in pathogenic bacteria.

Methods: We employed genetic analysis, fluorescent reporters, stimulated Raman scattering microscopy, and macrocolony and aggregate growth assays to investigate the crosstalk between lactate- and glycolate-associated physiology in *P. aeruginosa*.

Results: We found that *P. aeruginosa* endogenously produces lactate and glycolate at levels sufficient to influence gene expression. Deleting the fermentative lactate dehydrogenase (LdhA) or glyoxylate shunt protein (AceA) did not eliminate lactate or glycolate production. We also observed that, in multicellular aggregates, lactate- and glycolate-associated genes are induced in spatially segregated patterns, with glycolate production inversely correlated with metabolic activity. In aggregates in particular, LdhA and AceA do not contribute to the production of lactate and glycolate.

Conclusion: We propose that glyoxal/methylglyoxal detoxification, a pathway that converts toxic metabolic byproducts into non-toxic intermediates, is the primary source of lactate and glycolate. *P. aeruginosa* possesses homologs of this pathway, which is well-characterized in eukaryotes and non-pathogenic bacteria. Since *P. aeruginosa* forms aggregates during infection, understanding these metabolic pathways could provide insights into its persistence in host environments.

Biofilm structure responds differently to *Legionella* colonization under varying hydrodynamics'

Ana Rosa Silva[1,2], Charles William Keevil[3], 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. [3]School of Biological Sciences, University of Southampton, Southampton, Southampton, United Kingdom

Abstract

Legionella commonly colonizes biofilms in water systems, posing significant public health risks. Although several studies have explored *Legionella* ecology, the fundamental interactions between *Legionella* and biofilms remain unclear. This study investigates how biofilm structure responds to *Legionella* colonization under different hydrodynamic conditions.

To accomplish that, *Pseudomonas fluorescens* (*Pf*) biofilms were grown under dynamic conditions (80 RPM). The pre-established biofilms were then spiked with *Legionella pneumophila* (*Lp*) and reincubated at 80 RPM or under stagnation. A toolbox of analytical methodologies was applied to study biofilms and *Lp*. Biofilm mesoscale structure was evaluated through Optical Coherence Tomography (OCT). *Lp* spatial location was tracked via 16S rRNA PNA-FISH.

Mesoscale imaging revealed different biofilm structure responses to *Lp* colonization: 80 RPM biofilms became ~20% thinner upon colonization, while setting the biofilms to stagnation after the spiking promoted an increase in biofilm thickness (from 19 to 45 µm). The biofilm porosity followed the same trend as observed for the thickness. Molecular tracking coupled with microscale imaging revealed that *Lp* was located in the biofilm bottom layers, regardless of the hydrodynamic conditions. However, stagnation fostered *Lp* migration most likely due to the low oxygen concentrations in the bottom layers that fulfill *Lp* microaerophilic requirements. Water channels seem to facilitate *Lp* migration within the biofilm.

This work reinforces the role of biofilm structure and environmental conditions on *Legionella* colonization and proliferation and underscores the risk posed by stagnation for legionellae persistence in water systems.

Community composition and complexity drive *Salmonella* survival and adaptation under stress in mixed-species biofilms.

Eleftheria Trampari[1,2], Haider Al-Khanaq[1,2], Ryan Sweet[1,2], Maria Solsona Gaya[1,2], Mark Webber[1,3,2]

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

Abstract

Salmonella is the third most costly foodborne pathogen in the UK, imposing an annual economic burden of £200 M. It persists in the food chain through biofilms, which enhance its ability to survive and adapt under stress, posing a significant threat to food safety. My research uses an established biofilm evolution model to understand how *Salmonella* survives and adapts in food-related community settings under various antimicrobial stresses.

Expanding from single species to multispecies biofilms, we employed a Synthetic Community (SynCom) approach to simulate how *Salmonella* adapts in complex microbial communities resembling food processing environments. Communities of varying complexities, with and without *Salmonella*, were exposed to subinhibitory antibiotic concentrations. We tracked population dynamics over time, through metagenomic analysis, and used whole-genome sequencing and SNP identification to map *Salmonella*'s evolutionary trajectory in multispecies biofilms compared to single-species biofilms. Our findings revealed that community composition and complexity positively influence *Salmonella*'s survival, with higher pathogen recovery rates observed in more complex communities. However, this came with a trade-off: increased community complexity also heightened *Salmonella*'s susceptibility to antibiotics compared to single-species biofilms.

This research provides valuable insights into how foodborne pathogens survive and adapt within the food chain. It highlights the importance of community complexity and composition in shaping these adaptations. Understanding the impact of antimicrobials on mixed-community biofilms offers valuable information for designing interventions that target not only *Salmonella* but also key interactions within complex microbial ecosystems. This knowledge can help develop strategies to reduce pathogen resilience and enhance food safety in real-world settings.

Temperature and pH stimuli-responsive system delivers location-specific antimicrobial activity with natural products.

Yuri Diaz Fernandez[1,2], Ioritz Sorzabal-Bellido[1], Gareth Morris[1], Luca Barbieri[1], Adam Skelton[1], Chiara Milanese[2], Piersandro Pallavicini[2], Rasmita Raval[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Pavia, Pavia, Italy

Abstract

Smart materials with complex stimuli-response functions are key to future antimicrobial technology development. However, in the area of medical devices and health-care materials, stringent regulatory barriers limit the translation of emerging technologies into scalable processes with real industrial impact. Here we present a generic strategy that combines simple components, physicochemical responses and easy fabrication methods to achieve a dual stimuli-responsive system, capable of location-specific antimicrobial cargo delivery [1]. Out-of-equilibrium loaded systems were fabricated by combining a biocompatible inert polymeric matrix of polydimethylsiloxane (PDMS) and a bioactive cargo of saturated fatty acids, which are components already approved for use in humans.

The system responds to two control variables, temperature and pH, delivering two levels of antimicrobial response under distinct combinations of stimuli: one response towards the planktonic media and another response directly at the surface for sessile bacteria. Spatially resolved Raman spectroscopy alongside thermal and structural material analysis reveals that the system not only exhibits ON/OFF states but can also control relocation and targeting of the active cargo towards either the surface or the liquid media, leading to different ON/OFF states for the planktonic and sessile bacteria. This approach has led to the development of smart materials able to deliver complex functions resembling Boolean logic gates as a function of the experimental variables.

References

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epsSMASH predicts exopolysaccharides in environmental and human microbial communities

Anders Ogechi Hostrup Daugberg[1], Angie Waldisperg[1], Marie Riisgaard-Jensen[1], Sofie Zacho Vestergaard[1], Roberto Sánchez Navarro[2], Tilmann Weber[3], Kai Blin[3], Simon Shaw[3], Per Halkjær Nielsen[1], Morten Kam Dahl Dueholm[1]

[1]Aalborg University, Aalborg, Denmark. [2]Aalborg University (But left in 2023), Aalborg, Denmark. [3]Technical University of Denmark, Copenhagen, Denmark

Abstract

The vast majority of organic matter in biofilms does not reside in cells, but rather the extracellular polymeric substances (EPS) which make up the biofilm matrix. The EPS matrix is a highly dynamic system which provides many benefits to the microbial community. Despite the ubiquity of biofilms (and by extension EPS), research into the genomic potential for EPS production in microbial communities has been limited. A major component of EPS are exopolysaccharides (exoPS). The genes required for exoPS biosynthesis are known to cluster into biosynthesis gene clusters (BGCs), enabling prediction of exoPS potential through genome mining.

We present epsSMASH, a bioinformatic tool for detecting known exoPS BGCs and predicting novel ones. Based on the antiSMASH framework, we leverage custom-made Hidden Markov Models to craft detection rules for 26 different exoPS BGCs. In addition we employ less stringent rules in order to capture novel exoPS BGCs, which have not yet been characterised.

We have applied epsSMASH to four large genome databases, which represent the microbial communities of the human gut, the ocean, soil, and activated sludge, which showed a significant difference between the exoPS profiles of these communities.

epsSMASH will be available as both a user-friendly web service and a local command line tool for more experienced bioinformaticians. In future versions we plan to broaden the search to the other major biopolymers in the biofilm matrix. We believe epsSMASH will become an essential tool for microbiologists investigating the fascinating world of biofilms.

ECO-COATING: Harnessing Marine Bacteria for Biofilm Engineering

Cristina Amador[1], Amanda Seger Jakobsen[1], Nan Yang[1], Bristi Paul[2], Emma Harrington[2], Gabrielle McCarthy[2], Narges Sadat Shamabadi[2], Justin Leonhardt[3], Barbara J Campbell[3], Diptee Chaulagain[3], David Karig[3], Mette Burmølle[1]

[1]Department of Biology, University of Copenhagen, Copenhagen, Denmark. [2]Department of Bioengineering, Clemson University, Clemson, USA. [3]Department of Biological Sciences, Clemson University, Clemson, USA

Abstract

Microbial colonization of submerged marine surfaces plays a pivotal role in ecological processes and industrial applications. While biofilms contribute to nutrient cycling and habitat formation, they also drive biofouling and material degradation, posing challenges for maritime industries. Understanding biofilm formation on marine coatings is essential for both mitigating these challenges and leveraging microbial interactions to develop protective, functional biofilms.

This study explores biofilm engineering by repurposing marine microbial "building blocks" to construct stable, beneficial biofilms. We isolated 160 bacterial strains from unmanned underwater vehicle (UUV) parts coated with PPG PSX® 700, a durable siloxane-based coating. These isolates were screened for adhesion and growth on untreated (uPS) and treated (tPS) polystyrene under varying conditions. Results showed that uPS induced higher adhesion rates, reliably predicting bacterial attachment to PSX700.

From the top 30 adhering isolates, nine were selected based on adhesion profiles and taxonomy. These were assembled into seven multispecies biofilm communities, which were evaluated for biomass, stability, and resistance to microbial invasion. After five days, three communities remained stable, while four resisted external invasion, regardless of the surface material tested. Notably, *Alteromonas* sp. ABV4_151, a high-biofilm-biomass producer, emerged as a key species contributing to both stability and invasion resistance.

These findings demonstrate the potential of marine-derived bacteria in engineering stable biofilms, offering insights for industries aiming to control biofouling while harnessing microbial processes for improved material performance in marine environments.

Cosmic Quorums: Driving Biofilm Breakthrough using Space Biology Open Science.

Katherine Baxter[1], Vinothkannan Ravichandran[2]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Amity University, Mumbai, India

Abstract

Background: Open Science practices are a powerful way to accelerate innovation and make scientific research freely available to everyone in society. When applied, research outputs such as publications, protocols, data, physical samples and software are processed and curated in a way that makes them Findable, Accessible, Interoperable and Reusable (FAIR), promoting inclusivity, transparency and reproducibility.

A global research community has arisen around the NASA Open Science Data Repository (OSDR) (https://www.nasa.gov/osdr/). Nine Analysis Working Groups (AWGs) leverage both space flown and terrestrial Open Science data, gaining novel insight into fundamental biological processes in both environments.

Methods and results: The Microbes Analysis Working Group recently formed the Biofilms Subgroup to explore and leverage the microbial data held by NASA OSDR. This interdisciplinary group spans all career stages across multiple continents- a diversity which provides unique scientific perspectives and access to a variety of different funding pools. We are currently undertaking a project to characterise knowledge gaps in biofilm behaviour and identify NASA OSDR datasets which can be interrogated. Since 2017, the NASA OSDR AWG community has enabled 133 peer-reviewed publications, pre-prints and published student theses through data reuse, indicating the capacity for research advancement. These experiences have yielded valuable lessons on how to optimise Open Science practices and productivity.

Conclusion: The work of the Microbes Analysis Working Group is an exemplar for how Open Science can accelerate discoveries in biofilm research, both for space exploration and in translation to meet challenges on Earth.

Pseudomonas aeruginosa clinical isolates can encode functional plastic-degrading enzymes that allow survival on plastic and augment biofilm formation.

Ronan McCarthy, Sophie Howard, Ruben De Dios, Evgenia Maslova, Antonis Myridakis, Thomas Miller

Brunel University of London, London, United Kingdom

Abstract

Several environmental bacteria have been shown to encode functional plastic-degrading enzymes; however, thus far no clinical bacterial isolates have yet been reported with this capability. In environmental bacteria, biofilm formation on waste plastic plays a key role in the degradation process. Given the widespread use of plastic in healthcare and the notorious ability of pathogens to form biofilms on implanted medical devices, including plastic devices, we hypothesised that clinical bacterial isolates might also encode functional plastic-degrading enzymes. If so, this could render plastic-containing medical devices vulnerable to degradation and failure while simultaneously providing pathogens with a growth-sustaining substrate, potentially aiding their persistence within hospital environments or the host.

Here, we identify a clinical isolate of *Pseudomonas aeruginosa* capable of degrading the medically relevant plastic polycaprolactone (PCL), leading to a 78% reduction in weight within seven days. This level of degradation could compromise the integrity of PCL-containing medical devices such as sutures or implants. Crucially, we also demonstrate that this strain can utilise PCL as its sole carbon source for growth.Furthermore, we identify the novel enzyme responsible for this plastic-degrading activity and show that it is secreted via a Type II secretion-dependent mechanism. Notably, we establish a direct link between plastic degradation and virulence, demonstrating that encoding a plastic-degrading enzyme significantly enhances biofilm formation. We reveal that this enhancement is driven by the incorporation of plastic breakdown products into the extracellular matrix, ultimately leading to increased biofilm levels.

The Aggregate Advantage: Genetic and Biophysical Drivers of *Pseudomonas aeruginosa* Infection and Antimicrobial Resistance

Caroline Miller, Oriana Williams, Sophie Darch

University of South Florida, Tampa, USA

Abstract

Bacterial aggregates are present in both chronic and acute infections and can be formed by bacteria, archaea, and fungi. *Pseudomonas aeruginosa* (*Pa*), an opportunistic pathogen, primarily affects individuals with compromised immune defenses or barrier functions, including those with chronic and acute wounds, implanted medical devices, and people with cystic fibrosis (pwCF). In pwCF, *Pa* establishes chronic lung infections, where a substantial proportion of bacteria persist as small aggregates (~10–1000 cells) within airway sputum. Previous studies of *Pa* have largely relied on well-mixed flask cultures, which form macro-scale biofilm structures. While these models have greatly advanced our understanding of *Pa* growth, quorum sensing, and antibiotic tolerance, they do not accurately mimic in vivo infection conditions, where bacteria exist as aggregates. This discrepancy represents a critical gap in translating biofilm research into clinically relevant insights.To address this, we used synthetic CF sputum media (SCFM2) and genomics to identify a subset of genes essential for *Pa* aggregate formation and immune tolerance. Additionally, atomic force microscopy (AFM) revealed that aggregates possess distinct biophysical properties compared to planktonic cells. Disrupting these genetic and physical traits altered *Pa*'s tolerance to antibiotics, immune challenges, and mechanical disruption. These findings highlight previously unrecognized aspects of *Pa* aggregate biology that enhance our understanding of its physiology during infection. By uncovering genetic and physical properties unique to aggregates, our study identifies potential therapeutic targets for combating this multi-drug-resistant pathogen.

Studying gastric microbiota with nucleic acid mimics-fluorescence in situ hybridization (NAMs-FISH) combined with spectral imaging

Sonia Miranda[1,2,3,4], Andreia Azevedo[1,2], Nuno Azevedo[1,2]

[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 in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal. [3]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135, Porto, Portugal. [4]IPATIMUP-Instituto de Patologia e Imunologia Molecular, Universidade do Porto, 4200-135, Porto, Portugal

Abstract

Background: The 3D organization of gastric microbiota, which may influence an individual's health status, is still poorly studied. In this study, Spectral Imaging (SI), combined with Nucleic Acid Mimics - Fluorescence *in situ* Hybridization (NAMs-FISH) was developed to investigate the spatial distribution of relevant gastric pathogens.

Methods: Six different genus/species-specific LNA/ 2'OMe probes targeting *Campylobacter spp*., *Citrobacter spp*., *Clostridium spp*., *Fusobacterium spp.*, *Streptococcus spp*. and *Helicobacter pylori* were designed. Following this, the hybridization conditions for each probe, including hybridization solution, temperature, and time were individually optimized. Suitable hybridization conditions for all the probes were then selected. Subsequently, the method was validated by analyzing artificial mixtures with varying concentrations of each genus/species using spectral imaging.

Results: The hybridization condition selected for the probe mixture consisted of 2M urea, 4M NaCl, 50 mM Tris-HCl, 5 mM disodium EDTA, 10% (w/v) dextran sulfate, 0.1% (w/v) sodium pyrophosphate, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll , 0.1% (v/v) Triton X-100, pH – 7.5, for a hybridization period of 60 minutes at a temperature of 62-64 ºC. Subsequently, we successfully validated the method, demonstrating a good correlation between the input into the mixture of gastric bacteria with the output result from the FISH analysis.

Conclusion: These findings pave the way for future research on spatial 3D microbiota organization in gastric samples s. The main goal is to characterize the gastric microbiota profiles associated with health and disease from adult patients.

Persistence of *mcr* resistance following long-term absence of colistin selection in a biofilm environment

Jingxiang Wu[1], Jennifer Adams[1], Francesca Boardman[1], Mei Li[2], Ian Boostrom[1], Jordan Mathias[1], Lydia Powell[3], Manon Pritchard[1], Carles Tardío Pi[4], Rafael Peña-Miller[4], Timothy Walsh[2], Owen Spiller[1], David Thomas[1], Katja Hill[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]Oxford University, Oxford, United Kingdom. [3]Swansea University, Swansea, United Kingdom. [4]Universidad Nacional Autónoma de México, Cuernavaca, Mexico

Abstract

The alarming global spread of plasmid-borne colistin resistance (*mcr*) conflicts with the significant bacterial fitness costs associated with plasmid carriage in vitro. In vivo however, bacteria exist predominantly in biofilms, which influences acquisition of antimicrobial resistance (AMR). In long-term (51-day) *Escherichia coli* bead biofilm models, the effects of biofilm environment and colistin (at sub-inhibitory concentrations) on growth, *mcr* carriage and persistence were studied. In the absence of colistin, *mcr-1* was rapidly lost (Day 23), but *mcr-3* persisted (> 51 days). At 28 days following *mcr-1* loss, colistin resistance rapidly re-emerged on re-exposure to colistin (< 48 h), being associated with *mcr* compensatory mutations, including loss of a 28,256 bp multi-drug resistance region. Theoretical modelling of plasmid dynamics incorporating compensatory mutations supports the observed persistence of *mcr-1* in the absence of selection and its re-emergence following renewed antibiotic exposure. This work underscores the importance of the biofilm environment in the acquisition, persistence and evolution of AMR.

Developing an antimicrobial release system using nanoclay gels to treat osteomyelitis

Maria Khalique[1], Prof Richard Oreffo[2], Prof Jeremy Webb[1,3], Dr Agnieszka Janeczek[2], Prof Jonathan Dawson[1,2]

[1]University of Southampton, Southampton, United Kingdom. [2]Renovos Biologics Ltd., Southampton, United Kingdom. [3]National Biofilms Innovation Centre, Southampton, United Kingdom

Abstract

Background: Localised antimicrobial therapies are being developed to treat bone infections (osteomyelitis) as they 1) increase drug bioavailability at site of infection 2) reduce off target side effects and 3) minimise bacterial exposure to antibiotics. Nanoclay particles self-organise to form gels that are biocompatible, biodegradable, and act as a scaffold for molecules to bind to, including growth factors, to stimulate tissue regeneration and aid bone healing.

Methods: Synthetic nanoclay gels were prepared using Renovite®, a patented formulation designed by Renovos Biologics Ltd. Nanoclay gels were loaded with gentamicin and vancomycin and *in vitro* drug release assays were performed to characterise drug release in PBS. The effect of nanoclay gels on planktonic *Staphylococcus aureus* growth was studied using growth curve assays and confocal microscopy. *In vitro* assays were carried out to determine minimum drug concentration required to inhibit *S. aureus* biofilm growth.

Results: Nanoclay gels were able to retain antibiotics within their gel structure as there was only 40 – 70 % drug release after 48 hr incubation. 64 – 128 µg/mL gentamicin loaded within nanoclay gels showed 100 % inhibition in bacterial growth in 4 *S. aureus* strains over a 24-hr period. A 5-log reduction in biofilm growth was observed in nanoclay gels loaded with 128 µg/mL gentamicin, with 100 % inhibition of biofilm growth on polystyrene pegs compared to bacterial growth control.

Conclusion: Nanoclay gels loaded with gentamicin showed 100 % antimicrobial activity against planktonic *S. aureus*, and showed complete biofilm inhibition, offering a promising localised delivery system to treat bone infections.

Synthetic wound fluid enhanced the antimicrobial susceptibility and altered the biofilm formation ability of chronic wound bacterial isolates of *P. aeruginosa* and MRSA

Charlotte Morgan[1], Aleyna Yarranton[1], Lydia Powell[1,2]

[1]Swansea University, Swansea, United Kingdom. [2]Cardiff University, Cardiff, United Kingdom

Abstract

Chronic non-healing skin wounds are a significant cause of morbidity and mortality in patients. Bacterial biofilm-related infections formed within these wounds impair tissue healing, resist conventional treatment and harbour multi-drug resistant bacteria. Therefore, there is an urgent need to gain a greater understanding of how bacterial biofilms form within chronic wounds to enable the development of alternative therapies. Chronic wound bacterial isolates of *Pseudomonas aeruginosa* PAO1 and Methicillin-resistant *Staphylococcus aureus* (MRSA) 1004a were grown in two recipes of synthetic wound fluid (SWF), comprised of either fetal bovine serum (FBS) or bovine serum albumin (BSA) in comparison to a standard laboratory media, Mueller Hinton Broth (MHB). SWF containing FBS supported bacterial growth of both *P.aeruginosa* and MRSA, while SWF containing BSA reduced growth. Unexpectedly, MRSA grown in SWF containing FBS exhibited increased antimicrobial susceptibility to colistin in comparison to MHB, resulting in a reduced minimum inhibitory concentration (MIC) value from 256 µg/ml to 16 µg/ml. The biofilm forming ability of the bacterial isolates in SWFs were characterised by confocal laser scanning microscopy with COMSTAT analysis, which revealed significant alterations in *P. aeruginosa* and MRSA biofilm biomass and dead/live bacterial ratio in comparison to MHB ($p < 0.05$). Atomic force microscopy and electrophoretic light scattering assays revealed alteration of the bacterial mechanical properties and surface charge when grown in SWF containing FBS, in comparison to MHB ($p < 0.05$). This greater understanding will provide new insights into how bacterial biofilm-related infections are established in the presence of exudate within wound beds.

Rapid Detection of Central Line Related Biofilms by Infrared Spectroscopy

Amy R. Crisp[1], Mark Butcher[2], Georgina Lynch[3], Gordon Ramage[2], Craig Williams[3], Ihtesham Rehman[1]

[1]University of Central Lancashire, Preston, United Kingdom. [2]Glasgow Caledonian University, Glasgow, United Kingdom. [3]Royal Lancashire Infirmary, Lancaster, United Kingdom

Abstract

Indwelling medical devices like central venous catheters (CVCs) are widely used but their presence can be associated with infection. The rate of central line associated blood stream infection (CLABSI) is 4.5 per 1000 days of catheter use, with a greater risk in low-income countries. Existing diagnostic protocols rely on cultures from peripheral and line blood samples, taking up to 48 hours, with results lacking specific information about whether the infecting bacteria have a biofilm phenotype.

Fourier transform infrared spectroscopy (FTIR) can reveal the exact chemical composition of a live biofilm. Mid-infrared analysis, 4000-400 cm^{-1}, can show the ratio of specific bond vibrations (i.e. C=O, CH_2 and O-P-O) which correlate with groups of proteins, carbohydrates and phospholipids. This non-destructive label-free technique, along with machine learning, promises accelerated detection of biofilms from CVCs.

In our pilot study, *S. epidermidis* was cultured, partially dried and FTIR spectra periodically collected at each developmental stage of the biofilm. In a rapid biofilm-forming model, maturing within 4 hours, indicators of the biofilm were reported from 30 minutes. We postulate that the release of extracellular polymeric substances is related to spectral changes in the amide I (1645 cm^{-1}) region and a shift in the phospholipid peak from 1062 cm^{-1} in planktonic samples, to 1080 cm^{-1}, could be a spectral biomarker for early surface attachment.

In this preliminary work, we show the potential of FTIR for quickly and precisely analysing biofilm development. Further work is ongoing with the aim of directly monitoring CVCs and predicting CLABSIs.

Building and Quantifying Synthetic Biofilm

Kathryn Zimlich[1], Isaak Thornton[1], James Wilking[2], Matthew Fields[1]

[1]Montana State University, Bozeman, USA. [2]Mayo Clinic, Rochester, USA

Abstract

Biofilms are typically defined and studied as self-assembled systems and the underlying mechanisms that drive biofilm formation and behavior are often difficult to systematically manipulate. To understand these complex biomaterials, methods of controlling and manipulating biofilm structure and composition are needed. We have developed a novel laser lithography-based 3D bioprinting method that provides control over the structure and composition of living materials at the submillimeter scale. Hydrogels were 3D printed with the bacterium *Pseudomonas fluorescens* and growth was spatially and temporally quantified throughout the matrix based upon the expression of mCherry fluorescence. Cell populations encapsulated in the printed films developed drastically different biovolume spatial distributions depending on initial cell volume fraction and depth of the printed hydrogels. Through the development and refinement of quantitative confocal microscopy, we observed that the number of active colonies decreased before stabilizing at a steady state, with the final fraction of active colonies remaining consistent across three orders of magnitude initial cell density. We also observe that structural gradients, namely the distribution of active colonies, are strongly influenced by the initial cell density, demonstrating that synthetic biofilm structure can be modulated by adjusting initial system conditions. Overall, the synthetic biofilms developed heterogeneities in growth and active biovolume similar to those observed in natural biofilms, and the results indicate that imposed biofabrication parameters could provide a framework for engineering microbial systems with specified characteristics that could possibly match different stages of a natural biofilm life cycle.

Investigating strategies to manage biofilms formed by the emergent pathogen *Candida auris* in Scotland

Gordon Ramage[1], Alicia Ware[1], Christopher Delaney[2], William Johnston[1], Abhijit Bal[3], John Butcher[1], Ryan Kean[1]

[1]Glasgow Caledonian University, Glasgow, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom. [3]NHS Greater Glasgow & Clyde, Glasgow, United Kingdom

Abstract

Background: *Candida auris* is a problematic fungal pathogen, recently elevated to the Critical Priority group of fungal pathogens by the WHO. Of key concern is its ability to cause outbreaks within intensive and chronic care units, which is facilitated through its environmental persistence. This current study investigates the impact of *Candida auris* in Scotland, and aims to understand the role that biofilms play in surviving disinfection using a semi-dry surface biofilm (SDB) model.

Methods: Prospective surveillance of *Candida auris* is being conducted in Scotland in collaboration with NHS Scotland. Using two phenotypically distinct isolates, *C. auris* were grown in an optimised SDB model across a 12 day period. Disinfection was performed using clinically relevant protocols of sodium hypochlorite (NaOCl) treatment and evaluated using conventional plate counting and live/dead qPCR. RNA-sequencing was performed on NaOCl and untreated SDBs in comparison to planktonic cells.

Results: To date, only 4 confirmed cases have been reported and recorded. Experimentally, isolates were found to grow robust biofilms using the SDB protocol, and could tolerate all treatment parameters, with only 2-3 log10-reductions observed at highest concentrations. Transcriptional profiling identified genes corresponding to ABC transporters, heat shock proteins and metal acquisition were strongly upregulated in SDBs.

Conclusion: We have optimised a SDB protocol in which *C. auris* biofilms can mediate tolerance to adverse conditions such as NaOCl disinfection, suggesting a lifestyle through in which this problematic yeast can environmentally persist and transmit. Mechanistically, the upregulation of small-molecule and haeme-iron transport may support survival.

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

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