SCELSE
Singapore Centre for Environmental Life Sciences Engineering

A MICROBIAL WORLD

10 Years of SCELSE - Advances in Biofilm and Microbiome Research

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Wong Lan Li (SCELSE)

An anammox cell cluster (*Candidatus* Brocadia sinica cells, red) with borders edged with *Chloroflexi* (blue), and anammox S-layer protein coating bacterial cells (details: p. 55).

Date of Publication

June 2022

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CELEBRATING A DECADE OF RESEARCH EXCELLENCE

The Singapore Centre for Environmental Life Sciences Engineering (SCELSE) is a Research Centre of Excellence (RCE) established in 2011. SCELSE is funded by Singapore's National Research Foundation, Singapore Ministry of Education, Nanyang Technological University Singapore (NTU) and National University of Singapore (NUS), and hosted by NTU in partnership with NUS. Its life sciences engineering research is addressing a growing need for understanding microbial biofilms and microbiomes, to develop sustainable solutions and applications for industry, society and the environment, especially in increasingly urbanised settings amid global challenges such as climate change.

Director's message

SCELSE was founded as a Research Centre of Excellence (RCE) on the premise that biofilms – microbial communities and the extracellular matrix in which they are embedded – are integral to all ecosystems, both natural and anthropogenic. Biofilms, the oldest and most widespread life form, are highly complex systems, biologically, chemically and physically. They support and protect the microbial inhabitants, enabling community interactions and cooperation among member populations, while shielding against external stressors. The importance of this mode of life is such that our world would not exist as we know it without their presence and activities.

Microbes are everywhere, and our ability to manipulate and control them provides great advantages and helps to ensure sustainability across multiple domains. These deliverables generate profound, long-term environmental, human health, societal and economic benefits. The processes controlling this microbial life have, until recently, eluded scientists and engineers. However, our ability to harness microbial life has significantly advanced over the last decade, owing to a concerted effort to merge disciplines in environmental life sciences engineering.

Solutions to many environmental challenges articulated and faced by Singapore, and across the globe, are effectively based upon an understanding of biofilm communities and microbiomes, which have the metabolic potential to address diverse opportunities and challenges. SCELSE is therefore exploring, elucidating and managing biofilm- and microbiome-driven processes based on state-of-the-art genomic, molecular and analytical tools, as well as high-resolution imaging of microbial structures and functions. A comprehensive understanding of biofilms and microbiomes in natural and engineered systems is also significantly aided by computational biology for providing meaningful interpretation and analysis of enormous data sets now routinely generated by next-generation technologies.

SCELSE recognises that the ability to harness and control microbial biofilms and microbiomes rests with generating a holistic understanding of a system. This requires a multidisciplinary approach that encompasses a range of fields involving life sciences, chemistry, physics and engineering, among others. SCELSE is uniquely structured to accommodate such an approach, moving beyond a single discipline, academic department or even university, to capitalise on the strengths across the Singapore research landscape. Indeed, SCELSE has established multi-institutional collaborations, nationally and internationally, both academically and industrially. The centre is now recognised as a world-leading biofilm and microbiome research node that is embedded in Singapore's R&D ecosystem and addresses many of the nation's strategic objectives.

The importance of biofilms to all systems

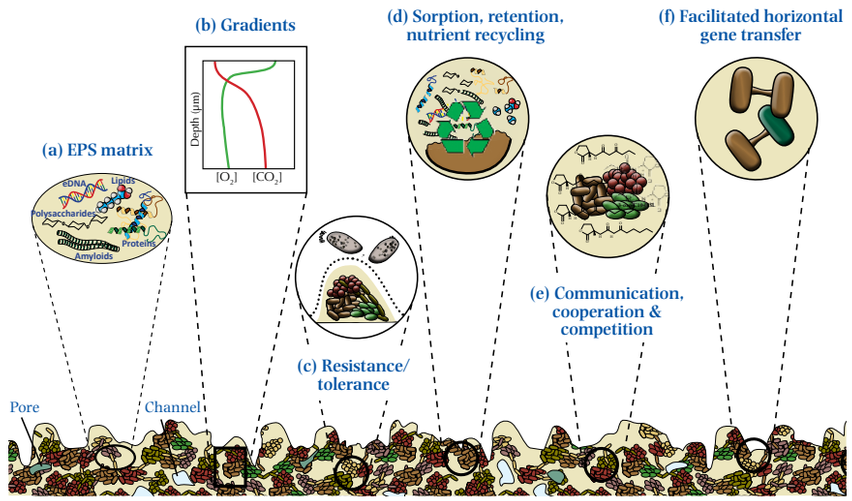
Biofilms and microbiomes – the basis of SCELSE's mission

Microbes are no longer viewed primarily as free-living, single-celled organisms. It is now widely acknowledged that they reside in dynamically structured communities of multiple species embedded in a self-produced polymeric biofilm matrix, collectively known as a biofilm. Similarly, the complex microbial consortia co-inhabiting a particular environment are now understood to act as interactive communities, known as microbiomes. Both biofilms and microbiomes associate with either living or inanimate environments, imparting effects on both, and in turn being influenced by them.

Biofilms have a distinctive architecture, forming fluid-filled channels that transfer nutrients, dissolved gases, chemical signals and waste to and from the embedded cells. Similarly, microbiomes have coevolved with higher organisms to form highly specialised associations.

While biofilms can be made up of microbial cells of the same species, they are far more commonly composed of multi-species consortia that cooperate to the advantage of member bacteria. For example, individual cells coordinate their behaviour to benefit the group, often showing altruism. This requires the production and detection of signal molecules to coordinate gene regulation and synchronise behaviour.

Astonishingly, until recently, the activities driving microbial community dynamics have



Biofilm emergent properties are those not predictable from the study of planktonic cells and are directly associated with the extracellular polymeric matrix (a). They provide architecture and stability, and include: (b) localised chemical, food and waste gradients providing habitat diversity; (c) tolerance and/or resistance to stressors; (d) resource capture by sorption, enzyme retention for digestive capabilities; (e) proximity between microbial cells to facilitate social interactions; and (f) conditions for sharing genetic information.

eluded scientists and environmental engineers. A thorough understanding of biofilm processes is needed if we are to control and harness them to our advantage.

In the urban environment, biofilms and microbiomes house the metabolic capabilities to convert and remediate waste material and pollutants in a sustainable and cost-effective way, and can improve or impair the air we breathe, both indoors and outdoors.

Biofilms and microbiomes are also key for human health and wellbeing. The emerging field of host-microbiome biology is only now revealing the extent to which microbes and biofilms are integral in host survival. Conversely, biofilms are implicated in the vast majority of bacterial infections and in the rising failure of conventional antimicrobial drugs, which were developed for planktic cells rather than cells protected within the biofilm.

Additionally, this dual role of biofilms is applicable in industrial settings. For example, biofilms are employed to produce fine chemicals, and humans have a long history of using microbes in biofilms for food preservation and micro-nutrient enrichment. On the other hand, unwanted microbes spoil food, and cause biofouling and biocorrosion in shipping and marine structures, with significant economic and environmental impact.

In terrestrial and aquatic environments, microbial biofilms and microbiomes recycle nutrients throughout an ecosystem to support the next generation of organisms from all domains of life. Microbes promote healthy functioning as well as cause disease in a disrupted system or weakened host. These traits form the basis of SCELSE's research in agriculture, aquaculture, air and marine systems, where microbial biofilm and microbiome community composition and function can be optimised to benefit the plant or animal host and promote a healthy and productive ecosystem; for example, for habitat resilience, or the efficient production of food.

The prevalence and impact of biofilms and microbiomes across all ecosystems present vast opportunities to manipulate the activities of microbial communities to our advantage, be it controlling unwanted biofilms or harnessing beneficial biofilms, as depicted in SCELSE's mission:

"To discover, control and direct the behaviour of microbial biofilm communities and microbiomes for sustainable environmental, engineering, public health and medical applications."

SCELSE is structured for multidisciplinary inter-domain research

SCELSE has a unique structure and organisation that spans universities, faculties, schools and departments. The expertise needed for SCELSE research is based across both Nanyang Technological University, Singapore (NTU) and the National University of Singapore (NUS). This engagement is further expanded to include multiple government agency and industry stakeholders to capture a substantial and broad collaborative base across Singapore. It also reflects the now rapidly increasing understanding and recognition of biofilm and microbiome biology, as well as its translational significance and potential.

Within SCELSE, the research structure takes a 'top-down/bottom-up' approach to understand biofilm and microbiome biology. The top-down and bottom-up components lie mainly in the Meta-'omics and Microbiomes, and Biofilm Biology clusters, respectively. These are guided by their own research output, together with that of the Environmental Engineering cluster, in an iterative approach involving comprehensive data collection and in-depth mechanistic analyses of microbial biofilm communities and microbiomes. This provides an ever-more detailed understanding of a biologically dynamic system.

From this knowledge, powerful tools such as biomarkers are developed for monitoring and intervention, environmental management and sustainable solutions. Successive rounds of such highly refined analyses also lead to the development of new ecosystem theories, database formation and understanding of systems biology. Research across SCELSE is supported by the Integrative Analysis Unit, which provides a hub for the analysis and interpretation of complex data sets.

SCELSE's research model ensures all facets of biofilm and microbiome research are rigorously investigated. Such comprehensive understanding is essential for converting biofilm research into practical applications. The expertise and capacities based in the research clusters are employed to answer key questions across a set of research themes, which highlight the interdisciplinary nature of the centre's work. These themes deliver biological insights and concepts, and identify commonalities applicable to any ecosystem, as well as generate knowledge for translational outcomes. Specifically, this research involves: biofilm biology and mechanisms; the urban water cycle; marine and air ecosystems; host-microbe interactions; microbiomes and food production; bioprocessing and circular economy; pathogen surveillance; and biofilm drugs, detection and control. Research projects within these themes are explored in Research Themes (p. 18) of this book.

By providing the expertise to support biofilm and microbiome research, SCELSE has become a catalyst for attracting broad and increasingly diverse stakeholder engagement, stemming from elevated appreciation of the value and importance of biofilm and microbiome research. This, in turn, boosts demand for biofilm and microbiome research, and stimulates further support for improved infrastructure and capacities.

SCELSE is positioned to keep abreast of developments across multiple fields, providing mechanisms to identify novel research as it arises, communicating with its interdisciplinary network members, both academic and non-academic, and responding to unforeseen threats and challenges for immediate understanding and solutions. Indeed, this has been aptly demonstrated during the COVID-19 pandemic, when SCELSE rapidly and effectively mobilised its research capacity to enable informed decision-making in Singapore based on high resolution SARS-CoV-2 surveillance in air, on surfaces and in wastewater. These analyses enabled intervention procedures to be immediately and effectively deployed. Importantly, SCELSE's effectiveness in addressing challenges and opportunities reflects the relevance of biofilms and microbiomes in all ecosystems, both natural and engineered.

Professor Staffan Kjelleberg
Centre Director
Singapore Centre for Environmental Life Sciences Engineering

SCELSE's narrative – biofilm and microbiome R&D, a decade and beyond

A research approach built on the commonalities of microbial biofilms and microbiomes across all ecosystems

The economic and societal impacts of biofilm and microbiomes are central to some of the most important global challenges, with potential benefits to be derived across multiple sectors for harnessing and controlling the activities of complex biofilms.

Microbial biofilms share a remarkable commonality in their structure, function and behaviour across diverse ecosystems. Such consistent characteristics provide a means by which microorganisms can be exploited, regardless of the habitat in which they reside, be it a biofilm involved in a chronic wound infection or one involved in treating wastewater. Importantly, the know-how created by SCELSE has the strength, breadth and flexibility to meet detailed research questions as they evolve.

The shift in emphasis in microbiological life sciences from single-species (population) investigations to understanding multi-species (community) biofilms and their functions is presenting distinct and significant opportunities across increasingly diverse systems. Examples illustrating the utility of this focus are innumerable and span every environment. In the context of SCELSE's research, these include the nascent field of holobiont biology (holistic study of host-microbiome interactions and host-system biology) that facilitates our understanding and therefore ability to manage ecosystems and their activities in various settings, from coastal marine settings to agriculture and aquaculture. Similarly, a focus on microbial community biofilms and microbiomes, both complex and simple, is elucidating the role of pathogens and host interactions in infectious disease for expedient novel antibiofilm and antimicrobial drug design, as well as pathogen surveillance, transmission mitigation and targeted health benefits.

Meeting national strategic objectives

Crucially, multidisciplinary biofilm and microbiome research is strategic to Singapore. Insights derived from biofilm and microbiome research benefit many aspects of society and economy. Advantages derived from such R&D range from the provision of safe water and waste removal through used water treatment, to public health and human wellbeing, as well as sustainable and safe food aquaculture and agriculture to help achieve Singapore's '30 by 30' food production target. SCELSE addresses such strategic objectives across natural, medical, public health and engineering domains. This approach is facilitated and strengthened by active engagement with government agencies, clinicians and industry.

Jurong Lake Gardens. SCELSE's original flagship research programme focused on aspects of the urban water cycle, including microbial bioremediation of waterways.
Image credit: Sharon Longford, SCELSE

Since its inception in 2011, SCELSE has evolved its narrative to reflect changes in local and international landscapes, creating new opportunities in concert with rapid technological developments. The centre's structure and inherent flexibility have accommodated these opportunities and enabled it to deliver on changing strategic needs.

In particular, in its early years, SCELSE focused on targeted core research themes, such as the urban water cycle. As the utility of biofilms and microbiomes has become more widely recognised through the availability of technological advances, the centre's research has expanded to accommodate a wider range of opportunities where biofilms and microbiomes are increasingly recognised as key. These include both well-established fields and mainstream areas (e.g., medicine, public health, maritime, urban and environmental sustainability, food science), as well as new fields arising from the emergence of biofilm and microbiome knowledge (e.g., circular economy, vertical agri- and aquaculture farming, host microbiomes and human wellbeing, conserving natural environments such as coastal marine habitats, GenomeAsia 100K and precision medicine). Moreover, the majority of these fields and areas are impacted upon by climate change. The microbial communities are integral to maintaining a well-balanced environment or host organism can be disrupted by the effects of climate change, with potentially detrimental consequences, including impairing ecosystem resilience, the emergence of new pathogens and increased prevalence of disease.

Alternatively, the solutions to mitigating climate change also reside in the activities and potential of microbial communities to promote carbon capture.

Biofilm and microbiome research is integrating Singapore R&D beyond the university system, incorporating industries, government agencies and other research organisations. This united platform is increasingly relevant to Singapore's strategic goals and capacity to deliver future value. Broader integration across Singapore is also facilitated by the Singapore National Biofilm Consortium (SNBC), an NRF technology consortium hosted at SCELSE and capitalising on SCELSE's translational capacity and multidisciplinary research on microbial biofilm biology and ecology and microbiology. SNBC serves as a coordinating platform at the convergence of health, engineering, technology and science, that integrates innovation and business across diverse industries throughout Singapore.

The excellence and world-class capacities established by SCELSE have positioned Singapore as a world leader in biofilm research, boosting the nation's ability to maintain relevance and international standing.

An increased drive for evidence-based policy and scientific solutions

SCELSE has built a solid foundation on which to deliver future value to Singapore. The centre's scientific imperative is to drive national, regional and global biofilm and microbiome research. SCELSE's societal impacts are founded on enhancing natural and urban environmental sustainability, improving public health, and creating new technologies and products, through its comprehensive scientific, experimental and technological platform of environmental life sciences engineering.

Understanding biofilms and microbiomes is key to providing solutions for urban and environmental sustainability.
Image credit: Sharon Longford, SCELSE

Singapore is presently facing significant change, affected by internal and external factors in broad domains. For example, there is a growing interest in supporting measures that promote public wellbeing and healthy ageing as a critical adjunct to medical interventions. Further, regional political and environmental uncertainty, accentuated by climate change, is driving a sharp focus on the need to internalise both food and water supplies. Food and water resources are increasingly susceptible to the effects of climate change, as are the emergence and management of infectious diseases. Given their metabolic potential and ability to cycle all elements, microbial communities and their properties are central to addressing such challenges. SCELSE is well placed to drive this R&D with its collective expertise in the understanding, analysis and control of microbial communities in ecologically relevant systems.

Multiple stakeholders are integral to SCELSE's success

Successful biofilm and microbiome research will continue to incorporate natural, medical, engineering and social sciences. The nexus of key infrastructure and the establishment of knowledge within SCELSE is combined with close governmental and industrial engagement to serve the changing needs of the centre's current and future stakeholders.

SCELSE research greatly benefits from the centre's model, where the RCE is hosted by both NTU and NUS, and synergises specific expertise and capacities available at each. This research model is further supported by national and international collaborations. Within Singapore, SCELSE has established an integrative network of partner organisations for complementary expertise and capacities, and effective R&D. Internationally, SCELSE has formal agreements and interactions with prominent biofilm and microbiome centres in the UK, other parts of Europe and the USA, as well as collaborative arrangements with research teams across Asia and Australia.

The close research ties SCELSE has developed within and beyond Singapore for environmental, industrial, public health and medical research extend benefits to multiple stakeholders, as well as the centre's host universities.

An integrated approach to sustainability for Singapore, the region and beyond

Biofilms and microbiomes have the potential to provide solutions to sustainability challenges. SCELSE is therefore ideally placed to deliver these much-needed outcomes for Singapore. SCELSE's host universities have both identified sustainability as a key focus in their long-term planning. Singapore continues to lead and coordinate efforts, including many major planning initiatives, with multiple platforms for climate change mitigation and adaptation, and sustainable urban living. Further, global governance and international cooperation have underscored these efforts, in particular with respect to the UN Sustainable Development Goals (SDGs).

SCELSE is an integral and essential organisation driving innovative sustainability R&D, including microbial-based solutions for carbon capture, carbon-neutral and cost-effective waste treatment and resource recovery, circular economy–based food production and maximising agricultural output by optimising host-associated microbiomes, and public health.

Sustainability cannot be achieved by research, science, engineering or technology alone, but requires multidisciplinary collaboration and an inclusive, equitable and innovative approach, such as that delivered by SCELSE and R&D networks.

The comprehensive microbial life sciences platform SCELSE delivers for Singapore provides a mechanism for promoting regional leadership, and further fosters collaborations abroad. The centre is driving the development and proliferation of local talent as future sustainability leaders who can, in turn, connect and draw upon relevant expertise and capacity across Singapore.

National and International Networks

SCELSE has established an extensive collaborative network both nationally and internationally to provide the skills and technologies necessary to achieve effective research outcomes, both fundamental and translational.

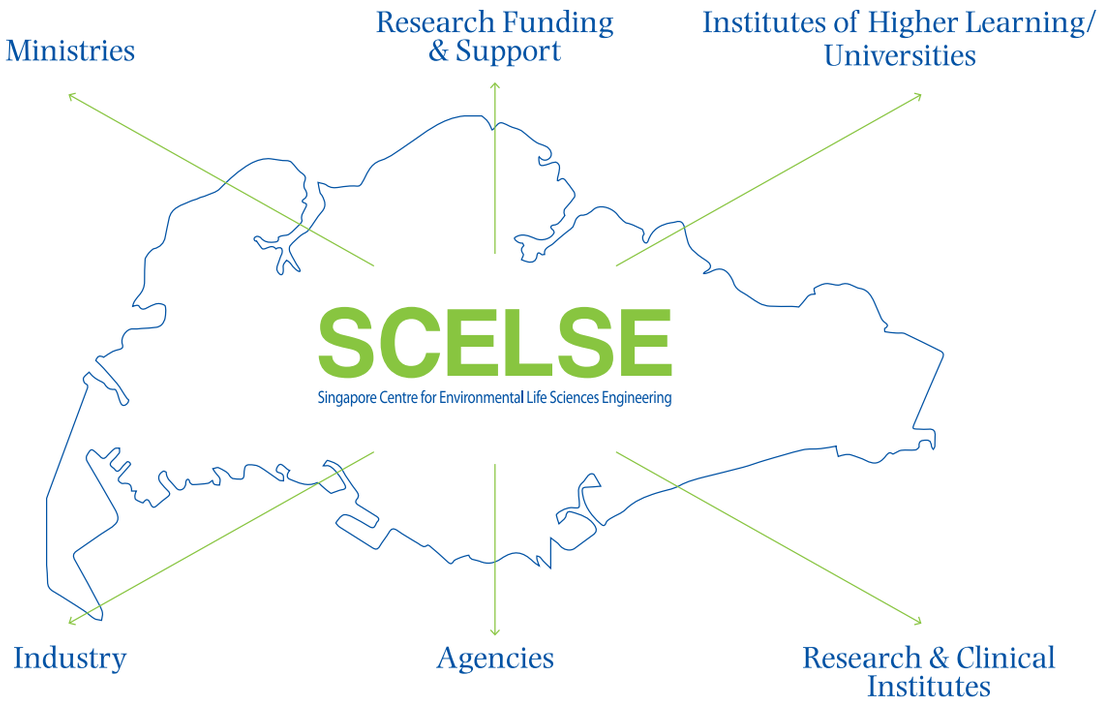
National networks

SCELSE has coupled the innovative technology and expertise available within its host universities with practical applications offered by agency and industry partners to create an integrative multidisciplinary centre at the forefront of biofilm- and microbiome-based research.

Within the Nanyang Technological University, Singapore (NTU), SCELSE faculty members and students have been affiliated with the following schools across campus: Materials Science and Engineering; Civil and Environmental Engineering; Mechanical and Aerospace Engineering; Chemical and Biomedical Engineering; Biological Sciences; Physical and Mathematical Sciences; Asian School of the Environment; and the Lee Kong Chian School of Medicine. Within these schools, SCELSE academics are heavily involved in both research and teaching, and bring together multiple perspectives.

SCELSE is a core component of the NTU Integrated Medical, Biological and Environmental Life Sciences (NIMBELS) cluster, which integrates and coordinates life sciences research and capacities at NTU.

Singapore Research Landscape



NIMBELS members collaborate to address global challenges in biomedical and life sciences, and utilise and further develop joint state-of-the-art technology platforms.

SCELSE is a principal member of NTU's Sustainability Office, along with the Nanyang Environment and Water Research Institute, Energy Research Institute @ NTU, and the Earth Observatory of Singapore, which facilitates, strengthens and integrates campus-wide sustainability initiatives. SCELSE participates in each of the research domains covered by the Sustainability Office: energy, food, water, waste, environment and earth; for which biofilm- and microbiome-based R&D can provide highly effective solutions.

At the National University of Singapore (NUS), SCELSE capitalises on its strengths in life sciences, particularly systems biology, chemical biology, metabolomics and public health. These collaborations are based both on joint research and shared equipment infrastructure. NUS members of SCELSE are affiliated with the departments of Biological Sciences; Chemistry; Microbiology and Immunology; Civil and Environmental Engineering; the Yong Loo Lin School of Medicine; and the Saw Swee Hock School of Public Health. Further, SCELSE is integrated across several NUS institutes, including the Life Sciences Institute, the Centre for Bioimaging Sciences, NUS Environmental Research Institute, Tropical Marine Science Institute and Lee Kong Chian Natural History Museum.

To take full advantage of the state-of-the-art technologies available at SCELSE and create an effective platform with notable added value, the centre has promoted a collaborative atmosphere of R&D partnerships, and is continuously expanding its research interactions. SCELSE has extensive reach across Singapore's local research organisations, clinics and hospitals; national agencies; and industry collaborations. The centre's engagement with local agencies and industry members is supported by the Singapore National Biofilm Consortium (SNBC). The need for biofilm and microbiome R&D is evidenced by the significant uptake the SNBC has enjoyed among stakeholders. SNBC facilitates effective and constructive connections among biofilm researchers and industry/agency members, providing the former with access to translational opportunities and the latter with science-based solutions to industry-relevant challenges. SNBC currently has 25 industry and seven academic-based members, and continues to evolve as an integral component of the R&D landscape and driver of knowledge and innovation aligned with Singapore's strategic objectives.

International networks

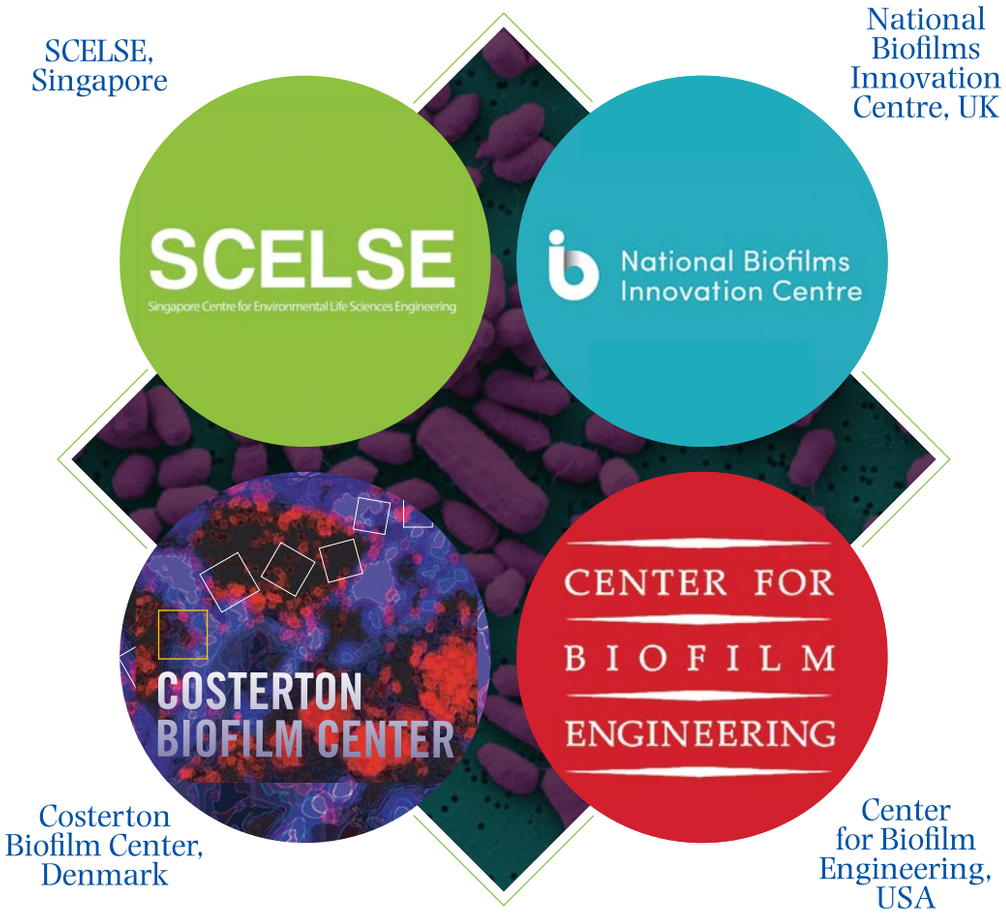
SCELSE has well-established international collaborations across its many research themes. As with local research partnerships, SCELSE's international collaborative engagements emerge from both bottom-up (PI initiated) and top-down interactions.

SCELSE's PI-driven participation in international research engagements encompass universities, research centres and institutes worldwide, acting in both collaborative and advisory capacities. The centre has forged strong R&D ties with more than 50 research groups globally, including alliances with industry partners, from start-up to MNC levels.

SCELSE is one of an elite group of biofilm and microbiome research centres worldwide, and the only in Asia, with the scale, knowledge and advanced capacity to advise the biofilm community and identify evolving issues in science, innovation and policy for the biofilm field. In a top-down manner, SCELSE has formally partnered with many research centres – including the National Biofilms Innovation Centre (NBIC; UK), the Costerton Biofilm Center (CBC; Denmark), and the Center for Biofilm Engineering (CBE; USA) – to leverage support for international initiatives and provide perspectives that capitalise on collective expertise.

At the international scale, this confers the advantages of accessing state-of-the-art technologies and expertise, while providing a voice for Singapore and the region in international dialogue on emerging challenges and opportunities. For example, the biofilm centre network forms the core of an international task group to develop standards for biofilm control products in health care settings, industrial systems and the built environment. Such engagement further strengthens SCELSE's, and hence Singapore's, reputation and recognition for innovative and strategic research.

Further, SCELSE, SNBC, NBIC and the Indian Biofilm Society have created a network for collaboration and knowledge transfer. SCELSE's involvement in the international arena also extends to establishing the *npj Biofilms and Microbiomes* journal, a leading publication in its field, in partnership



with Nature Partner Journals and NTU. The open-access journal provides a platform for communicating scientific advances in understanding the mechanisms governing biofilm and microbiome structure and function, and their impacts in natural and engineered settings.

SCELSE is a coordinating node for international biofilm colloquia held in Singapore and abroad. Examples of these include co-organisation of: the International Water Association (IWA) Membranes and Biofouling Workshop (Singapore, 2011); 2nd International Water Research Conference (Singapore, 2013); Developmental Biology of Microbial Biofilms (Singapore, 2013); Nature Conference: Environmental Microbial Biofilms and Human Microbiomes: Drivers of Future Sustainability (Singapore, 2017); and the Nature Conference: Biofilms and Microbiomes – Global Impact on Public and Ecosystem Health (Singapore, 2022).

>600
International Conference Presentations

>900
Peer-Reviewed Publications
(66% in top ranked journals)

>40
Industry Partners

14
Technology Disclosures

48
Current PhD Students

50
PhD Graduates

Collaborators From
>60
International Universities/Institutes

Co-founded a Leading International Journal
(*npj Biofilms and Microbiomes*)

>130
Research Fellows Trained

47
Research Fellows Currently at SCELSE

87
Now in Academic or Industry Positions

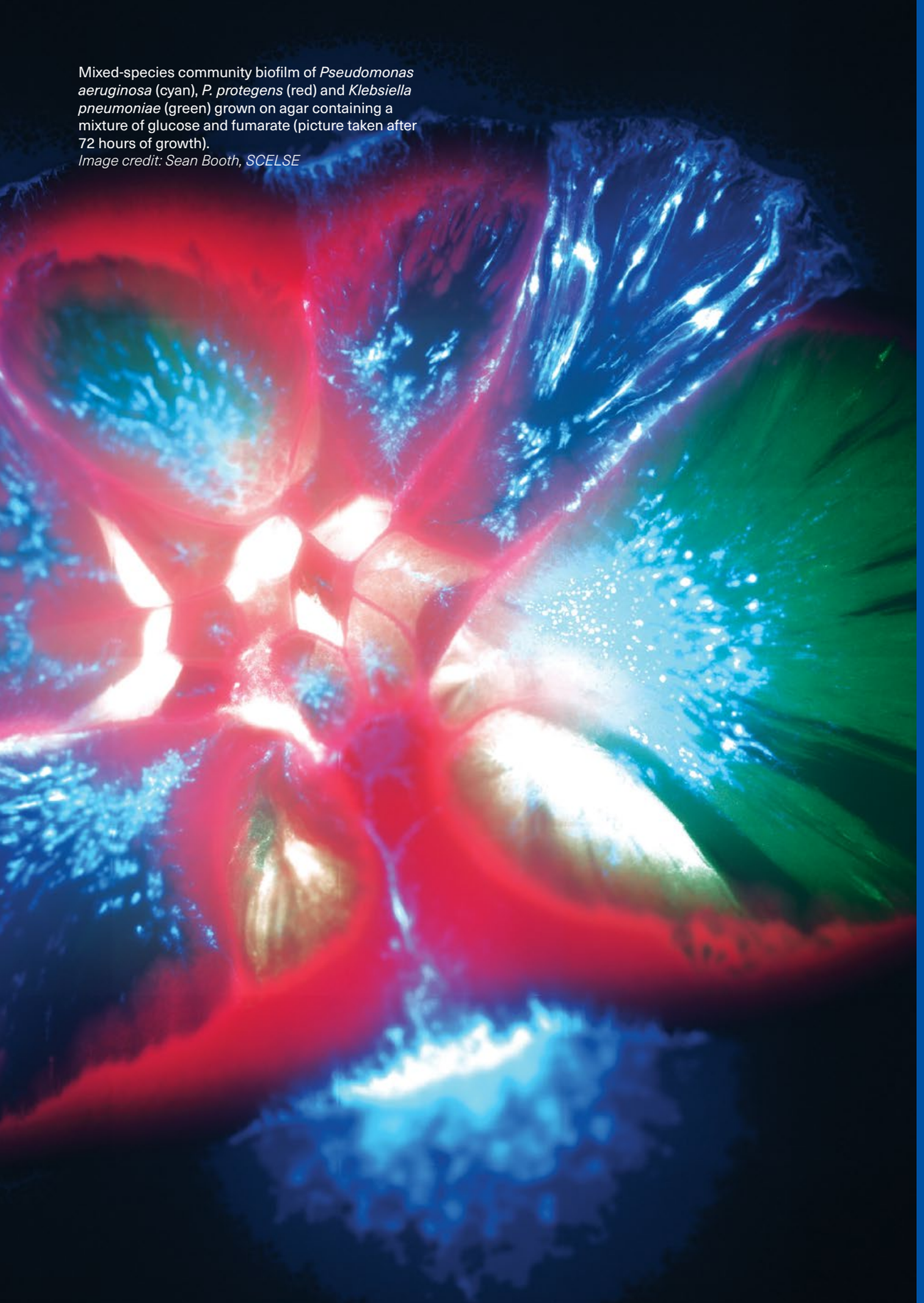
Activated sludge showing members of the chloroflexi (magenta) and the eubacteria (blue).
Image credit: Wong Lan Li, SCELSE

RESEARCH THEMES

SCELSE's research themes highlight the interdisciplinary nature of the centre's activities, much of which is integrated across multiple research clusters. These themes deliver biological insights and concepts, and identify commonalities applicable to any ecosystem, as well as generate specific knowledge for translational outcomes.

The projects presented under the banner of Research Themes reflect the scope of SCELSE's research activities. However, it is not an exhaustive account, owing to the format of the book.

A full list of SCELSE's publications is available on the centre's website (www.scelse.sg) and also via the QR code:



Mixed-species community biofilm of *Pseudomonas aeruginosa* (cyan), *P. protegens* (red) and *Klebsiella pneumoniae* (green) grown on agar containing a mixture of glucose and fumarate (picture taken after 72 hours of growth).
Image credit: Sean Booth, SCELSE

RESEARCH THEMES

Biofilm biology and mechanisms

Many aspects of biofilm research are severely limited by our inability to employ experimentally tractable and reproducible mixed-species biofilm communities as model systems. The various existing population-based models have been invaluable in uncovering much we now know about biofilms and their functions, and are still an indispensable part of biofilm research. However, these are not very representative of biofilms in natural systems and observations of limited mixed-species experimental communities to date have revealed unanticipated, emergent properties as a function of the stepwise increase in species diversity. To further progress this knowledge, SCELSE employs reproducible mixed-species biofilm communities as model systems to better understand the functions performed by such communities in their natural environments.

Moreover, the matrix is a relatively understudied component of the biofilm, classically limited to chemical extraction of extracellular polymeric substances (EPS). Understanding biofilms in their entirety requires a sound knowledge of both the biological cells and the extracellular matrix environment. Given that the matrix properties are altered with different EPS components, the matrix composition is significant for the physiology of enmeshed cells. Unravelling these interrelationships requires a strong interdisciplinary platform.

SCELSE utilises several biophysical approaches to understand and quantify biofilm structure/function by means not possible using classical microbiological approaches. Research advances involve the role of structural molecules, such as filamentous phage, polysaccharides and eDNA, as well as soluble signalling molecules, in biofilm formation, structure and function.

UNDERSTANDING THE MECHANISMS OF BIOFILM AGGREGATION FORMATION

Harikrishnan A. S. Nair[1], Sujatha Subramoni[1], Poh Wee Han[1], Nabilah Taqiah Binte Hasnuddin[1], Martin Tay[1], Michael Givskov[1,2], Tim Tolker-Nielsen[2], Staffan Kjelleberg[1,3], Diane McDougald[1,4], Scott A. Rice[1,5]

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Introduction

Mature biofilms disperse in response to environmental cues such as nutrient limitation, oxygen limitation, or in response to chemicals such as nitric oxide. This process is thought to be mediated by altered levels of the signalling molecule cyclic di-GMP (c-di-GMP) in bacterial cells, with the dispersed cells having lower amounts. However, biofilm dispersion under these conditions is always incomplete, with up to 40% of *Pseudomonas aeruginosa* biofilm biomass resistant to starvation-induced dispersal. Resistance to dispersion is thought to be due to physiological heterogeneity among cells within the biofilm. Since biofilms are more resistant to removal by antibacterials, their formation is undesired in several situations. Hence, mechanisms that support biofilm formation and maintenance are of particular interest.

Surface-attached biofilms differ from biofilm aggregates that are suspended in aqueous media and form in the absence of a substratum. Examples of such aggregates are samples isolated from the sputum of cystic fibrosis patients. The formation of *P. aeruginosa* aggregates in response to detergent stress involves the *sia* operon, *psl* operon and c-di-GMP signalling. Additionally, this process is energy-dependent, and aggregation is abolished when the cells are deprived of energy under various conditions, leading to the hypothesis that aggregation may be controlled by nutrient availability or starvation. As such, it is of interest to investigate the mechanism of aggregate formation, which can also define targets for therapies in human infections.

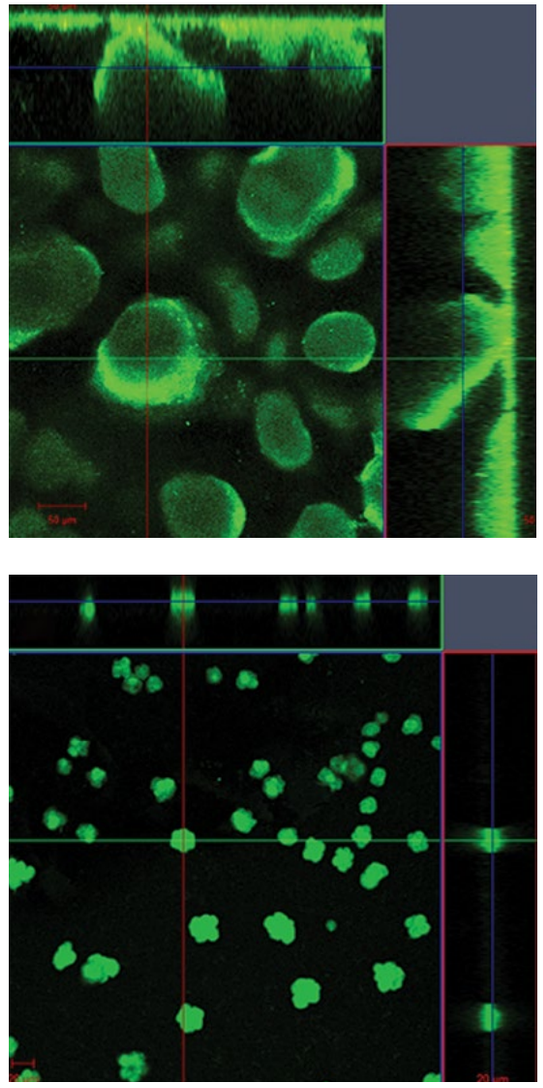

Figure 1:
Orthogonal view of *P. aeruginosa* microcolonies before and after starvation. (a) Biofilm before starvation, (b) colonies after 72 hours of starvation. *P. aeruginosa* contained the c-di-GMP responsive reporter construct as described. Orthogonal view showing top panel *x-z* plane, right *y-z* and middle corresponding *x-y* plane. Magnification 20x. Scale bar: 50 µm (a) or 20 µm (b).

Main findings

Effect of nutrient limitation on biofilm formation and dispersal

In this study, prolonged starvation or cycles of starvation-induced partial resistance to dispersion in *P. aeruginosa* biofilms. Furthermore, the resistant component contained a higher proportion of mutants that have enhanced biofilm capabilities than wild-type cells. To understand this process better, the growth features of *P. aeruginosa* cells were investigated using biofilm effluents with susceptible cells and resistant residual biofilms. Small colony variants (SCV) and wild-type *P. aeruginosa* were present in all samples tested: unstarved control biofilms, starved biofilms and their effluents. However, only biofilms resistant to dispersion after prolonged starvation gave rise to rugose small colony variant (RSCV) upon culturing, which was absent in the effluents of these biofilms. This suggested that this variant phenotype is associated with resistance to starvation-induced dispersion. Genome sequencing of the RSCV showed single nucleotide mutations in several genes associated with biofilm formation, such as *wspF*, *pilT*, *fha1* and *aguR*. These genes are important for resistance to dispersal as they render them susceptible to starvation-induced dispersal.

Dispersion of biofilms by several agents are c-di-GMP-mediated. Further, *wspF* was identified as an inhibitor of c-di-GMP formation. This suggested a role for c-di-GMP in starvation-induced dispersal as well. To test this hypothesis, a c-di-GMP-specific reporter fused to a green fluorescent protein (GFP) was used to monitor cellular c-di-GMP levels. After starvation, significantly high levels of brightness were observed in the remaining microcolonies. Furthermore, RSCVs isolated from biofilms that were resistant to starvation-induced dispersal also showed high levels of

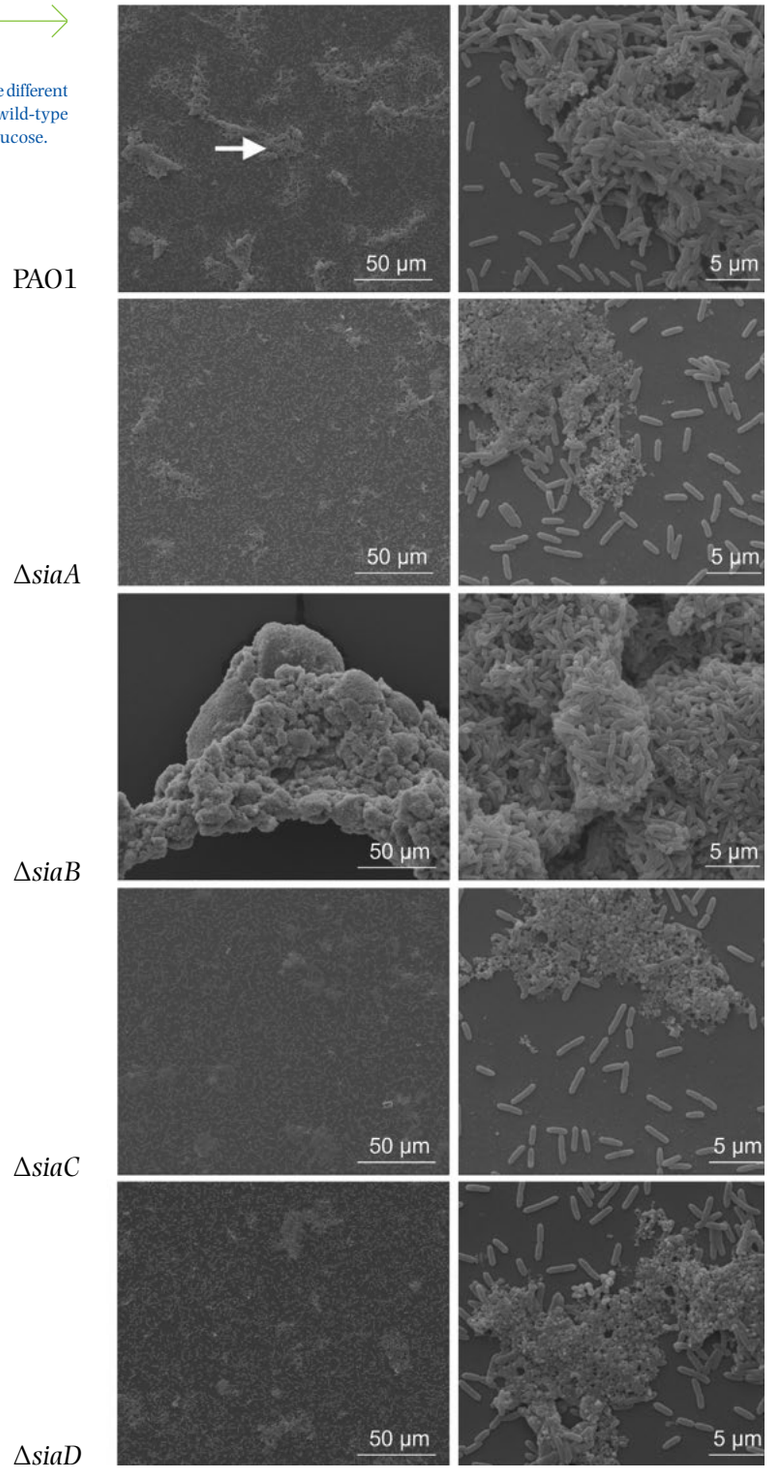

Figure 2:
Scanning electron micrograph displaying the different aggregation phenotypes of *P. aeruginosa* wild-type and mutant strains during growth with glucose.

c-di-GMP, while other variants and wild-type colonies did not. These observations have implications at an applied level. Starvation conditions are likely to give rise to variants that resist dispersion, and repeated cycles of starvation are likely to enhance this process.

Molecular mechanisms of biofilm aggregation formation

These studies demonstrate that SiaABC represent a signal response pathway that functions through a partner switch mechanism to regulate biofilm aggregate formation in *P. aeruginosa*. SiaA and SiaB are, respectively, a phosphatase and kinase acting on their cognate substrate SiaC. In turn, SiaC can interact and regulate the activity of SiaD, a diguanylate cyclase to regulate intracellular c-di-GMP levels. Carbon availability was one of the key signals integrated by the SiaABCD system, and biofilms of the Δ*siaB* mutant were found to be defective in dispersal upon depletion of carbon sources such as ethanol. This finding was interesting given that some of the carbon substrates evaluated have been reported to influence microbial colonisation of epithelial cells or the persistence of microbes during infection.

Crystallographic studies of SiaA-PP2C (phosphatase domain) and SiaC were carried out. Based on structural data, SiaC presents as a dimer. Dynamic modelling of SiaA with SiaC suggested that SiaA interacts strongly with phosphorylated SiaC and dissociates rapidly upon dephosphorylation of SiaC. Further, the known phosphatase inhibitor fumonisin inhibits SiaA mediated phosphatase activity *in vitro*.

This study also found that aggregation is regulated by the SiaABCD operon and that the Δ*siaB* showed resistance to starvation-induced dispersal. Additionally, SiaA was identified as a target for control of biofilm aggregate formation through inhibition of its phosphatase activity. It would be of interest to determine how *sia* mutants, which showed similar defects in starvation-induced dispersal, may differ from the starvation-adapted mutants identified above. Such knowledge would improve the identification of targets and fine-tune strategies for biofilm control.

Future directions

The genetic heterogeneity of *P. aeruginosa* biofilms plays an important role in maintaining the non-dispersing population after nutrient starvation. Further work in this direction involves identifying mechanisms that generate and select for these mutations during biofilm starvation. More studies on the antibiotic resistance of these variants would be important from a clinical perspective. Starvation-adapted biofilms provide a good model system to study antimicrobial resistance.

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Pf4 PHAGE SUPERINFECTIVITY AND CONTROL OF HOST GENE EXPRESSION

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Introduction

Pseudomonas aeruginosa is a pathogen that causes much of the morbidity and mortality in chronic cystic fibrosis airway infections, and also found in the majority of human *Pseudomonas* wound infections. A *P. aeruginosa* bacteriophage, called the Pf4 phage, plays an essential role in biofilm biology, eliciting a dispersal response, the formation of genetic variants during dispersal, cell death within biofilms and virulence. The ability of *P. aeruginosa* to establish chronic infections relies, in part, upon its capacity to form biofilms. The maturation of a biofilm involves five stages, and a key developmental process is the hollowing of microcolonies and dispersal. In a mutant defective in type IV pili and flagella, this cell death was not observed, leading to the discovery of the role of filamentous phage activity in cell death during microcolony hollowing. These effects correlate with the Pf4 filamentous phage's acquisition of a superinfective (SI) phenotype (a variant phage that form plaques on otherwise lysogenic bacterial host). SI phages produced by *P. aeruginosa* have mutations in a gene (located in the intergenic region of PA0716 and PA0717) that has homology to an immunity protein of the P2 and lambda phage, called the repressor C protein. This protein, Pf4r, is also responsible for *Pseudomonas* immunity to Pf4 infection. However, the specific mechanism by which Pf4r controls superinfection was unknown.

Main findings

The molecular mechanism of superinfection was investigated, as well as how the Pf4 phage controls host gene expression to modulate host behaviour. Pf4 exists as a prophage in *P. aeruginosa* PAO1 and encodes a homolog of the P2 phage repressor C protein (Pf4r). A combination of molecular techniques and gene expression analyses was used to identify a critical site in Pf4r where a mutation, 788799A>G (Ser4Pro), causes Pf4r to lose its function as the immunity factor against reinfection by Pf4. X-ray crystal structure analysis shows that Pf4r forms symmetric homodimers homologous to the *E. coli* bacteriophage P2 RepC protein (Figure 1a). A mutation, Pf4r*, associated with the superinfective Pf4r variant, found at the dimer interface, suggests dimer formation may be disrupted, which derepresses phage replication (Figure 1b). This is supported by multi angle light scattering (MALS) analysis where the Pf4r* protein only forms monomers. The loss of dimerisation also explains the loss of Pf4r's immunity function. Phenotypic assays showed that Pf4r increased LasB activity (a virulence factor) and was also associated with a slight increase in the percentage of morphotypic variants. Molecular and transcriptomic analyses suggest that Pf4r also likely functions as a transcriptional regulator for other host genes, including those that contribute to *Pseudomonas* virulence. Collectively, these data suggest the mechanism by which filamentous phages play such an important role in *P. aeruginosa* biofilm development.

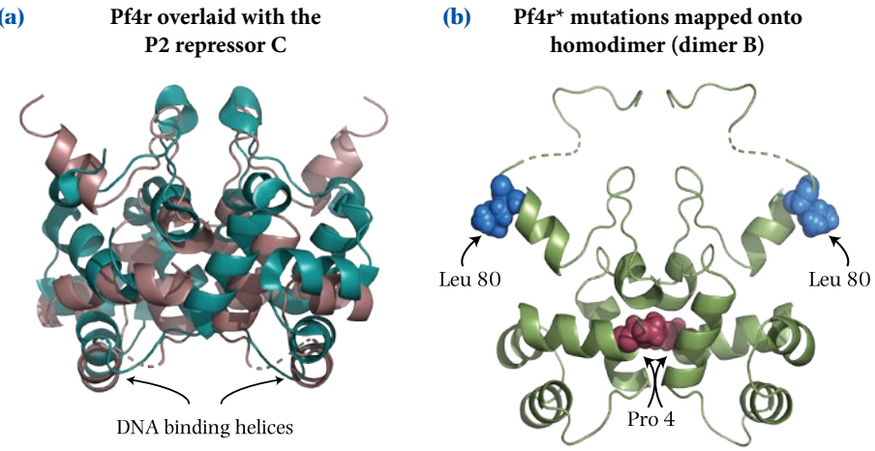

Figure 1: (a) Resolved structure of the Pf4r protein. The Pf4 filamentous phage Pf4r protein overlayed with its P2 phage homolog. (b) Space-filling spheres for the two mutations in Pf4r*, and the serine to proline mutation (red spheres) located at the dimerisation interface causes a loss of function of Pf4r as the immunity factor.

Future directions

The data in this work suggests that Pf4r is not only important in the control of superinfection but may also be an important regulator of virulence factor-related genes. It is of interest to further elucidate the molecular mechanisms of Pf4r in affecting the expression of these genes, either indirectly by interacting with other transcriptional regulators or directly by binding to the promoters of these genes. Filamentous phages are pervasive in bacteria and the work here suggests that filamentous phages in other host species are also important in regulating biofilm and virulence responses of their host.

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UNDERSTANDING A MIXED-SPECIES BIOFILM COMMUNITY

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Introduction

Bacterial biofilms are composed of aggregates or surface-attached bacteria encased in extracellular polymers. Although mechanisms of the development and impact of biofilms on bacterial properties have been studied extensively, much of these studies focused on monospecies biofilms of a few model organisms. In nature, however, complex biofilm communities containing a variety of bacterial species are more common. The assembly, structure and function of such mixed species communities are governed by competitive and co-operative interactions among members that constitute the community, and such interactions remain largely uncharacterised for complex biofilms.

Studies on such multispecies biofilms are difficult because of the complexities in identifying contributions of particular members to the whole community and in genetically tracking specific bacterial species in communities. It is useful to have defined simple communities to carry out such studies that can then be tested and generalised to more complex communities. Towards this end an experimentally robust three-species biofilm community consisting of *Pseudomonas aeruginosa* (PAO1), *Pseudomonas protegens* (Pf-5) and *Klebsiella pneumoniae* (KP1) has been established and investigated. These three species are found in the gut of the domestic silk moth *Bombyx mori* and in other environments with co-occurring bacteria. This community is highly reproducible in structure and viable cell counts, and well-suited for studying community dynamics. Compared to monospecies biofilms, this community, which is composed of 60–75% *K. pneumoniae*, 10–15% *P. protegens* and 1–5% *P. aeruginosa*, generated higher biomass and resistance to stress conditions such as detergents and antibiotics, and accumulated reduced genetic variation. These phenotypes were apparent only when they grew as spatially organised biofilms and not as planktonic cultures, suggesting emergent community behaviour.

Main findings

Omics-based analyses of communities

A combination of transcriptomics, metabolomics and genome-wide mutagenesis was used to understand genes and pathways involved in adaptations of the three species in the community. Transcriptomic analyses were conducted for the developmental stages of the three-species community. Gene expression of the three species differed significantly between single and mixed-species biofilms, but there was no difference in gene expression between different days of biofilm growth, suggesting an absence of significant developmental stages. However, metabolism remodelling was observed in two of three species in the community. *K. pneumoniae* in the three-species community had increased expression of glycolysis and pentose phosphate pathways. Genes of these pathways were suppressed in *P. protegens* in the three-species biofilm, while glyoxalate cycle genes showed increased levels. Overall, the data suggested that *P. protegens* is likely to rely on TCA cycle intermediates for energy.

These observations show a reorganisation of the metabolic pathways when these bacteria are in the mixed-species community.

Each species of bacteria communicates, competes and cooperates with other members, specialising in their environmental niche and enhancing biofilm formation, to form the final stable mixed-species biofilm. The contribution of *P. protegens* to this process was characterised using transposon directed insertion sequencing (TraDIS), a genome-wide approach. Here, the genes essential for interspecies interactions and mixed-species biofilm formation were investigated. TraDIS involves generating a *P. protegens* transposon mutant library and testing them for essentiality in a mixed-species biofilm model.

In the mixed-species biofilms, 96 genes were significantly enriched or depleted, relative to the single species *P. protegens* biofilm. The enrichment of a mutant gene in the community suggests that a functional gene might impair the ability of Pf-5 to compete during biofilm formation. Conversely, when mutants of a gene are observed below expected levels, the gene function is suggested to contribute to

Function	Number of genes	TraDIS analysis
Flagellar assembly	33	Enrichment
Chemotaxis	23	
Two-component system	18	
Oxidative phosphorylation	22	Depletion

Table 1: Breakdown of *Pseudomonas protegens* genes in mixed-species biofilm.

maintenance of Pf-5 in the biofilm. The analysis identified several genes involved in flagellar assembly, bacterial chemotaxis, and two-component systems as not essential for both single and mixed-species biofilms (Table 1). This observation supports the view that motility reduces considerably as bacteria aggregate to form mature biofilms.

Twenty-two genes involved in oxidative phosphorylation were found to be essential for mixed-species biofilm formation, suggesting that oxygen-dependent energy production is important in the community biofilm. Oxidative stress, generated as a by-product, may also contribute to cell death, biofilm development and competition. The approach provides insight into the regulation and signalling driven by *P. protegens*, not easily uncovered using conventional methods, into the complex biological interactions occuring during biofilm development.

Testing physiological predictions from global analyses

The transcriptomics analysis showed that bacteria are likely to cross-feed metabolites, leading to greater resource utilisation as mixed-species consortia. *K. pneumoniae* is the primary consumer of glucose to generate TCA cycle intermediates, which are then used by pseudomonads. Planktonic growth experiments were conducted to further understand growth requirements and metabolite sharing. While *K. pneumoniae* grew poorly in malate, citrate or succinate, and not at all in acetate, the pseudomonads grew well in citrate, malate or acetate. However, in mixed-species biofilms where citrate, succinate or acetate were the sole carbon source, *K. pneumoniae* still dominated the community composition. This suggests that *K. pneumoniae* used metabolic by-products of pseudomonads as an energy source.

The three-species biofilm showed emergent properties of stress resistance. Although *P. protegens* monospecies biofilm was sensitive to the surfactant SDS, the mixed-species biofilm composition was unaffected when exposed to SDS. A transcriptomic comparison of *P. aeruginosa* as single and mixed-species biofilms exposed to SDS showed that the most highly expressed gene was *sdsA1*, a gene coding for an alkyl sulfatase, involved in SDS degradation. Deletion of *sdsA1* rendered *P. aeruginosa* biofilms sensitive to SDS, suggesting a functional role for this gene under these conditions. Moreover, mixed-species biofilms containing *sdsA1* mutants instead of wild-type *P. aeruginosa* were also more sensitive to SDS. Hence, SdsA1, a secreted protein, is important for the cross protection observed in the mixed-species biofilm community.

Identification of genes involved in mixed-species biofilm growth

P. aeruginosa exopolysaccharides contributes to mixed-species biofilm development and stress resistance. Loss of Psl polysaccharide reduced the proportion of *P. aeruginosa* in the mixed-species community, while overproduction of alginate resulted in mixed-species biofilms having a higher proportion of *P. aeruginosa*. Importantly, mixed-species biofilms that included the *psl* mutant of *P. aeruginosa* showed a significant loss of biomass upon SDS treatment. Moreover, biofilm associated traits such as *P. aeruginosa* type IV pilus and *K. pneumoniae* matrix production influenced interspecies interactions and spatial distribution in colony biofilms.

Other experiments have also shown that although *P. aeruginosa* is present at low proportions in the mixed-species community, it is pivotal for community function. The *N*-acyl homoserine lactone-dependent quorum sensing (QS) system

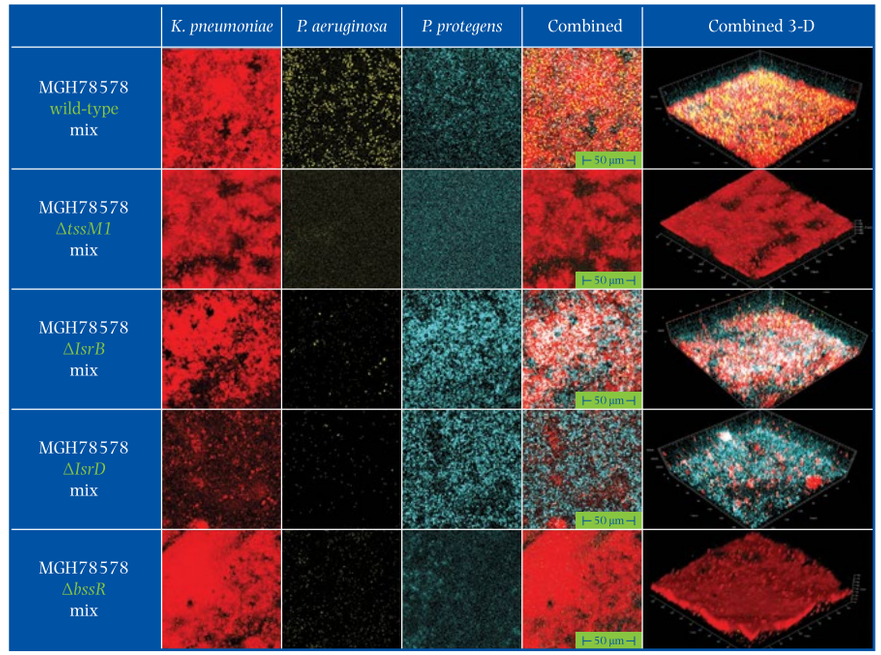

Figure 1:
Mixed-species continuous-culture biofilms containing *K. pneumoniae* mutants.Representative CLSM images of biofilms consisting of *P. aeruginosa* (yellow), *P. protegens* (cyan) and *K. pneumoniae* (red) grown in M9CasGlucose. Magnification 20x. Scale bar represents 50 µm.

was used to determine the *P. aeruginosa* factors that regulate community structure and function. This process regulates community-specific functions such as biofilm formation and secretion of effectors such as rhamnolipids, siderophores and proteases. When QS mutants of *P. aeruginosa* substituted the wild-type strain in mixed-species biofilms, the proportion of *K. pneumoniae* and *P. protegens* was significantly altered. The relative proportion of the QS mutant remained unchanged in the mixed species, compared to the wild-type. This change in proportion was largely due to competitive interaction between these two species, as demonstrated by cultivating dual-species biofilms consisting of *P. aeruginosa* and *P. protegens*. This QS-dependent resistance of *P. aeruginosa* to *P. protegens* is likely mediated at least in part by rhamnolipids and siderophores. The roles of *P. protegens* GacA-dependent secondary metabolites in QS-dependent competitive interactions with *P. aeruginosa* are currently under investigation.

The genes and signals that mediate *K. pneumoniae* function in mixed-species biofilms were also analysed. Transcriptomic experiments have identified several *K. pneumoniae* genes that are differently expressed when cultured as mixed-species biofilms compared to monospecies biofilms. Autoinducer-2 (AI-2) transporter genes, *lsrB* and *lsrD*, and the biofilm regulatory gene, *bssR*, were upregulated by 3.9, 2.9 and 2.8-fold, respectively, in *K. pneumoniae* mixed-species biofilms. The LsrB and LsrD proteins function as the membrane-bound AI-2 transporter complex where they recognise (LsrB) and internalise (LsrD) the AI-2 signal. Deletion of *lsrB* and *lsrD* genes in *K. pneumoniae* increases the extracellular AI-2 concentration of planktonic cultures by 75-fold but did not significantly affect monospecies biofilm formation. However, when grown as mixed-species biofilms, the loss of *lsrB* and *lsrD* resulted in a significant change in composition of the three-species biofilm community with significant decrease in the proportion of *K. pneumoniae* and *P. aeruginosa*, with a concomitant increase in *P. protegens* (Figure 1). Dual-species biofilms grown with *K. pneumoniae* Δ*lsrB*/*lsrD* mutants showed that this was likely due to the combined effect of pairwise interspecies interactions of the *K. pneumoniae* mutant with both *P. aeruginosa* and *P. protegens*. Inactivation of the *bssR* gene resulted in increased monospecies biofilm formation, suggesting it is a repressor of *K. pneumoniae* biofilm formation. Interestingly, deletion of *bssR* significantly reduced the proportions of *P. protegens* and *P. aeruginosa*, with a concomitant increase in *K. pneumoniae* proportion. This suggests that *bssR* in *K. pneumoniae* supports survival of *P. aeruginosa* and *P. protegens* in the mixed-species biofilm community (Figure 1). Phenotypic assays of the Δ*bssR* mutant showed that the *bssR* gene upregulates motility and inhibits surface attachment and formation of non-surface attached floating aggregates. These changes could account for the increased monospecies biofilm formation in the Δ*bssR* mutant and its role in supporting three-species biofilm formation.

The outcomes of this project demonstrate the importance of various genes and signals from different members of the community in modulating the composition and spatial organisation of mixed-species biofilm community.

Protection of mixed-species biofilms from predation

Predation by other organisms is a major regulator of bacterial mortality and shapes bacterial communities in nature. Three-species biofilm protects its susceptible members from predation when exposed to the heterotrophic ciliate, *Tetrahymena pyriformis*, a ciliate that feeds both on planktonic cells and biofilms. This protection was conferred to the whole community by *P. aeruginosa*, primarily through the secretion of rhamnolipids that can intercalate into the cell membrane. *T. pyriformis* cells underwent lysis when they came in contact with *P. aeruginosa* (Figure 2). Interestingly, the two bacterial species gained associational resistance from the grazing-resistant *P. aeruginosa* when the three species were grown together in a biofilm.

However, a secondary mechanism is also involved in this defence process since absence of rhamnolipids did not completely abolish protozoa killing by the bacteria (Figure 2). An untargeted gene deletion screen was used to identify the source of this additional protozoa toxicity. Initial results suggested that the *P. aeruginosa* quinolone signal (PQS) pathway, including PqsA, PqsB, PqsC, PqsD and PqsR genes, is involved in protozoa killing. PQS is a small molecule that mediates cell-to-cell communication during quorum sensing in *P. aeruginosa*. Exposing *T. pyriformis* to PQS reduced their survival within 24 hours, suggesting that PQS, secreted by *P. aeruginosa*, plays a protective anti-grazing role.

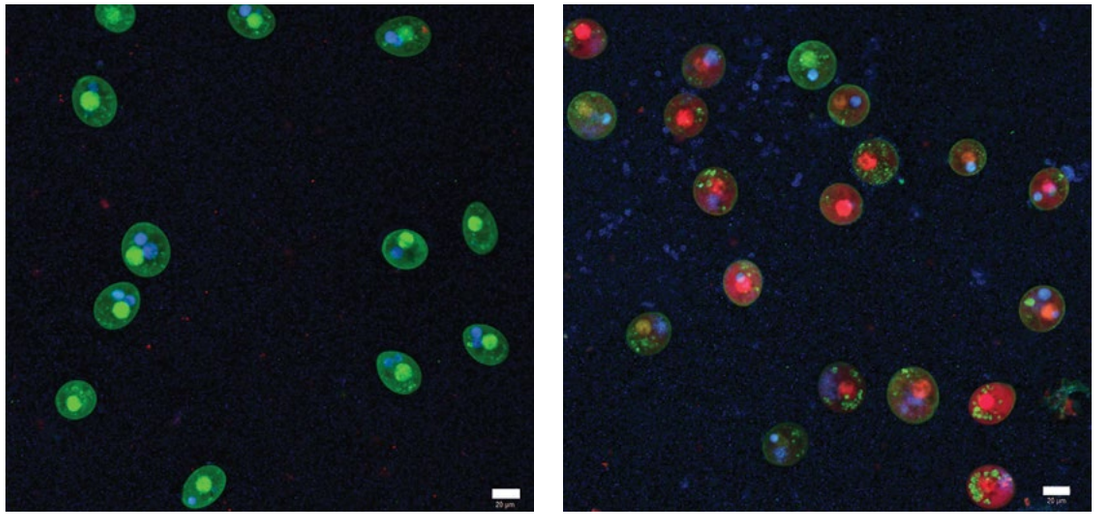

Figure 2:
T. pyriformis predation of PAO1Δ*rhlA* biofilms after 2 h. Confocal images of protozoa after incubation with eYFP tagged (blue) *P. aeruginosa* PAO1ΔrhlA biofilms for 0.5 h (left) and 2 h (right). Cells were stained with live/dead assay. Green cells represent live bacteria or protozoa, while red cells are bacteria or protozoa with damaged membrane. Magnification: 20 x. Scale bar: 20 µm.

Future directions

These observations suggest that the three-bacteria biofilm model generated is ideal for analysing contribution of different factors to biofilm growth and emergent properties. Several genes that were identified as differently regulated in biofilm using transcriptomics analyses have not yet been characterised for their role in spatial organisation and structure of mixed species community. Future work will also extend transcriptomic studies with detailed metabolome analyses to identify compounds that confer cross-feeding and other biofilm-specific characters. Quantification of energy metabolites would help to define the co-operative energy balance of the community relative to single-species populations. The role of *P. aeruginosa* QS system in maintaining its competitive relationship with *P. protegens* in biofilms is being investigated. Additional studies are also being directed towards unravelling the mechanisms of PQS-mediated protozoa toxicity. The overall aim of these projects is to advance the understanding of interspecies interactions within a mixed-species biofilm and their role in mediating emergent properties of the community.

The ISME Journal (2014) 8: 894–907.

The ISME Journal (2015) 10: 846–857.

Frontiers in Microbiology (2015) 6: 851.

Biofilm (2020) 2: 100035.

Frontiers in Cellular and Infection Microbiology (2021) 11: 646991.

THE ROLE OF QUORUM SENSING IN COMPLEX BIOFILM COMMUNITIES

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Introduction

Contrary to popular belief, bacteria are predominantly social microorganisms that constantly interact with each other via exchanging chemical signals. Quorum sensing (QS) is a phenomenon whereby the bacteria employ autoinducers, chemical signals to survey the population density in their surrounding environment and integrate the information to regulate the expression of certain sets of genes. By doing so, a group of bacteria can synchronise gene expression and carry out cooperative behaviour at a population level. QS-based signalling is responsible for triggering a wide array of phenotypes in different bacterial species, such as bioluminescence or swarming migration. Most of the time however, QS acts as a "switch" to tell the free-living (planktonic) bacteria to convert into a matrix-encased sessile biofilm lifestyle. The QS bacteria divert resources into signal production and QS phenotypes in exchange for the survival advantages of the population. Some bacteria, however, have evolved strategies to interfere with the QS interaction of other species to maintain competitiveness within the community, or simply consume the signal molecules as a source of nutrients. Such interference in QS activity is generally referred as quorum quenching (QQ) and is widespread among bacteria.

Most environments harbour biofilms that consist of not only one or two species, but often hundreds to thousands of different microorganisms living in a highly structured community. The complex biofilm structure and community composition are influenced by chemical crosstalk among the biofilm members, including via the QS interaction. Yet, the impact of QS signalling and the interaction between QS and QQ bacteria in the context of such complex communities remain understudied. This project aimed at unravelling the role of acyl-homoserine lactone (AHL)-based QS signalling and quenching pathways that drive the assembly of a complex biofilm community.

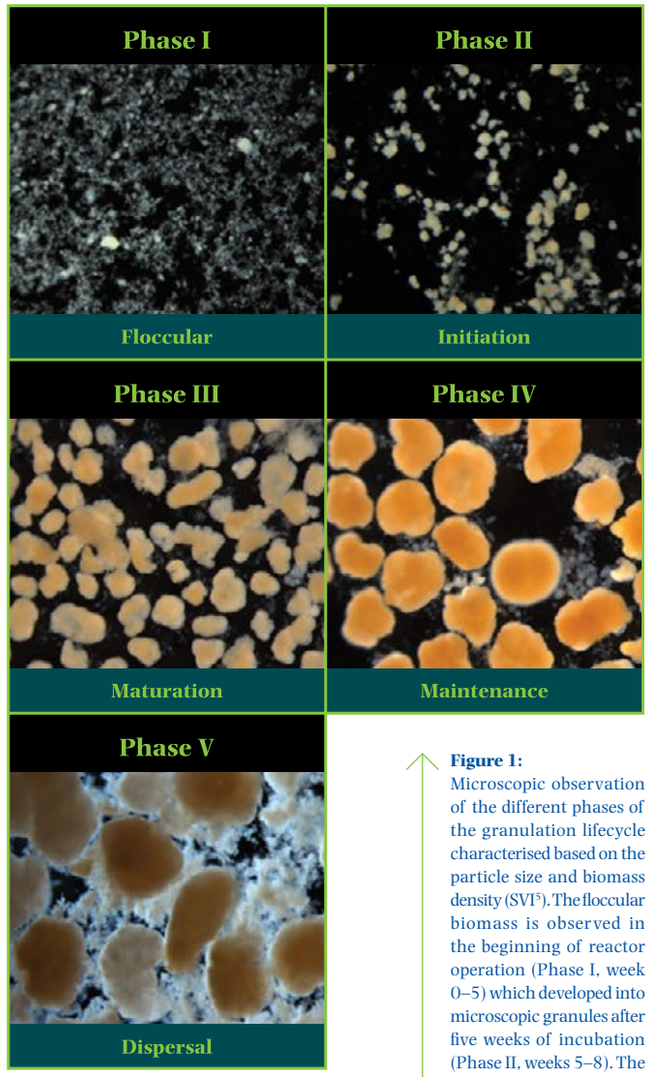

Figure 1: Microscopic observation of the different phases of the granulation lifecycle characterised based on the particle size and biomass density (SVI[5]). The floccular biomass is observed in the beginning of reactor operation (Phase I, week 0–5) which developed into microscopic granules after five weeks of incubation (Phase II, weeks 5–8). The sludge granules then slowly increased in size over the subsequent weeks (Phase III, weeks 8–15) and stabilised at the maximum size for another five weeks (Phase IV, weeks 15–20). Afterward, the granules disintegrated back into floccular biomass which was accompanied with a decrease in the particle size (Phase V, weeks 20–22).

Main findings

Aerobic granular sludge (AGS) is a biological-based wastewater treatment technology that relies on the activities of microbial biomass in the form of suspended spherical aggregates (granules) to remove nutrients from the wastewater. The highly complex microbial granules were used to investigate the role of AHL-based QS and QQ activities in a replicated experimental bioreactor seeded with floccular sludge.

Over 154 days, the sludge biomass underwent morphological changes from the floccular form into granules, and eventually disintegrated back to the floccular form again, thereby enabling the entire process of the granulation lifecycle to be investigated. This process can be divided into five key phases (Figure 1), with each transition marked by a change in the particle size and density.

Indeed, the AHL-based QS is closely linked to the granulation process. A strong correlation between AHL signal concentration and the sludge particle size was observed, where the AHLs accumulated during the maturation of the microbial granules, and the disintegration of the sludge granules was accompanied by the disappearance of particular AHL signals. The AHLs could potentially induce the granulation via triggering the production of extracellular polymeric substances (EPS, the main structural component of biofilm matrix), which also increased during the formation of the granular sludge. In fact, the sludge community responded rapidly by overproducing EPS when treated with exogenously added AHL signal in a concentration-dependent manner. Overall, these findings imply that

the AHL-based QS could be a key regulator of the granulation process, potentially via regulating the EPS production.

Regulation of AHL concentration during this process was subsequently investigated. Testing sludge community bacterial isolates revealed that many members were actively involved in AHL-based QS, either producing or degrading the AHL signals. Hence, the prevalence of both QS and QQ members are the key determinant for the AHL concentration in the system. During the floccular phase, the microbial community exhibits a high level of quenching activity, with more than half of the community members capable of degrading AHL signals. A shift in the sludge community was observed, however, when the floccular biomass transitioned into the granular phase, with a significant decrease in the prevalence of QQ bacteria while the QS bacteria were enriched. The de-repression of the QQ activity was immediately accompanied with an increase in the half-life and concentration of various AHL signals in the system, which coincide with the formation of granules. In a complex biofilm community, the balance between QS and the counteracting QQ activity is proposed as the crucial determinant for the expression of QS phenotypes.

Future directions

It is challenging to study the role of social interactions in shaping the structure and composition of a complex biofilm community. Nevertheless, this project demonstrated that granulation, the developmental process of a multi-species biofilm, is intimately connected with QS interactions among microbial community members. This is aligned with studies at the single-species level where QS has been reported to drive biofilm development in many bacteria that are commonly used in the laboratory. At a community level, QS drives similar biofilm phenotypes but importantly, the QS signal concentration is controlled by the arms race between the QS and QQ bacteria in the community. While this work elucidates the importance of QS signalling in the context of a complex microbial community, several questions do remain. For example, while QS was proposed to drive the granulation process, the opposite scenario, i.e., that QS is promoted by the formation of microbial granules, could not be precluded. The possibility of interspecies crosstalk via QS in a complex biofilm community was also considered with regard to its potential role in driving the assembly of complex biofilms. Resolving these questions will provide valuable insights in understanding the assembly of a complex microbial ecosystem and will enable novel biofilm control strategies to be developed.

The ISME Journal (2014) 8:1186–1197.

npj Biofilms and Microbiomes (2015) 1:15006.

COMMUNITY ECOLOGY THEORY IN COMPLEX MICROBIAL SYSTEMS

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Introduction

Microorganisms typically exist as diverse, complex and dynamic communities and are involved in all biogeochemical cycles. These microbial communities or microbiomes provide crucial functions for global climate regulation, human health, biotechnology and bioremediation. Community structure, often described in terms of α- and β-diversity, is understood to affect ecosystem function. However, our capacity to predict and manage the functions of microbial communities and how they are linked to community structure is still limited. In this regard, engineered systems like sludge bioreactors for wastewater treatment constitute model systems for microbial ecology studies, with measurable ecosystem functions, such as carbon and ammonia removal. This is important in plant operation practices and involves complex microbial communities in a controlled environment. Theory from ecology can help to draw generalised conclusions from specific observations of organisms in their environment, by allowing us to classify, interpret and predict the world around us. This research project aims to test and develop community ecology theory for complex microbial systems, using wastewater treatment bioreactors as a model system.

Microbial diversity is often related to the ability of a community to withstand environmental fluctuations that typically occur as disturbances. While a disturbance may result in inhibition, injury, or death

for some individuals in a community, it also creates opportunities for other individuals to grow or reproduce. Indeed, disturbance is considered a major factor influencing species diversity, but a clear understanding of the underlying mechanisms is lacking. Community assembly processes shape community structure, which in turn affects ecosystem function. Both deterministic and stochastic processes are known to simultaneously influence the assembly of communities. Although disturbance is believed to be an important driver of community assembly processes, its effects on their relative importance are not well understood.

Main findings

The knowledge generated by integrating experimental sludge bioreactor research with ecological theory has elucidated the role of disturbance in diversity and performance of complex microbial communities. Two types of environmentally relevant disturbances were employed: 3-chloroaniline, a xenobiotic compound derived from agricultural pesticides and herbicides; and organic loading shocks in the form of double chemical oxygen demand. Experiments were performed at a microcosm scale with replicated sequencing batch reactors subjected to different frequencies of disturbance, either pulses or continuous for periods of five to six weeks. These were then scaled up for volume (100-fold) and operation time (five to six months), to accommodate the multidimensional nature of disturbance.

The research uncovered a link between diversity and the underlying mechanisms of assembly for a complex microbial system for the first time. This causal relationship was coined the intermediate stochasticity hypothesis (Figure 1). Further, novel empirical evidence supported the application of a three-way life strategy framework based on the effect of disturbance in bacterial communities, accounting for trade-offs in community aggregated traits. It further showed that higher community diversity does not always imply better ecosystem function, and that community assembly at the taxonomic and genotypic levels varies in terms of diversity and underlying mechanisms. Additionally, resilience and resistance of ecosystem function can be variable across identical replicates, and recovery in function does not imply a return to the initial community structure state. An alternative method to consistently achieve partial nitrification by modifying the organic loading on bioreactors was demonstrated. These findings have implications for biotechnological applications where it is desired to control the aggregated functions microbial communities provide, even after perturbations. They are also relevant for managing other complex microbial systems that could display similar responses to perturbations, like oceans, lakes, soils or the human gut.

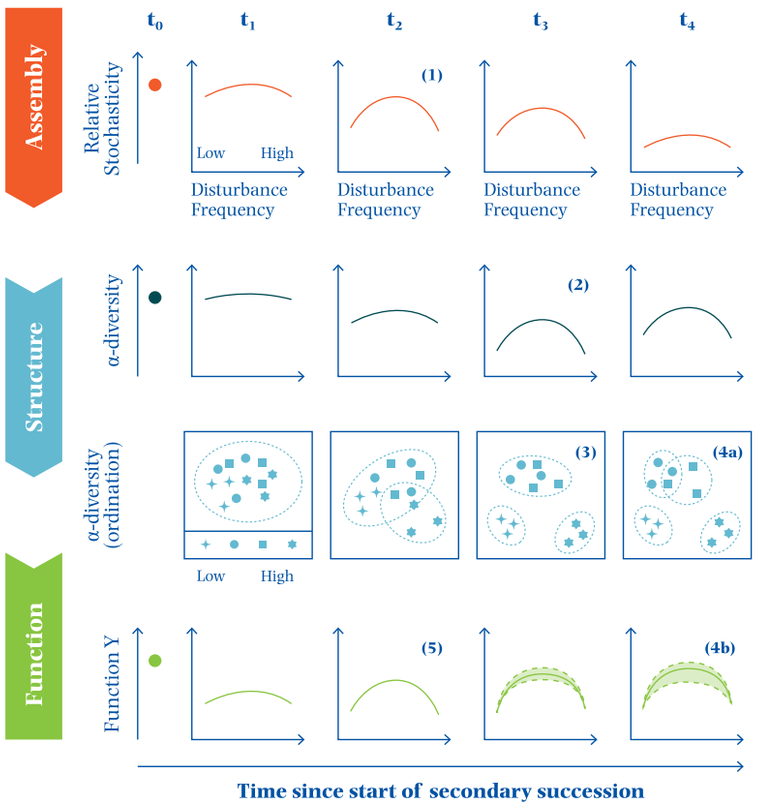

Figure 1:
Conceptual representation of the intermediate stochasticity hypothesis (ISH) to describe patterns of assembly and structure along a disturbance frequency gradient, for communities in secondary succession (starting at time point t_0). (1) Initially, stochastic assembly mechanisms are favoured at intermediate disturbance frequencies, promoting re-colonisation processes from the low-abundance fraction of the community or seed bank. (2) Subsequently, these are followed by changes in the community structure that manifest as a peak of alpha-diversity at intermediate levels of disturbance. (3) Over time, three separated clusters of alpha-diversity ordination may form comprising low, intermediate and high levels of the disturbance range. Furthermore, stochasticity operating at intermediate disturbance levels may lead to variable within-treatment (4a) alpha-diversity, visualised as partially overlapping cluster similarity (dashed ovals), and (4b) community function, displayed as wider confidence intervals (shaded area between dashed lines). (5) The output of some functions (e.g., nitrogen removal) could be higher at intermediate disturbance frequencies during initial successional stages due to higher stochastic assembly. Additionally, the overall relative contribution of stochasticity decreases with succession time (see top row of subpanels).

Future directions

The theories and models outlined above are being explored on different microbial communities using open anaerobic bioreactors. Overall, the predictions from these ecological frameworks help to identify cases when disturbance-induced stochastic assembly promotes alternative states of community structure that compromise or enhance ecosystem function. This information enables the design of mitigation or intensification strategies, or to promote community resistance and resilience to future disturbances via increased alpha-diversity and functional-gene diversity. Moreover, these theoretical frameworks inform the design of functionally resilient bespoke communities, through the stochastic mechanisms that are initially elicited at intermediate frequencies of disturbance and provide an advantage to rare or low-abundance taxa.

npj Biofilms and Microbiomes (2019) 5: 8.

Scientific Reports (2020) 10: 21388.

mSystems (2020) 00471-00420.

MICROBIAL PREDATION IN GRANULATING REACTORS

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Introduction

To ensure that treated wastewater can be safely discharged to the environment, contaminants and nutrients must be removed. In a water reclamation plant that uses engineered systems such as activated and aerobic granular sludge, important processes like nutrient removal and flocculation are largely conducted by bacterial communities. Bacterial predators such as bacteriophage and protozoa exert significant predation pressure and can cause death of bacteria within such communities. However, the roles of bacteriophages and protozoan predation in impacting the process of granulation remain limited. The roles of bacteriophage and protozoan communities throughout the granulation process were investigated using a combination of bacteria, bacteriophage, and protozoa sequencing as well as chemical treatment to remove protozoa.

Main findings

In this project, four independent reactors were seeded with activated floccular sludge from Ulu Pandan Water Reclamation Plant, Singapore, and operated to achieve aerobic granulation for 11 weeks. Using genomic sequencing, the changes in the bacteriophage, protozoa and bacterial communities were characterised throughout the granulation process. The aerobic sludge underwent initiation of granulation in weeks 4 to 6, before increasing in size in the maturation phase from week 7 (Figure 1a). There was a positive correlation between the number of lytic bacteriophages (e.g., Microviridae) and the increase in granule particle size and initiation of granulation (weeks 4 to 7). Furthermore, the Inoviridae viral family (filamentous bacteriophages) also had an effect during the maturation phase (weeks 8 to 9) of aerobic granulation (Figure 1b). For the protozoan community, ciliated protozoa from the class *Oligohymenophorea* were the dominant group of protozoa during the formation of granules, having a strong positive correlation with the initial formation of compact aggregates (Figure 1c).

A more direct study to investigate the role of protozoa in granulation was subsequently performed. The chemical thiram was used to inhibit protozoan growth and in these reactors, there was a delay in the initiation of granule formation by two weeks. Furthermore, recovery of '*Candidatus* Accumulibacter' was positively correlated with the formation of compact aggregates and granules, showing the importance of this species in a granulating reactor system.

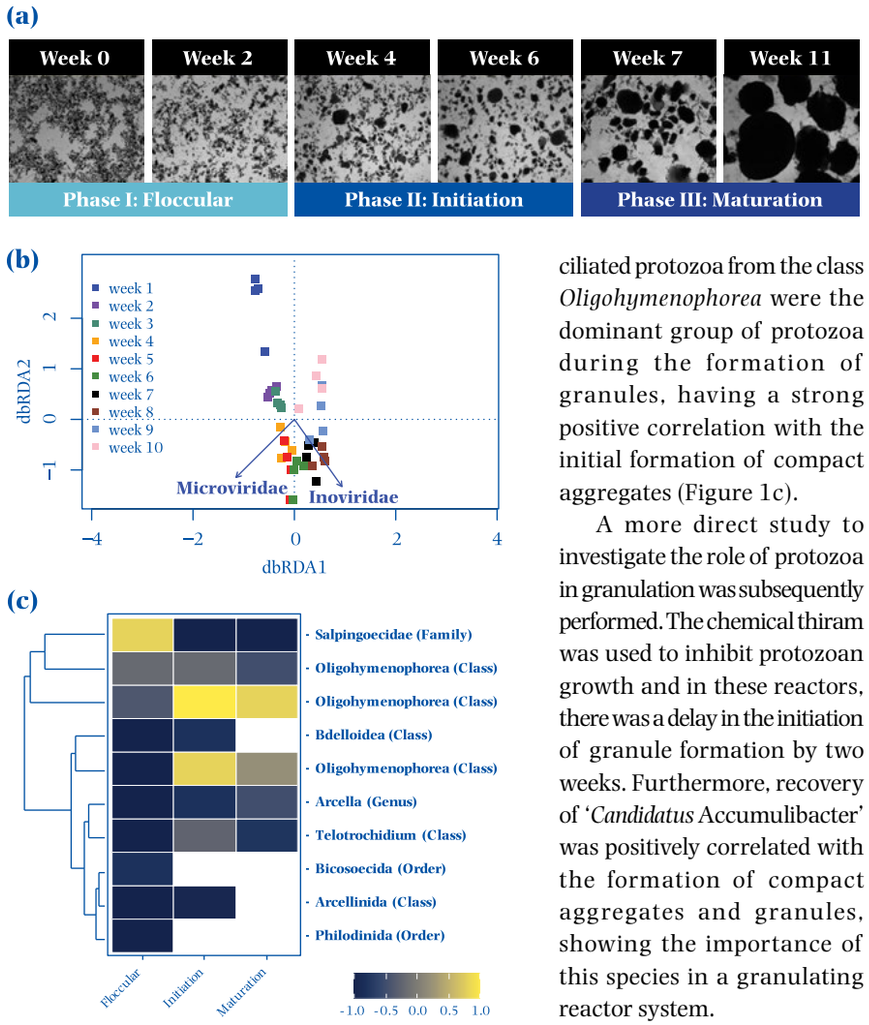

Figure 1:
(a) Microscopic visualisations of the development of small granules from floccular activated sludge over 11 weeks. (b) Distance-based redundancy analysis of the effect of bacteriophage dynamics on the changes in the bacterial community during aerobic granulation showed Microviridae having an effect during granular initiation, while Inoviridae played a role during maturation of granules. (c) Spearman correlation of the top 10 most abundant protozoa with the sludge particle size during the different phases of granulation showing *Oligohymenophorea* being positively correlated to particle size during initiation and maturation.

Future directions

Predation by bacteriophages and protozoa influences the diversity and the composition of the bacterial community. While physical parameters such as settling time can promote granulation of sludge, the potential role of bacteriophages and protozoa in promoting granulation was also demonstrated. Using the observations in this work, the different bacteriophages and protozoa can be isolated and characterised. Further, using these isolated predators, a controlled change in bacterial community of activated sludge into one that promotes granulation can be achieved by: (1) increasing the proportion of *Ca.* Accumulibacter through removal of unwanted species; or (2) adding filamentous bacteriophages or protozoa that promote granulation through physical means of increasing bacterial attachment on phage filaments or sessile ciliates.

BMC Microbiology (2021) 21: 91.

THE EVOLUTION OF VIRULENCE IN PROTOZOAN PREDATION

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Introduction

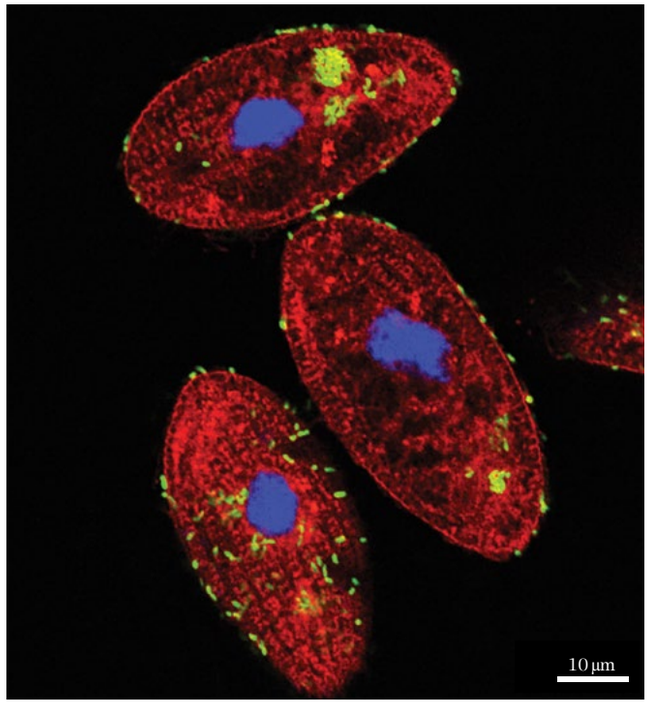
Protozoa are unicellular eukaryotic organisms that feed on bacteria, controlling their population structure and composition in the environment. Protozoa also serve as hosts for many opportunistic pathogens in various environments and play crucial roles in modulating pathogenic traits of these pathogens. Patho-adaptation of opportunistic pathogens in response to protozoan predation is now widely recognised. Often these adaptive strategies are associated with phenotypic and genetic diversity, leading to the evolution of virulence. An understanding of the mechanisms driving the phenotypic and genetic diversity in response to long-term protozoan predation pressure is generally lacking. Unravelling the molecular mechanisms of how predation shaped the evolution of virulence in response to long-term predation pressure by protozoa is essential in establishing the virulence potential of evolved pathogens.

Main findings

The evolution of virulence in two model opportunistic pathogens *Pseudomonas aeruginosa* (a model chronic pathogen) and *Vibrio cholerae* (a model acute pathogen) were investigated in response to protozoan predation. *P. aeruginosa* and *V. cholerae* were cultured with and without the amoeba *Acanthamoeba castellanii* over 500 generations using an experimental evolution approach. Phenotypic diversity was quantified in evolved isolates using various microbiological assays. The genetic variations resulting from the long-term evolution experiments were identified by sequencing the evolved isolates.

Adaptation with amoeba resulted in considerable phenotypic and genetic diversities in both *P. aeruginosa* and *V. cholerae*. The late-stage adapted isolates showed a reduction in motility and biofilm formation phenotypes compared to the ancestral strain. Altered production of several secondary metabolites in late-stage adapted isolates was also observed. In particular, *P. aeruginosa* showed reduced pyoverdine and rhamnolipid. However, a trade-off between the production of proteases and hemolysins resulted for *V. cholerae*. The late-stage *V. cholerae* isolates showed increased protease and decreased hemolysin production compared to the ancestral strain. Genome sequencing and mutational analysis of the evolved isolates revealed key phenotypes linked to several missense mutations in essential regulatory genes. The genes involved in motility-chemotaxis showed many mutations compared to other genes in sequenced isolates. Several genes in quorum sensing pathways were mutated in *P. aeruginosa*. These mutations reduced the production of secondary metabolites, and intracellular survival assays with the amoeba host revealed that the altered phenotypes increased the survival of the late-stage adapted isolates compared to the ancestral strains. Interestingly, most of the observed phenotypes in amoeba-adapted *P. aeruginosa* were similar to isolates obtained from chronic cystic fibrosis patients, suggesting the pathogen adopts similar adaptation strategies in response to both amoeba and human hosts immune response.

The findings in this study increase our understanding of how virulence evolves in response to predatory pressure in the environment. The mechanisms used by amoeba and human macrophages for the killing of bacterial cells are very similar and thus, protozoa are considered to be a 'training ground' for opportunistic pathogens.



Vibrio cholerae (green) co-incubated with *Tetrahymena pyriformis*. *T. pyriformis* nucleus (blue) and membranes (red). Intracellular food vacuoles contain *V. cholerae* (green).

Applied and Environmental Microbiology (2022) 88:e01665-21.
Applied and Environmental Microbiology (2022) 88:e02322-21.
The ISME Journal (2022) 16:856-867.
BMC Microbiology (2021) 21:91.
Frontiers in Microbiology (2020) 11:17.
Nature Microbiology (2019) 4:2466-2474.
FEMS Microbiology Ecology (2017) 93:fix147.
The ISME Journal (2015) 9:1812-1820.

Future directions

The findings obtained in this study further our understanding of the complex interactions of opportunistic pathogens and their hosts. Investigating the interactions of bacteria and protozoan hosts will inform our understanding of amoebae in the origin and transmission of infectious diseases. The knowledge gained here is being applied to other bacterial pathogens and eukaryotic hosts.

PROTOZOAN-BACTERIAL INTERACTIONS AND STRESS RESISTANCE IN *VIBRIO CHOLERAE*

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Introduction

Vibrio cholerae interacts with many organisms in the environment, including heterotrophic protists (protozoa). Protozoa and bacteria have been conducting 'evolutionary warfare' for billions of years. Protozoa in the environment graze on biofilm-based and planktonic bacteria, taking bacterial prey into phagosomes that become acidified and filled with enzymes, resulting in digestion. However, bacteria have developed traits that have allowed them to survive ingestion by protozoa, which can package and release 'unpalatable' bacterial pathogens. The undigested pathogens are protected in cavities in the protozoan gut and ejected into the environment in expelled food vacuoles (EFVs). This project investigated the biological role of EFVs as a vector for pathogen transmission in higher organisms. Several ciliated protozoa, e.g., *Tetrahymena pyriformis*, were investigated as grazers of *V. cholerae*, the aetiological agent of the acute diarrheal disease cholera, which is endemic in many countries and responsible for an estimated 1.3–4.0 million cases and up to 143,000 deaths worldwide annually.

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Main findings

This study has expanded our understanding of the interplay between protozoan grazers and bacteria. It demonstrated that the *V. cholerae* outer membrane protein, OmpU (involved in *V. cholerae* pathogenesis), also mediates production of EFVs in *T. pyriformis*. EFVs, in turn, confer survival advantages under stressful conditions, such as pH stress and antimicrobial compounds encountered following ingestion. Once expelled from the protozoa, EFVs are stable in the environment and protect the bacterial cells within from stresses. Further, the EFV-encased cells acquire growth and colonisation advantages over free-living planktonic bacteria in both *in vitro* and *in vivo* studies, such that toxic strains of *V. cholerae* in EFVs expelled into the environment are primed for human host colonisation and infection. At 37°C, neutral pH or high nutrient concentrations, the EFVs release their contents, discharging a large number of *V. cholerae* cells.

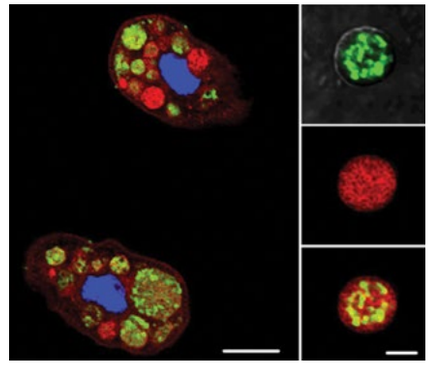

Figure 1:
Production of EFVs containing *V. cholerae*. Left: GFP-tagged *V. cholerae* (green) co-incubated with *T. pyriformis* (DAPI, blue) showing EFV membranes (red). Intracellular food vacuoles contain GFP-tagged *V. cholerae* (left; scale bar, 15 µm.) EFV (right panels): green *V. cholerae* cells (top); red EFV membrane (middle); merged image denoting *V. cholerae* cells in EFV (bottom) (right scale bar, 5 µm).

In the context of human disease, EFVs also enhance the survival traits of *V. cholerae* ingested by a human host as they pass through the acidic environment of the stomach. On reaching the non-acidic, nutrient-rich small intestine, the EFVs release *V. cholerae* with enhanced colonisation traits, which trigger disease progression in the host. Hence, for pathogenic bacteria such as toxic strains of *V. cholerae*, interactions with protozoa such as *T. pyriformis* may have made them more resistant and significantly more infectious.

These results elucidate some of the mechanisms of persistence and the modes of transmission of *V. cholerae* and may further apply to other opportunistic pathogens that are released by protists in EFVs.

Future directions

There are currently no detection systems for EFVs that would allow for the monitoring of potential pathogenic outbreaks. Future research directions involve identifying biomarkers, such as proteins on EFV surfaces, and developing antibodies to enable real-time monitoring. Identifying targets to prevent the production of EFVs would provide another solution to mitigate disease outbreaks. This requires understanding how and why the cells in EFVs acquire enhanced growth and colonisation traits, compared to free-living cells.

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A NOVEL MECHANISM OF DEFENCE AGAINST PROTOZOAN PREDATION OF *VIBRIO VULNIFICUS*

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Introduction

Vibrio spp. are among the most common bacteria found in marine and estuarine waters throughout the world. Despite their environmental origin, several *Vibrio* spp. – including *V. cholerae, V. vulnificus* and *V. parahaemolyticus* – have emerged as human pathogens. The global rise in *Vibrio*-related human infections is associated with increases in the relative abundance of the bacteria in the world's oceans, linked to warming waters. These bacteria in the environment are exposed to protozoan predation, which selects for persistence of specific genetic variants. In response to this constant predation pressure, bacteria evolve defence mechanisms ranging from changing morphology, biofilm formation, and secretion of toxins or virulence factors. Some of these adaptations may result in the emergence of potential human pathogenic strains. Therefore, it is important to study predator defence strategies of environmental bacteria, especially in light of climate change and the consequences of warming coastal waters on the proliferation of specific bacterial species. *Vibrio vulnificus* is a Gram-negative, halophilic bacterium that inhabits warm marine and estuarine environments globally. Despite its environmental origin, the bacterium is associated with opportunistic infections in humans. Very little is known about the defence mechanisms of *V. vulnificus* against protozoan predation, maintenance and proliferation in coastal waters.

Main findings

Thirteen strains of *V. vulnificus*, representing different genotypes and isolation sources, were assessed for grazing resistance against the ciliated protozoa *Tetrahymena pyriformis*. Investigation of the cell-free supernatant showed that *V. vulnificus* strain (ENV1) acidifies the environment by the excretion of organic acids, which are toxic to *T. pyriformis*, a mechanism dependent on the presence of iron. Transcriptomic analysis of the strain with and without iron revealed that ENV1 ferments pyruvate and the resultant acetyl-CoA leads to acetate synthesis under aerobic conditions, a hallmark of overflow metabolism. The global anaerobic respiration regulator, *arcA*, was upregulated when iron was available, suggesting that the strain has adapted to anaerobic respiration, despite growth under aerobic conditions. Deleting *arcA* led to reduced acetate accumulation and loss of grazing resistance. Transcriptome and metabolite analyses showed that ENV1 has rewired the central carbon metabolism, enabling anaerobic respiration and production of excess organic acid that is toxic to *T. pyriformis*. This is a previously unknown mechanism of predation defence that protects against protozoan predators.

Anaerobic respiration induces virulence factor production in many *Vibrio* species, including *V. cholerae*. Given that the overflow acetate metabolism of ENV1 is a natural variation of the central carbon metabolism, it is likely that *V. cholerae* and other potential human pathogens can acquire such metabolic adaptation in their natural habitats. These results present a compelling case for active environmental surveillance of such natural variants for their emergence as human pathogens.

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Future directions

Further studies exploring the genetic modifications that underlie the altered central carbon metabolism are required to elucidate the interactions between environmental pathogens and the human host. This is particularly relevant in light of a potential increase in virulence of human pathogens inhabiting climate-change-affected marine coastal waters.

BIOFILM BIOPHYSICS AND POTENTIAL CONTROL

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Introduction

Biofilms are complex and exhibit a range of properties different from the planktonic cells, such as enhanced resource capture and antibiotics resistance. These are largely due to the presence of extracellular matrix in biofilms. Structural elucidation of biofilm exopolymers is challenging due to their complexity and recalcitrance. Functional characterisation of exopolymers has tended to be qualitative rather than quantitative and has neglected inherent biofilm heterogeneity. To establish structure-function relationships for constituent biofilm matrix exopolymers, both problems need to be tackled. The extracellular matrix of biofilms is viscoelastic; however, the importance of this trait to the survival and persistence (including antimicrobial drug resistance) of the microbial communities is yet to be fully understood. The matrix comprises biophysical materials, where the cells are equivalent to colloids and the encasing extracellular polymeric substance (EPS) can be considered as a cross-linked polymer gel. This framework has allowed parallels to be drawn from soft matter physics, which enables an understanding of viscoelastic biofilms. A range of techniques was developed to account for the contribution of specific exopolymers to critical biofilm functions. Elucidating biofilm mechanical properties, and how the biofilm responds to mechanical forces in its surroundings, offers new insights into the establishment, survival and potential control of biofilms.

Main findings

Understanding biophysics of biofilms and extracellular matrix

A passive microrheology method based on video particle tracking rheology was used to describe the rheological heterogeneity in biofilms, and how this contributes to dynamic biofilm remodelling and antibiotic resistance. This method works by tracking the mean square displacement of polystyrene microspheres incorporated into the biofilm matrix. The elastic storage modulus and the viscous loss modulus can then be extracted from the mean square displacement of the beads. This identified the role of different individual exopolymers in *P. aeruginosa* biofilms (specifically the putative polysaccharides alginate, Pel, Psl as well as extracellular DNA). Psl increased the elasticity and effective cross-linking within the matrix, which strengthened its scaffold and facilitated the formation of microcolonies. Conversely, Pel reduced effective cross-linking within the matrix. Without Psl, the matrix becomes more viscous, which facilitates biofilm spreading. The effective cross-linking of wild-type biofilm decreased over time, which would be advantageous for the spreading and colonisation of new surfaces. This suggests regulatory mechanisms control production of the exopolysaccharides that serve to remodel the matrix of developing biofilms. The exopolysaccharides also have profound effects on the spatial organisation and integration of *P. aeruginosa* in a mixed-species biofilm model of *P. aeruginosa*-*Staphylococcus aureus*. Pel was required for close association of the two species in mixed-species microcolonies. In contrast, Psl was important for *P. aeruginosa* to form single-species biofilms on top of *S. aureus* biofilms. These results demonstrate that Pel and Psl have distinct physical properties and functional roles during biofilm formation.

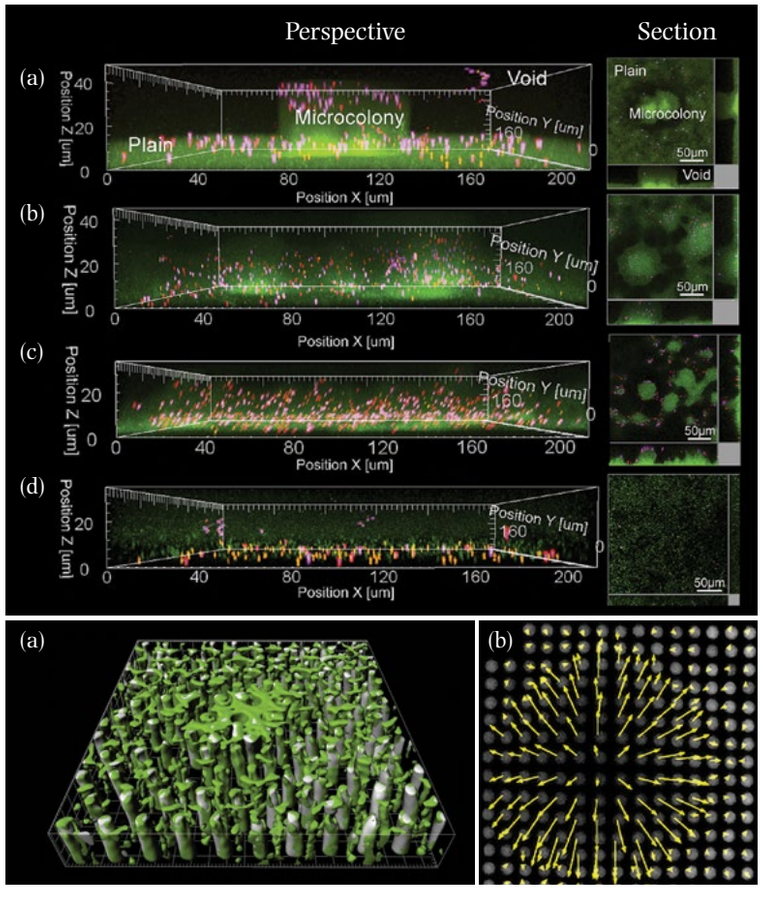

Figure 1:
Confocal images (perspective and sectional view) of five-day biofilms formed in a flow-cell after continuous feeding with particles of sizes 1.0 μm (purple), 0.5 μm (red), and 0.2 μm (orange). The biofilms are formed using *P. aeruginosa* (a) wild-type, (b) with Pel-minus mutant, (c) with Psl-minus mutant, and (d) with Psl- and Pel-minus biofilms.

Figure 2:
(a) Confocal micrograph of *P. aeruginosa* biofilms (green) formed on a micro-pillar array (vertical grey posts). The biofilm cells and matrix adhere to the posts that comprise the micro-pillar array and substratum of the array. As the biofilm grows and spreads laterally, they exert a force on the posts, causing them to bend. (b) The degree of bending is determined and then translated into force measurements. The length of the arrows indicates the magnitude of deflection.

Cells in biofilms sense and interact with their environment through the extracellular matrix. The physicochemical properties of the matrix, particularly at the biofilm-environment interface, determine how cells respond to changing conditions. Using atomic force microscopy (AFM), the mechanical properties of these interfacial regions were determined to elucidate how key matrix components contribute to the physical sensing by the cells. Young's modulus of microcolonies differed according to the size and morphology of microcolonies, as well as the flow rate. The Young's modulus increased as a function of microcolony diameter, which was correlated with the production of the polysaccharide Psl at later stages of maturation for hemispherical or mushroom-shaped microcolonies. Thus, changes in the specific polysaccharide components imbue the biofilm with distinct physical properties that modulate the way in which bacteria perceive or respond to their environment.

While the AFM applies a force on the biofilm to determine the elastic response, it is also important to understand and measure the force exerted by the biofilms on the surroundings as they grow and mature. Micro-pillars were developed and used to study the net mechanical forces (differential pressure) exerted during biofilm formation *in situ*. Pressure from the biofilms are transferred to the micro-pillars via the extracellular matrix, and reduction of major matrix components decreases the magnitude of micro-pillar deflections. The spatial arrangement of micro-pillar deflections caused by pressure differences in different biofilm strains could thus be used as mechanical signatures for biofilm characterisation (Figure 2).

Using the various methods and techniques developed to study the mechanical properties of biofilms, the biofilms were characterised as viscoelastic, exhibiting variations in properties depending on the matrix composition. The differential expression of various matrix components helps to maintain the structure of biofilms and to establish structure-function relationships.

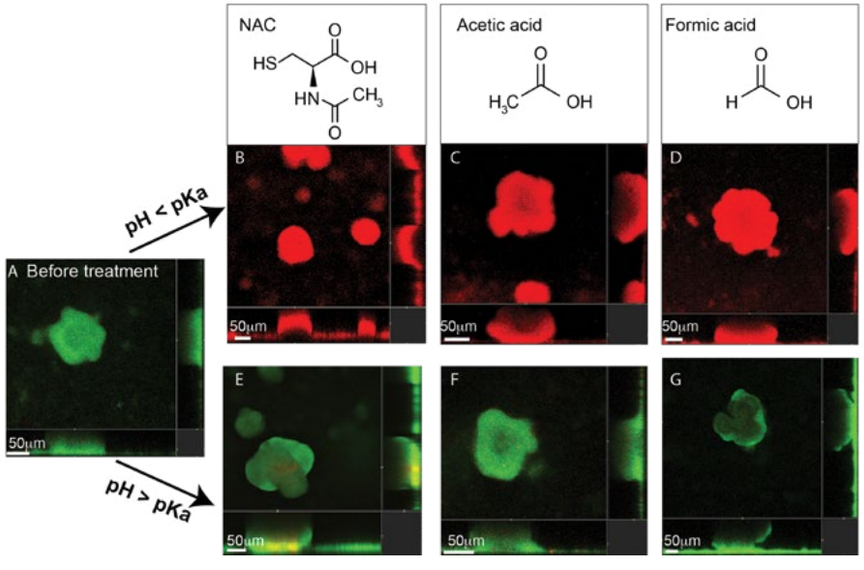

Figure 3:
Mucoid *P. aeruginosa* biofilms treated with monoprotic weak acids at pH below their pKa values kill all the bacteria in biofilms. (a) GFP-tagged biofilm (green) treated with dead stain (red) before treatment with drugs shows only a few dead bacteria. No significant killing was observed when biofilms were treated with (b) NAC, (c) acetic acid and (d) formic acid at pH > pKa. All the bacteria in the biofilm were killed when treated with (e) NAC, (f) acetic acid and (g) formic acid at pH < pKa. *Image credit: Biofilm (2020), 2: 100019*

Weak acid drugs as an alternative anti-microbial therapy

Given that biofilm matrices are viscoelastic and selectively permeable, they prevent most drugs from diffusing into the matrix and are responsible for developing antibiotic resistance. Alternative drugs that could diffuse through the matrix without degrading it were explored.

The weak organic acid drug, N-acetyl-L-cysteine (NAC), is used to eradicate mucoid *P. aeruginosa* biofilms. NAC at pH < pKa can penetrate the biofilm matrix and eventually kill 100% of the bacteria embedded in the biofilm. Comparison of the efficacy of various monoprotic weak acids such as N-acetyl cysteine (NAC), acetic acid and formic acid as well as triprotic acids such as citric acid showed that monoprotic weak acids can kill mucoid *P. aeruginosa* biofilm bacteria, provided the pH is less than their pKa, demonstrating that the extracellular biofilm matrix does not protect the bacteria from the activity of the weak acids. Triprotic acids, such as citric acid, kill biofilm bacteria at pH < pKa1. However, at a pH between pKa1 and pKa2, citric acid is effective in killing the bacteria at the core of biofilm microcolonies but does not kill the bacteria on the periphery (Figure 3). Weak acids have a broad spectrum of activity against a wide range of bacteria, including *Klebsiella pneumoniae, Pseudomonas putida, Staphylococcus aureus*, *P. aeruginosa* DK1-NH57388A, a mucoid cystic fibrosis isolate, and *P. aeruginosa* PA_D25, an antibiotic resistant strain. Thus, weak acids or weak acid drugs can be used as an alternative anti-microbial drug to eradicate antibiotic resistant bacteria and the persister cells in biofilms that are tolerant to other conventional methods of biofilm eradication and can be safely used with human cells.

Further investigation of the biofilm matrix after killing the bacteria provided more insight on the role of bacteria in maintaining the structure and the viscoelastic properties of the matrix. Here, live bacteria were shown to maintain the structure by acting as cross-linkers. Once the bacteria are killed, the cross-links are broken and the remnant matrix swells significantly to 40% and behaves as a pH-switchable polyelectrolyte hydrogel.

Future directions

Understanding biofilm mechanical properties, and how the biofilm responds to mechanical forces in its surroundings, provides new insights into biofilm formation and survival. Measuring biofilm mechanics offers a way of describing biofilms that is complementary to current quantification measures such as microscopy.

Having initially focused on single species, future research will address complex, multispecies biofilms, including interspecies competition, the role of the matrix, and the coexistence of multiple species in different environments and applications. Understanding the mechanical properties of the biofilm matrix enables the synthesis of similar materials for biofilm control, from biofouling mitigation to engineering processes.

Weak acid drugs can be used as an alternative therapy for chronic infection, especially wound infection, where the drugs can be topically applied. Understanding the mechanistic action of drugs on the biofilm matrix will be applied for drug design and biofilm control technologies.

MBio (2014) 5: 4.
Soft Matter (2016) 12: 5718.
Science Reports (2017) 7: 4783.
Biofilm (2020) 2: 100019.

eDNA AS FOUNDATION MATRIX POLYMER FOR *P. AERUGINOSA*

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Introduction

In many habitats, either natural or engineered, microorganisms attach themselves to surfaces, either abiotic or biotic, forming biofilms that protect them from environmental hazards. Traditional antimicrobial agents in the clinical and environmental arena were developed to act on planktonic cells and are significantly less efficient against biofilms due to the presence of extracellular biopolymers like extracellular DNA (eDNA), proteins and polysaccharides secreted by bacteria. eDNA was first identified as a key biopolymer of *Pseudomonas aeruginosa* biofilm matrix in the early 2000s and later found to have a vital role in initial biofilm attachment.

There has been a shift recently in eDNA research towards describing factors allowing DNA to form network structures, specifically in terms of higher order structures and associations with other biopolymers (extracellular RNA, proteins, etc.). This becomes crucial due to differences in the physical behaviour of eDNA outside cells in comparison to intracellular DNA. The structural role, physical property and nature of eDNA in the biofilm matrix of *P. aeruginosa* biofilms were investigated by employing a series of biophysical, biochemical and confocal microscopy techniques.

Main findings

This project has highlighted the importance of eDNA biopolymers as networking and structural components in *P. aeruginosa* biofilm stability. eDNA from *P. aeruginosa* biofilms was extracted using a 'green' solvent ionic liquid and further processed and purified using size exclusion chromatography (separation based on size) and dialysis.

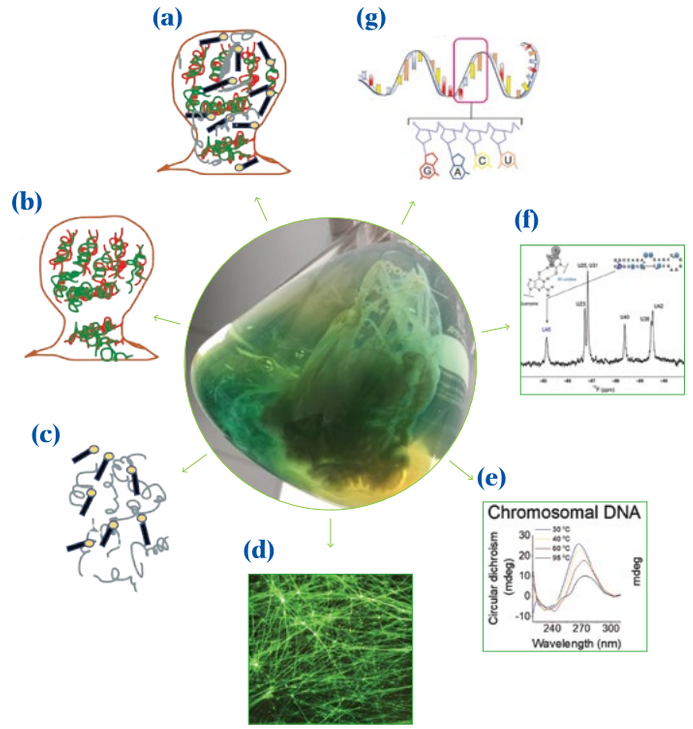

Figure 1:
The overall process of extracellular DNA extraction, biochemical and biophysical characterisation. (a) *P. aeruginosa* biofilms with different extracellular biopolymers (e.g., proteins, nucleic acids and polysaccharides). (b) Isolation of extracellular DNA from other biopolymers using green solvent-based ionic liquid extraction and concentration using perchloric acid and further purified using gel permeation chromatography. (c) Separation of other extracellular biopolymers such as proteins and polysaccharides. (d) Confocal laser scanning microscopy of eDNA fibres (in green) stained by eDNA specific stain TOTO-1. (e) Secondary order structural conformation and thermal stability using circular dichroism (CD). (f) Biochemical characterisation of biofilm and purified nucleic acid gel using nucleic acid resonance (NMR). (g) Sequencing of purified nucleic acid gel to identify the genes and RNA involved in biofilm matrix building of *P. aeruginosa*.

The purified eDNA biopolymer was found to form viscoelastic structures as key for building biofilm matrices. Nuclear magnetic resonance (NMR), a biochemical technique to identify inter- and intra-molecular interactions, was performed on *P. aeruginosa* biofilms and eDNA. This revealed the presence of non-canonical Hoogsteen base pair interactions in the biofilm matrix of *P. aeruginosa*, suggesting that the eDNA formed G-quadruplex structures. Confocal microscopy of both static and flow cell *P. aeruginosa* biofilms (different mode of biofilm growth) identified the distribution of G-quadruplex structures along eDNA fibres.

Phosphorous NMR of purified eDNA biopolymer identified ribonucleotide peaks, suggesting the presence of extracellular RNA (eRNA) in association with eDNA in *P. aeruginosa* biofilms. RNA sequencing of extracted eRNA revealed the enrichment of specific RNA species, but not total RNA, in the biofilm matrix of *P. aeruginosa*, suggesting the eRNA expression is independent of intracellular RNA expression. Significantly, eRNA was also identified in clinical sputum biofilm samples. Specific RNA co-existed with eDNA fibres implicating a possibility of RNA contribution to biofilm matrix building, in addition to its role in transcription and translation.

Future directions

Understanding the biophysical properties and higher order structure of key matrix biopolymers such as extracellular nucleic acids provides insights into *P. aeruginosa* biofilm formation and control in a range of conditions. Further studies will focus on fully elucidating how extracellular RNA is released into the extracellular environment and its contribution to eDNA fibre formation. Ongoing pertinent questions involve when and where the binding of RNA to DNA occurs, and how RNA expression becomes decoupled from the central dogma.

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ANAMMOX BACTERIAL EXTRACELLULAR PROTEIN MEDIATES BIOFILM COMMUNITY ORGANISATION

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Introduction

Anthropological introduction of nitrogen (e.g., urine, industrial discharge, agricultural run-off) into water bodies (marine, sea, lake, fresh water) has resulted in water quality degradation as well as adverse ecological effects such as eutrophication. The anaerobic ammonium oxidation (anammox) process that was identified about 20 years ago owns a central position in the global nitrogen cycle where this microbial process converts nitrite and ammonium ions directly into nitrogen gas under anoxic conditions. Anammox accounts for up to 50% of N_2 production from marine ecosystems into the atmosphere. Regardless of the habitat, anammox bacteria consist mainly of *Planctomycetes*, which aggregate through the expression of extracellular polymeric substances (EPS), and form polymicrobial biofilm granules together with a lower abundance microorganisms such as *Chloroflexi* and *Proteobacteria*. Although research on the direct relation between anammox and eutrophication is still in its infancy, the anammox bioprocess has been adopted extensively in bioreactors to treat side-stream wastewater for its high nitrogen removal efficiency, robustness and sustainability in the absence of aeration and organic substrate.

The nitrogen removal efficiency of anammox relies heavily on EPS-mediated granule formation; however, the structure-function mechanisms of the complex EPS are poorly understood.

To address the knowledge gap, a designer solvent (ionic liquid 1-ethyl-3-methylimidazolium acetate (EMIM-Ac)) was used to dissolve the recalcitrant anammox biofilm. This enabled downstream protein characterisations and resolution of EPS structure-function relationships, and allowed for the role of EPS role in biofilm ecology to be addressed.

Main findings

Using EMIM-Ac, anammox biofilm EPS was extracted and recovered. Solution nuclear magnetic resonance (NMR) and gel electrophoresis approaches revealed the identity of the dominant anammox biofilm EPS as a glycoprotein. This protein was sequentially identified to be homologous to surface-layer proteins, (BROSI_A1236), that were found to have intrinsically disordered (ID) C-terminal that can direct protein phase transition into liquid droplets and mediate bacterial cell adhesion. Additionally, BROSI_A1236 formed envelopes on anammox bacteria cell surfaces. These findings are in line with the proposed function of S-layer proteins as cellular adhesives. Furthermore, filamentous *Chloroflexi* demarcated anammox bacterial clusters with the highest BROSI_A1236 association at the edge of the biofilm, away from anammox bacteria. BROSI_A1236, as an EPS, therefore directs the spatial organisation in polymicrobial biofilms and provides an organic substrate to support filamentous *Chloroflexi* gluconeogenesis, and mediate optimum syntrophic interactions within the anammox biofilm.

This study provides comprehensive insight into extracellular protein identification, extraction, purification and characterisation in environmentally and industrially important anammox bacteria biofilms.

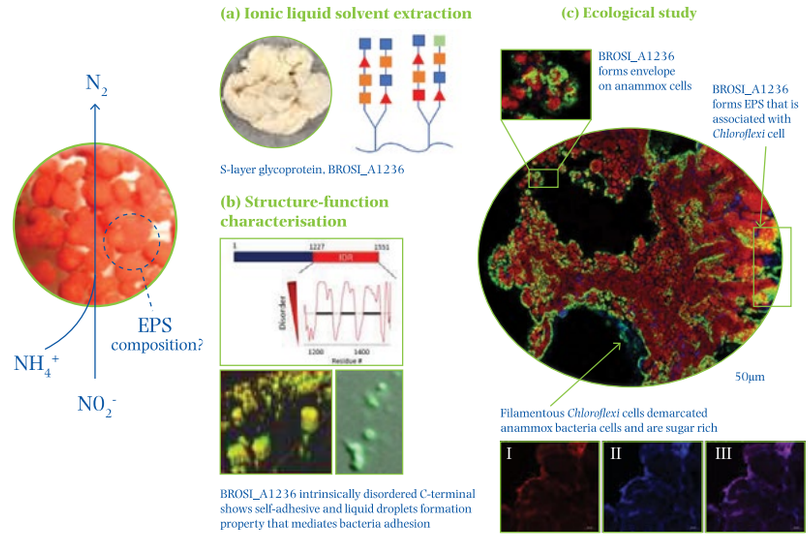

Figure 1:
Biofilm forming traits of anammox bacteria support nitrogen conversion from ammonium and nitrite into nitrogen gas. (a) An ionic liquid enabled anammox biofilm EPS extraction and biopolymer identification as a glycoprotein that is homologous to the S-layer protein, BROSI_A1236. (b) The recombinantly expressed short peptide sequence of the ID C-terminal of BROSI_A1236 showed (b, bottom left) a self-adhesive property as demonstrated by the coalescence of ID peptide tagged red and green fluorescent microspheres and (b, bottom right) a liquid droplet formation property that mediated *P. aeruginosa* (green) adhesion. (c) Confocal laser scanning microscopy demonstrating an anammox cell cluster (*Candidatus* Brocadia sinica cells, red) with borders edged with *Chloroflexi* (blue, green arrow) with the anammox S-layer protein, BROSI_A1236 (green rectangles) coating on anammox bacterial cells. Filamentous *Chloroflexi* cells are sugar rich as verified by superimposed fluorescence image (Ciii) of *Chloroflexi* FISH staining (Ci) and Concanavalin A staining (Cii) on an anammox biofilm thin section.

Future directions

The observation of heterotrophic bacterial association in mixed species anammox biofilm system suggests the exoprotein moonlighting role as an EPS and bacteria biofilm framework builder. Future directions will focus on understanding how this protein is assembled by studying the crystal structure and is important to bridge the gap and gain deeper insights between EPS and anammox granulation mechanism, thereby extracting useful information to be applied in various fields of science.

mBio (2020) 11: e02052-02020.

Water Research (2019) 151: 1-7.

Applied Microbiology and Biotechnology (2020) 104: 3643–3654.

BESPOKE MICROSCOPY:

MEETING THE NEEDS OF BIOFILM RESEARCHERS

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Introduction

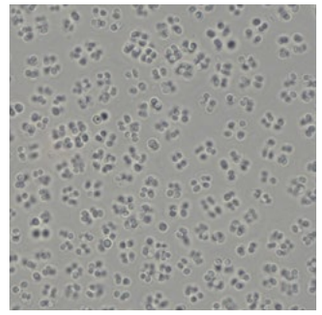
Microorganisms perform multiple functions in wastewater treatment, either to the benefit or detriment of the water treatment process. The highly complex and diverse nature of these microbial communities makes it difficult to pinpoint specific processes undertaken by certain members of the community. As a consequence, microbial participants tend to be categorised according to their potential influence on the effectiveness of removing key nutrients, e.g., whether they are polyphosphate accumulating organisms (PAOs) or glycogen accumulating organisms (GAOs). As cultured isolates from wastewater systems are extremely rare and difficult to maintain, studies have relied on 'omics-based profiling of bioreactor communities.

Having a model organism based in pure culture provides insights and understanding of the interactions and outcomes of particular cellular functions that hold importance for specific bioprocesses. The alphproteobacterial species *Defluviicoccus vanus*^{T}, a culturable organism exhibiting the GAO phenotype, is being used to develop new methods for studying molecular functions underpinning environmentally relevant functions. The genome of *Defluviicoccus vanus*^{T} was sequenced and the ecophysiology explored, with particular focus on the use of functional imaging.

As a proof of concept, Raman micro-spectroscopy was employed on plate cultures of the GAO model organism *Defluviicoccus vanus* to quantitatively characterise glycogen concentrations in live cells sampled at different growth stages. Spectra measured from individual cells provide not only the typical chemical composition of the culture but can also identify cell-to-cell variations.

Main findings

Raman spectra of individual live bacteria were measured using a custom-designed confocal Raman micro-spectroscopy system. Spectra were acquired for 20 s in each cell and approximately 20 cells were randomly selected in each sample. The baseline, fitted as a polynomial spline through portions of the spectrum excluding peaks, was subtracted from each spectrum and the spectra were normalised to the area under the largest peak (2,900–2,975 cm^{-1}). This peak contains contributions from most biomolecules and can be therefore considered indicative of the total biomass in the confocal detection volume. Spectra of individual cells were then averaged for each sample. Figure 1 shows average spectra of samples collected from one of the cultures at different times after seeding.



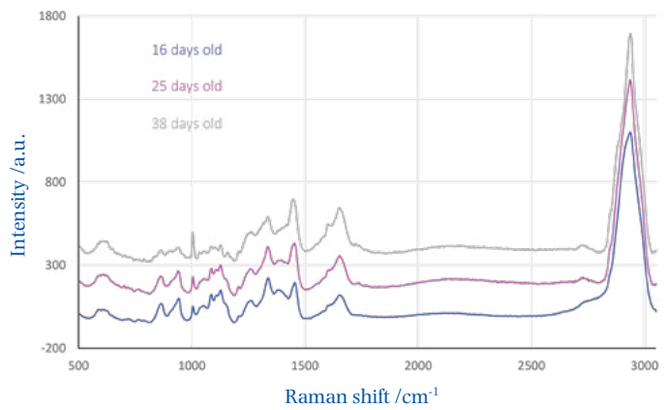


Figure 1:
A brightfield image of the sample and average Raman spectra of samples collected from the same culture at different times after seeding; 16, 25 and 38 days, respectively. The spectra have been processed and normalised as described in the text and an offset has been applied for better visibility of individual spectra in the plot.

Spectra of younger cultures (e.g., 16 days in Figure 1) contain prominent peaks typical for polysaccharides such as glycogen (e.g., between 850 and 980 cm^{-1} or 1,080–1,160 cm^{-1}). This observation has been corroborated by confocal imaging with fluorescent stain against polysaccharides (Figure 2). These peaks diminish in older cultures indicating depletion of glycogen reserves. Single-cell spectra in samples of intermediate age showed increased variability, including cells with high glycogen concentration as well as cells with nearly fully depleted glycogen reserves.

This proof of concept demonstrates the potential to track and monitor live cells under experimental conditions and without destructive sampling. The results obtained demonstrate the capabilities of Raman micro-spectroscopy to follow changes in specific metabolite concentration in live bacteria, making it a valuable tool for studying various processes of interest. The Raman experiments performed within this study have an additional significance of being the first application of the Raman micro-spectroscopy system designed and built at SCELSE.

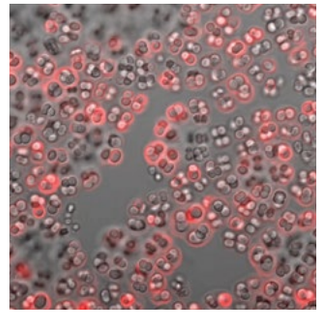


Figure 2:
Confocal imaging confirms the presence of polysaccharides in younger cultures, corroborating the findings from Raman spectroscopy.

Future directions

To fully understand the pattern of changes in glycogen concentration in the cells, additional experiments will be conducted under different conditions, such as aerobic and anaerobic, using different media and varying growth parameters. This technology complements SCELSE's capacities and expertise in biofilm and microbiome research, and can be translated to other environmentally and medically relevant microbial systems.

SHEDDING NEW LIGHT ON BIOFILM BEHAVIOUR AND MICROCOLONY DIFFUSION

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Introduction

Biofilm behaviour is substantially governed by diffusion driven processes, and direct observation of both the dimensions of interactions and of gradients themselves is necessary to account for the properties of biofilms. Light microscopy has undergone significant changes in the last decade; in particular, the introduction of light sheet microscopy has allowed for the first time the long-term observation of samples over hours and days with limited phototoxicity and bleaching. This technology ensures that only regions that are recorded are also illuminated, unlike confocal or widefield microscopy, which illuminates the whole sample irrespective of whether it is observed. In this project, a light sheet fluorescence microscope was modified to allow for quantitative spectroscopy, e.g., single particle tracking, transient state monitoring and fluorescence correlation spectroscopy.

Main findings

This research project leveraged on the hyperbolic flow cell designed at SCELSE, utilising purpose-designed flow chambers that can generate specific and controlled gradients. These flow cells, coupled with novel imaging techniques and reporters, were used to better define the physiological and chemical stratification of biofilms.

(a) Determining the distribution of oxygen concentrations in biofilm colonies

The distribution of oxygen within a biofilm can be heterogeneous and may result in bacterial cells exhibiting distinct physiological states depending on their local oxygen availability. Transient state (TRAST) monitoring is a non-invasive method that can measure the relative populations of triplet states by time-modulated illumination, to determine the distribution of oxygen concentrations in biofilm colonies. TRAST was implemented on a single plane illumination microscope that can perform optical slicing, making it a suitable approach for imaging thick samples. A map of triplet relaxation times for *Pseudomonas aeruginosa* biofilms was generated at micrometre resolution, and calibrated estimate oxygen concentrations. The results demonstrated that anoxic zones exist within the colonies, and oxygen consumption extends outside the areas of high cell densities, establishing a gradient until levels return to air saturation.

(b) Single molecule tracking for quantifying diffusion within a biofilm

The light sheet imaging platform was used to perform single molecule tracking experiments combined with fluorescent molecules of different molecular weight and charges, to quantify their diffusion through *P. aeruginosa* biofilms. The diffusional properties were compared with microcolony size as well as time of development. A spatial map of diffusion coefficients over whole biofilms in 3D was established to determine the mesh size of the matrix, heterogeneity across the biofilm and the impact of charge on diffusion. Negatively charged molecules did not penetrate the biofilm microcolonies, suggesting a charge-based repulsion, which would be consistent with a biofilm matrix largely composed of eDNA. In contrast, a 2 MDa molecule (dextran) penetrated the biofilm with a reduction in diffusion coefficient, suggesting that the biofilm acts as a molecular sieve and that a small molecule (e.g., antibiotic) would be able to penetrate the biofilm based on its size.

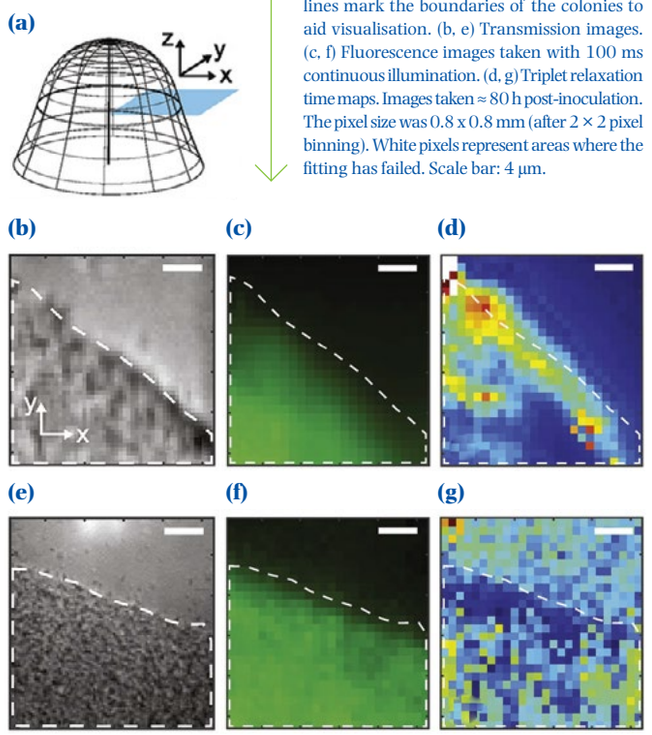

Figure 1:
(a) Schematic showing the optical sectioning of a colony. *P. aeruginosa* biofilm colonies imaged in air-saturated (b–d) and deaerated (e–g) solutions of Eosin Y (2 µm) in minimal medium. Dashed lines mark the boundaries of the colonies to aid visualisation. (b, e) Transmission images. (c, f) Fluorescence images taken with 100 ms continuous illumination. (d, g) Triplet relaxation time maps. Images taken ≈ 80 h post-inoculation. The pixel size was 0.8 x 0.8 mm (after 2 × 2 pixel binning). White pixels represent areas where the fitting has failed. Scale bar: 4 µm.

(c) Measuring biofilm stiffness

Brillouin imaging uses changes in the frequency of light interacting with a substance to reveal fine detail about the material's mechanical properties. The stiffness of biofilm colonies of different sizes over time was measured. In young colonies, stiffness increased towards the interior of the biofilm, while mature colonies had less-stiff interiors. The older biofilms may therefore have hollow interiors or may have been moving towards a phase of bacterial dispersal from the biofilm state. This non-disruptive method to study mechanical variations within and between living biofilms will help efforts to combat biofilms in clinical and industrial situations.

npj Biofilms and Microbiomes (2019) 5: 35.

Biomedical Physics & Engineering Express (2017) n3: 035020.

npj Biofilms and Microbiomes (2017) 3: 20.

Journal of Biological Chemistry (2016) 292: 477–487.

Future directions

The ability to understand biofilm behaviour and quantitatively monitor diffusion properties in microcolonies presents many opportunities for pharmacokinetic modelling of drugs and in mass transfer modelling in bioreactors for wastewater treatment. Moreover, such new approaches to biofilm imaging that do not require labelling have significant potential for further studies of mechanical properties and transport of signalling and nutrients through the microcolony.

EVALUATING GRADIENTS IN MICROBIAL BIOFILMS

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Introduction

Biofilms are inherently heterogeneous and their development is closely correlated to environmental gradients, both in the environment that they reside and within the biofilms themselves. The study of biofilms in flow cells is important as biofilms rarely grow in static environments in nature. A limitation of such flow cells is that nutrient gradients, e.g., carbon, oxygen, as well as metabolic waste products, accumulate along the length of the biofilm, based on the amount of biomass present. Such gradients are largely uncontrolled and cannot be readily defined, and the consequence is a high degree of heterogeneity of the biofilms along the length of the flow chamber. Consequently, depending on the position of imaging or biomass sampling, community organisation can vary drastically as the bacteria undergo different types of metabolism and hence, their interactions change.

To address this challenge, novel biofilm flow chamber systems were developed, based on the implementation of two-dimensional flow fields that can be used to tune gradients of nutrients in the flow cell and do so such that the concentration gradients are known.

Main findings

(a) Biofilm dispersal as a function of flow rate, colony size and position

A hyperbolic flow cell was designed to control chemical and flow gradients along the mid-line of the flow chamber. Flow patterns were computationally predicted and validated using particle image velocimetry. Using this tool, it was observed that the biofilms began to disperse after peak biomass accumulation, and this was initiated at the distal end (outlet) of the flow cell and subsequently moved towards the proximal end (inlet) of the flow cell (Figure 1). This behaviour was strongly correlated with flow rate, where at higher flow rates, more biofilm biomass accumulated and dispersal occurred later than at lower flow rates. At low flow rates, propagation of the dispersal front (from the distal towards the proximal end) was slower than when flow rates were high. These results were consistent with mass transport and nutrient limitation effects, which drive biofilm accumulation and dispersal, respectively.

(b) Development of a multichannel 3D thermopile for chip calorimeter applications

A chip calorimeter with a large sensing area and multichannel capacity was developed based on a 3D thermopile layout, for applications requiring a large sensing area or high throughputs. The temperature and heat-power sensitivity devices were characterised, and potential improvements identified for future development. Using this chip, specific temperature-dependent differences in biofilm structure development were demonstrated with *Pseudomonas putida* and *P. aeruginosa* biofilms.

(c) The effect of temperature and chemical gradients on gene expression in biofilms

Combining next generation flow cell platforms with membrane substrata enabled different sites within developing biofilms to be isolated using a laser microdissecting scope. The transcriptome response to controlled environmental cues generated within the flow chambers can be subsequently investigated through RNA sequencing.

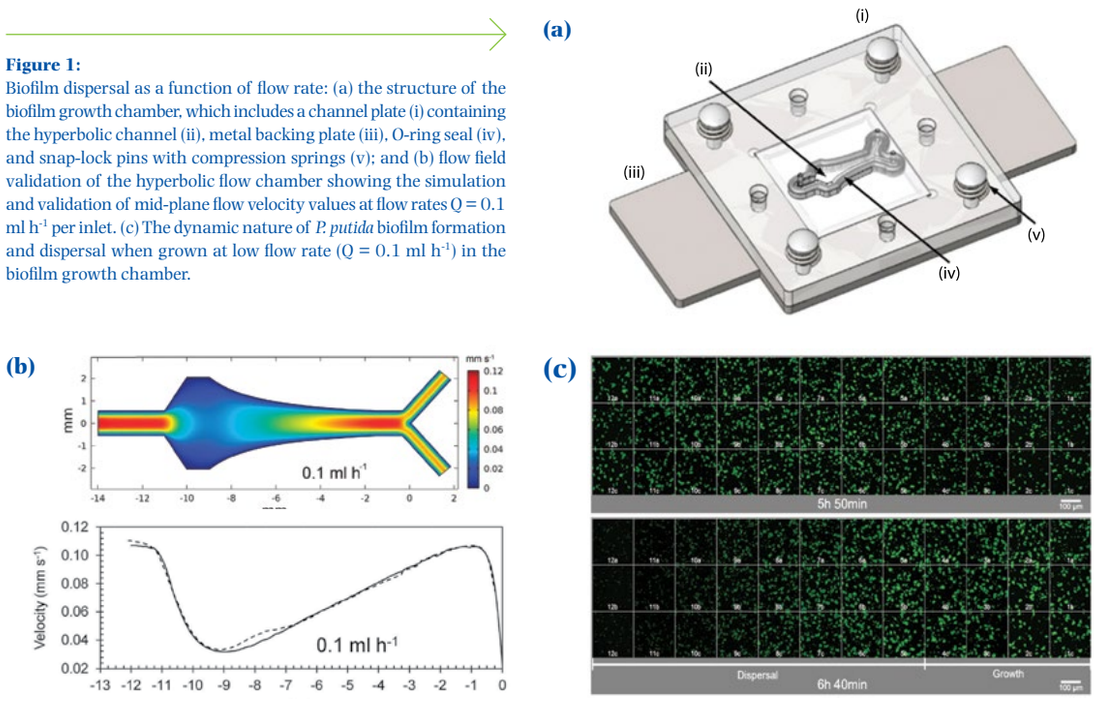

Figure 1:
Biofilm dispersal as a function of flow rate: (a) the structure of the biofilm growth chamber, which includes a channel plate (i) containing the hyperbolic channel (ii), metal backing plate (iii), O-ring seal (iv), and snap-lock pins with compression springs (v); and (b) flow field validation of the hyperbolic flow chamber showing the simulation and validation of mid-plane flow velocity values at flow rates $Q = 0.1$ ml h^{-1} per inlet. (c) The dynamic nature of *P. putida* biofilm formation and dispersal when grown at low flow rate ($Q = 0.1$ ml h^{-1}) in the biofilm growth chamber.

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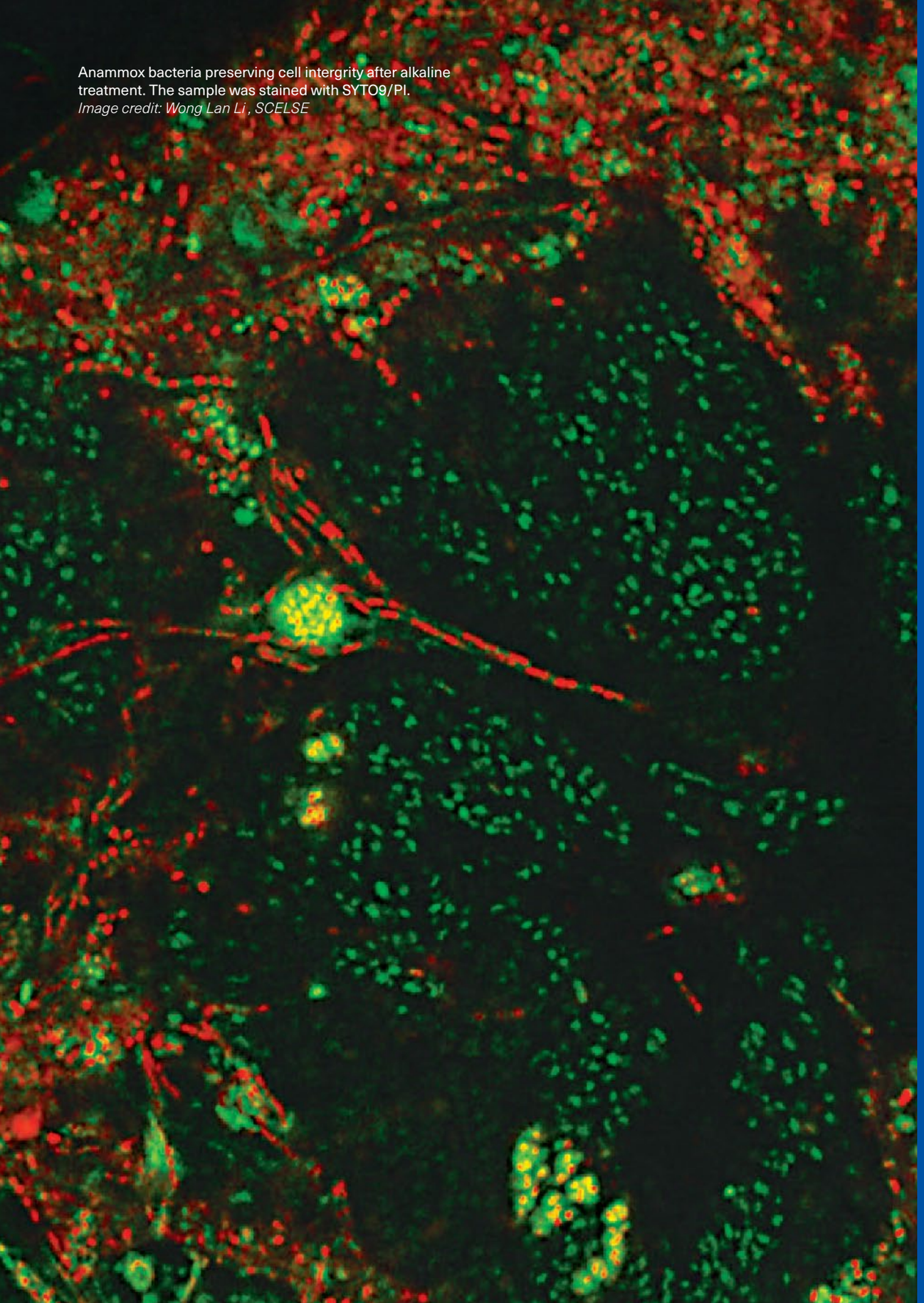
npj Biofilms and Microbiomes (2016) 2: 16023.

Sensors (2015) 15, 3351–3361.

Future directions

Flow chambers will be designed to enable temperature control. Additional design features can accommodate biofilm microdissection to collect cells for transcriptomic analysis. The flow chambers will also be designed to be compatible with various imaging platforms, such as the light sheet and Brillouin systems.



Anammox bacteria preserving cell intergrity after alkaline treatment. The sample was stained with SYTO9/PI.
Image credit: Wong Lan Li , SCELSE

RESEARCH THEMES

Urban water cycle

SCELSE's urban water cycle research covers the microbial processes associated with major components of the urban water cycle – used water treatment; engineered waterways (concrete open storm water canals); and drinking water distribution. Using comprehensive bioreactor biology, meta-'omics and systems biology analysis, and an ecological framework, this research systematically assesses microbial community composition, structure, function and resilience. The focus is directed towards elucidating the key role microbes play in the removal of excess organic carbon/nutrients and pollutants in wastewater treatment, bioremediation of engineered waterways, and safe supply of water to the city. The research outcomes drive the translation of fundamental biological insights into sustainable water management and practices.

ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL IN TROPICAL CONDITIONS

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Introduction

Enhanced biological phosphorus removal (EBPR) is one of the most sustainable processes to remove phosphorus (P) from wastewater. In EBPR, phosphorus is accumulated intracellularly by polyphosphate accumulating organisms (PAOs) in the presence of oxygen. The accumulated polyphosphate in PAOs can then be removed when the sludge containing the PAOs is from the tank. It is conventionally assumed that the EBPR process cannot thrive in tropic regions due to the higher ambient temperature since temperature >25°C typically favours glycogen-accumulating organisms (GAOs), which are competitors of PAOs. EBPR also typically requires anaerobic/aerobic or anaerobic/anoxic cycling conditions to proceed. This research investigated the presence of EBPR observed in a Singapore wastewater treatment plant (WWTP) with an ambient temperature range of 30–32°C that adopted the Modified Ludzack-Ettinger (MLE) system, which contains only anoxic/aerobic zones without a defined anaerobic zone.

Main findings

The presence of EBPR activities in the laboratory-batch study using sludge obtained from the activated sludge (AS) tank in a local WWTP was confirmed. This was evident from the P release during the anaerobic or anoxic stage and P uptake during the aerobic stage, a cycle that is indicative of EBPR activity. Moreover, EBPR activities were observed in the AS tank as well. PAOs and GAOs were constantly detected in the sludge collected from the plant (Figures 1).

As EBPR activities occurred in the tank without defined anaerobic zones, the organisms responsible for these activities were investigated. The presence of *Ca.* Accumulibacter as the only PAO group in the sludge was confirmed, with Type II Accumulibacter consistently detected at higher abundance than Accumulibacter Type 1. Among the multiple Accumulibacter strains observed, Clade IIC was also detected as the most abundant PAO in the sludge at both sampling dates. Type II Accumulibacter taxa are considered non-denitrifying PAOs (non-DPAOs) since they cannot reduce nitrate while Type I was identified as DPAO since these taxa are able to couple P uptake with nitrate reduction.

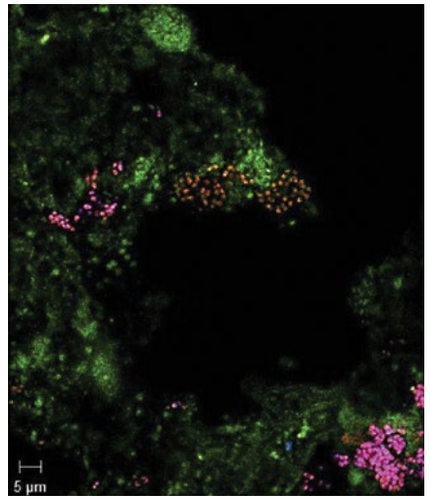

Figure 1: Fluorescence imaging of activated sludge from local WWTP. Bacteria hybridised with EUBMix probe(green), Accumulibacter-PAOs hybridised with PAOmix (Red), and Accumulibacter-PAOs Type II hybridised with ACC-II-444 (blue). Cells hybridised to both PAO probes appear magenta.

Non-DPAOs were more abundant than DPAOs in the samples. Hence, it was postulated that non-DPAOs were the organisms driving the EBPR observed in the tank without the anaerobic zones. Indeed, when either acetate or propionate was used in batch laboratory experiments, EBPR activities occurred under anaerobic/aerobic conditions using acetate or under anoxic/aerobic conditions using either acetate or propionate. The presence of EBPR in the anoxic/aerobic reactor fed with propionate suggested the participation of non-DPAOs since DPAOs could only use acetate and not propionate. The ability of non-DPAOs to use both acetate and propionate provides a competitive advantage over DPAOs.

Comparable EBPR activities between anaerobic/aerobic and anoxic/aerobic conditions when acetate was used as a carbon source further supported the hypothesis that non-DPAOs could perform EBPR under anoxic/aerobic conditions. Hence, non-DPAOs in the sludge recognised the anoxic condition as pseudo-anaerobic.

EBPR was demonstrated to occur in conditions conventionally believed to be unfavourable to the process (i.e., higher ambient temperature and lack of a defined anaerobic zone). Hence, this discovery has opened up the possibility for wider application of this method in tropical countries like Singapore.

Future directions

Different WWTPs adopt different treatment methods/designs. A wide variety of carbon sources is also present in these plants. Since these differences might lead to different microbial community profiles, future studies should aim to explain how these differences affect the EBPR activities in the plants.

Water Research (2017) 111: 393-403.
Scientific Reports (2016) 6: 25719.

ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL AT 35 °C

Qiu Guanglei[1], Law Yingyu[1], Rogelio Zuniga-Montanez[1], Deng Xuhan[1], Lu Yang[1], Samarpita Roy[1], Sara Swa Thi[1], Hui Yi Hoon[1], Thi Quynh Ngoc Nguyen[1], Kaliyamoorthy Eganathan[1], Liu Xianghui[1], Per H. Nielsen[1,2], Rohan Williams[1], Stefan Wuertz[1,3]

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Introduction

Our previous studies have shown why enhanced biological phosphorus removal (EBPR) from municipal wastewater can be stable at temperatures as high as 30 °C in both laboratory-scale reactors and full-scale treatment plants. In the context of a changing climate, the ability to perform EBPR at even higher temperatures is of interest. Hence, EBPR at 35 °C was explored, using laboratory-scale sequencing batch reactors (SBRs).

Main findings

Stable and nearly complete phosphorus (P) removal was observed in two SBRs operated at 30 °C and 35 °C (Figure 1) and 16S rRNA gene metabarcoding analysis showed a diverse range of *Candidatus* Accumulibacter (*Ca*. Accumulibacter) amplicon sequence variants (ASVs). These polyphosphate accumulating organisms (PAOs) are closely related to those found in temperate environments, suggesting that EBPR at elevated temperatures does not require a specialised PAO community.

A slow-feeding strategy effectively limited the carbon uptake rates of glycogen accumulating organisms (GAOs), allowing PAOs to outcompete GAOs at both temperatures. *Candidatus* Competibacter was the main GAO, along with cluster III *Defluviicoccus* members. These organisms withstood the slow-feeding regime, suggesting that their bioenergetic characteristics of carbon uptake differ from those of their tetrad-forming relatives. Comparative cycle studies revealed higher carbon and P cycling activity of *Ca*. Accumulibacter when the temperature was increased from 30 °C to 35 °C, implying that the lowered P removal performance at 35 °C was not a direct effect of temperature, but a result of higher metabolic rates of carbon (and/or P) utilisation of PAOs and GAOs, the resultant carbon deficiency, and escalated community competition. An increase in the total organic carbon (TOC)-to-PO_4^{3-}-P ratio (from 25:1 to 40:1) effectively eased the carbon deficiency and benefited PAOs. In general, a slow-feeding strategy and sufficiently high carbon input favoured high and stable EBPR at 35 °C, representing basic conditions suitable for full-scale treatment plants experiencing higher water temperatures.

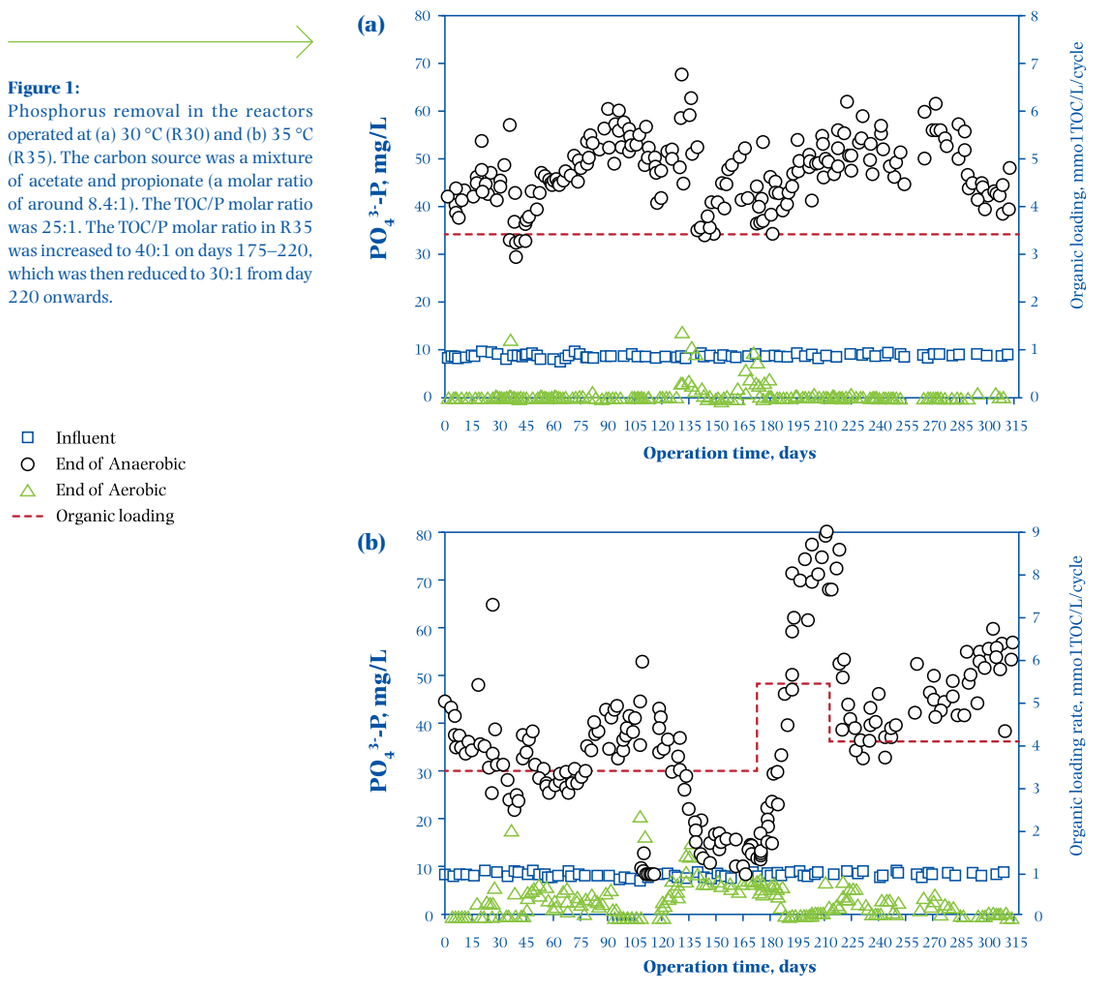

Figure 1:
Phosphorus removal in the reactors operated at (a) 30 °C (R30) and (b) 35 °C (R35). The carbon source was a mixture of acetate and propionate (a molar ratio of around 8.4:1). The TOC/P molar ratio was 25:1. The TOC/P molar ratio in R35 was increased to 40:1 on days 175–220, which was then reduced to 30:1 from day 220 onwards.

Future directions

Individual GAOs had a different response to the slow-feeding strategy. Hence, deeper investigations into the bioenergetic characteristics of these organisms and the PAOs involved in EBPR 35 °C would help prepare wastewater treatment plants for new challenges in the context of a warming climate.

DIVERSE CARBON SOURCES IN WASTEWATER TREATMENT PLANTS

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Introduction

SCELSE research has demonstrated the presence of enhanced biological phosphorus removal (EBPR) activities in a wastewater treatment plant (WWTP) in Singapore, despite the higher temperature and lack of defined anaerobic zone, two conditions that were typically viewed as unfavourable for EBPR. Diverse carbon sources might also be present in these WWTPs. Hence, a further comparison was made of the composition of key-functional polyphosphate-accumulating organism (PAO) communities in three full-scale WWTPs in Singapore with high *in situ* EBPR activities, and EBPR-associated carbon usage characteristics. How carbon usage influences community assemblies in these WWTPs was also elucidated.

Main findings

Using 16S rRNA gene amplicon sequencing, each WWTPs was determined to have a signature community composed of diverse putative OTUs related to *Candidatus* Accumulibacter, *Tetrasphaera* spp., *Dechloromonas* spp., and *Ca.* Obscuribacter (Figure 1). The distinct relative abundances of these OTUs at different WWTPs suggested that each WWTP had its own signature EBPR community.

Different carbon sources can be used by the PAOs in the sludge, as observed in the batch-scale laboratory tests using sludge collected from WWTPs, volatile fatty acids (VFAs) led to the highest phosphorus (P) release patterns across the different plants indicating the importance of VFAs in tropical EBPR systems.

Pearson correlation was employed to discern the preferences of different PAOs to the different carbon sources. A strong correlation (Pearson correlations >0.91 with P <0.0001) resulted between the total *Ca.* Accumulibacter and all VFAs, pyruvate and fumarate. A strong correlation was also detected between *Ca.* Accumulibacter and some amino acids. Additionally, different lineages of *Ca.* Accumulibacter were associated with P release induced by distinct carbon sources suggesting the presence of diverse members with versatile carbon metabolism. *Ca.* Obscuribacter showed no correlation between P release and any of the carbon sources tested, indicating the need for further study of its ability to utilise different carbon sources. *Dechloromonas* operational taxonomic units (OTUs) could not be unambiguously assigned to the P release stimulated by any of the tested carbon source.

Selected carbon sources were used in anaerobic-aerobic cycle studies to further investigate their EBPR capabilities. Highest P release was observed in WWTP2 sludge that contained highest relative abundance of PAOs (especially *Ca.* Accumulibacter). Highest P release was also observed when wastewater and VFAs were used as carbon sources. Acetate led to the highest P-uptake possibly because acetate was the major VFAs in all these plants. Interestingly, propionate-fed system displayed the lowest P release possibly due to the needs for the PAOs to acclimate to different intracellular storage polymer content.

These results showed the presence of characteristic PAO and glycogen-accumulating organism (GAO) communities in the WWTPs. Although *Ca.* Accumulibacter, the most abundant PAO, was capable of P release using a wide range of carbon sources, VFAs remained the most effective carbon source.

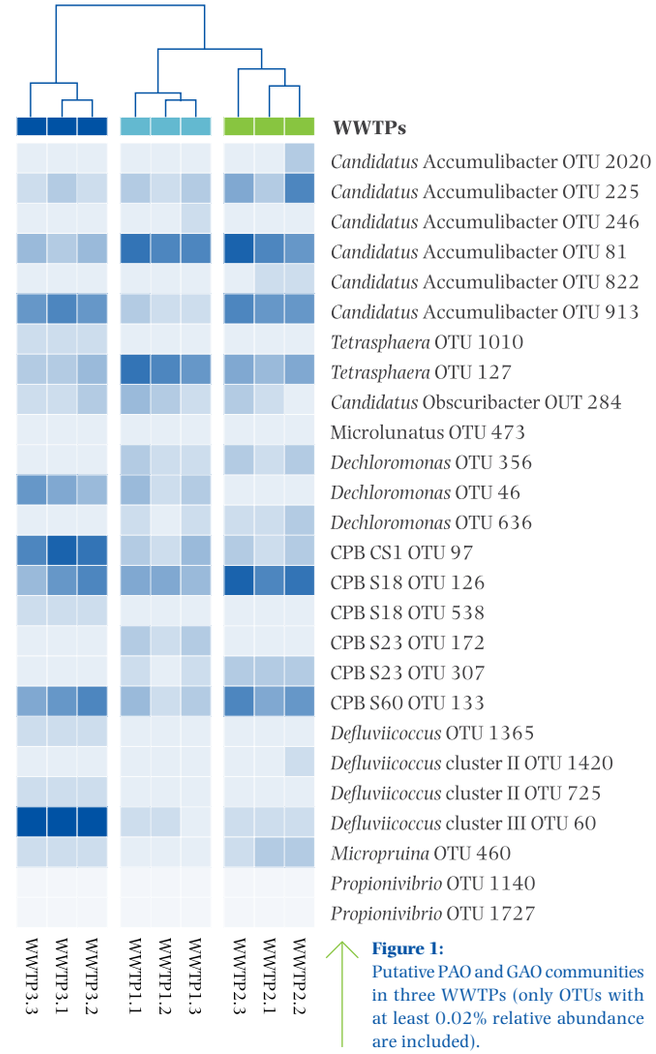

Figure 1: Putative PAO and GAO communities in three WWTPs (only OTUs with at least 0.02% relative abundance are included).

Water Research (2019) 149: 496-510.

Future directions

The dominant presence of *Dechloromonas* in the three WWTPs highlights the potential of PAOs other than *Ca.* Accumulibacter to drive EBPR at even higher wastewater temperatures than currently found in Singapore, a likely scenario if global warming trends are not abated.

UNDERSTANDING ECOLOGICAL FUNCTIONING OF URBAN FRESHWATER ECOSYSTEMS

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Introduction

The pressures on urban water resources in megacities have led to a growing interest in engineering ecologically friendly urban waterways that allow and enhance microbial community-derived services. These ecological services are critical to self-cleaning capacities of waterways. In megacities, ecological approaches for such purposes face challenges arising from complex interactions within microbial communities, densely interspersed land-use patterns and anthropogenic influences. Therefore, to inform and develop such ecological approaches, it is necessary to gain an understanding of the interactions between microbial communities and urbanised environments at various scales, ranging from sediments to large catchments and regions. These are being investigated using a combination of multiple culture-independent, meta-omics and data-intensive computational approaches.

In the tropics, rain events are a major source of disturbance. They introduce sediments of different types, complex mixtures of inorganic and organic molecules/nutrients and microbial communities associated with sediment and water phases. The characteristics of rain events and hydrodynamics in the stormwater canals, which in turn, are based on the canal design, influence the extent of disturbance and the ensuing changes or microbial community succession in the canal system. Microbial communities develop biofilms both on biotic (plants) and abiotic surfaces (sediments and other solid structures). Sedimentary biofilms are the major sources of the microbial load and biodiversity that provide multiple ecosystem services in the waterway system. In this project, field-based investigations were combined with the development and use of novel experimental tools to combine observational and hypothesis-driven studies. These approaches have elucidated sediment microbial communities, their functions and their environmental drivers across different spatial and temporal scales.

The initial studies using a model catchment identified sediment as the dominant microbial habitat, which prompted the investigation into the influence of habitat type on their assembly and behaviour. These were complemented in parallel with studies on post-rain microbial dynamics/succession using novel mesocosm-scale reactor systems that corroborated the influence of similar environmental drivers across different scales.

Main findings

Microbial services in engineered waterways: an ecogenomics approach

A field study was initially conducted to understand the interactions between microbiomes and the environmental parameters under different land-use pressures. This study addressed the influence of pressures from urbanised catchments with mixed and interspersed land-use patterns on the composition and functions of sedimentary and suspended microbial communities of these waterways. Physical separation of the same land-use types did not influence the assembly of microbial communities. Differences were apparent between land-use types, both at the level of functional potential and phylogenetic taxa but not at the level of chemical markers. Sediments harboured more diverse and complex communities than did particles suspended in the water. In the well-managed urban waterways of Singapore, it was found for the first time that where levels of physicochemical parameters are low, metals such as aluminium, copper and potassium, rather than organics, are the major driver of microbial community composition and potential function.

Sedimentary microbial communities dominate microbial life within urban waterways, with capacity to drive ecosystem services. Hence the influence of sediment particle size on the structure and function of such communities was investigated. Size separation revealed that the sand fractions dominated sediment size composition. Microbial communities on the dominant size fractions (coarse and fine sand) were substantially different. Preferential enrichment of bacterial lineages on either of the two size fractions was apparent, even at broad phylogenetic levels (e.g., class level). The fine scale variation in sediment size likely supports the co-existence of a number of closely related taxa through niche partitioning and specialisation.

Gene-centric analysis showed that communities attached to the two size fractions also differed in their capacity to carry out important nutrient transformations. Key marker genes involved in the transformation of inorganic nitrogen and sulfur compounds were partitioned across the size fractions. The genes involved in the metabolism of aromatic compounds and response to stressors also showed a significant association with particle size fractions, establishing association with niche functioning.

To link phylogeny to function, 202 strain-resolved draft genomes from the different size-specific niches were constructed. These genomes span both abundant and rare bacterial lineages in the sediment microbiomes. Functional analysis showed that a majority of taxa only possess the ability to carry out singular redox transformations of inorganic nitrogen and sulfur compounds, for energy metabolism.

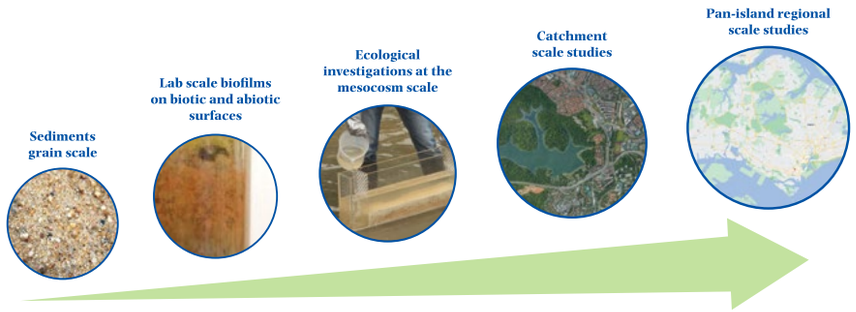

Figure 1:
Research framework to understand the ecological functioning of microbial communities in urban waterways.

Effects of land use and rain perturbation on microbial communities in tropical urban waterways

Two representative industrial and residential locations were used to determine the effects of land-use (residential versus industrial) and rain perturbations (pre- versus post-rain) on the microbial communities of the sediments of Singapore's urban waterways. The hypothesis proposed that changes in microbial community structure and function are triggered by perturbations due to rain and/or differences in land-use types. This represents the first reference metagenome from tropical urban waterways. This large data set, using a combination of network and multivariate analyses, yielded important insights into the role of land-use and, to a lesser extent, rain perturbations in causing shifts in the abundance of specific microbial populations.

The combined metagenome of benthic microbial communities in urban waterways was composed of 75 phyla and 4,163 species (OTUs). At genus level, the most reads were from Nitrospirae (*Nitrospira*) and Cyanobacteria (*Coleofasciculus*). More than 99% of the mapped reads in the sediments of urban waterways were represented by 23 phyla (~30%). Benthic microbial communities in residential land-use were more diverse and dispersed than in industrial sediments. The functions of microbial communities were less affected by environmental factors, compared to the structure of microbial communities. This indicates that core microbial functions do not change with changes in taxa composition to survive in challenging environments. The study showed for the first time that urban waterways with low contaminant levels can maintain the microbial diversity akin to undisturbed freshwater systems.

Four aspects of post-rain succession of microbial communities, their functions and impact in relation to the water and sediment environment were studied. (1) Using a novel reactor system that mimics a linear channel flow first, succession was explained in terms of shifts in the metabolically active member of the microbial community compared with the full community. (2) The metal and ion interactions during biogeochemical successions and their associations with microbial communities were identified. (3) The biogeochemical successions were explained through functional successions, and (4) the members of microbial communities involved in these transformations were identified.

Three major phases in biogeochemical succession after a rain event were identified: acclimatisation, reductive and oxidative phases. The highest number of parameters were involved in the acclimatisation phase. The second reductive phase appeared with high levels of nutrient (nitrogen and/or sulfur species) and metal combinations. Finally, the oxidative phase showed the emergence of some metal, oxidised nutrients, such as sulfates, redox potential and cell counts.

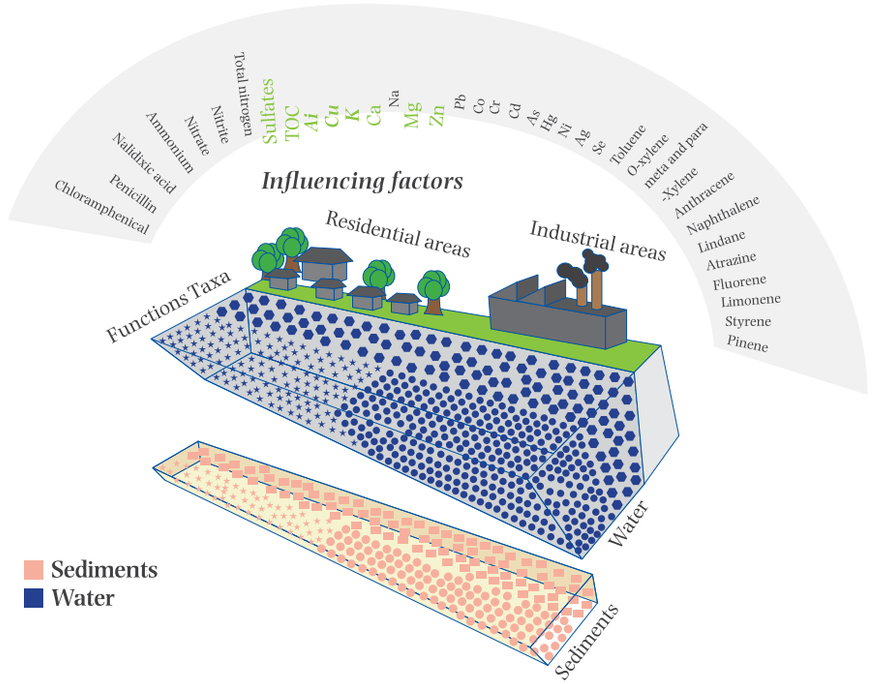

Figure 2:
Key drivers of microbial community composition in urban waterways at the catchment scale.

Future directions

Having identified the taxa groups and selected functions leading these coupled nitrogen-sulfur-metals transformations, the project will pursue the mechanism underlying the coupling of metals with these transformations through controlled manipulative experiments.

This study has revealed a deeper understanding of change in microbial community composition succession in sediments following rain disturbance. Further investigation will involve the spatial contribution of land cover types (geochemical and microbial load contribution) to waterways in Singapore.

The Government of Singapore has embarked on an ambitious 'Singapore Green Plan 2030' to achieve long-term sustainability goals such as net zero emission by 2030. One of the key pillars of the plan is the greening of Singapore aptly called City in Nature. By 2030, the government aims to increase nature parks' land area by more than 50% from the 2020 baseline as well as plant more than one million trees in Singapore. At an island scale this will lead to an increased contribution of organics through run-off onto the waterways. As such, studying the effects of different functional groups of organics on microbial community structure and function will be investigated. Changes in the bioavailability of metal ions to microbes will be investigated as these processes determine key biogeochemical pathways, e.g., those linked to greenhouse gas emissions.

Environmental Science and Technology (2015) 49(3): 1462–1471.

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Sarcophyton sp., a species of soft coral in Singapore's intertidal waters.
Image credit: Lindsey Deignan, SCELSE

RESEARCH THEMES

Marine biofilms and microbiomes

SCELSE integrates marine research across microbiology, ecology/ecological theory, chemistry, genomics and engineering, with inter-institutional collaboration in a nationwide programme. Research projects capitalise on Singapore's unique urban setting and tropical marine diversity, and harness the capacity available at the St John's Island National Marine Laboratory, a national infrastructure facility.

SCELSE's marine research focuses on ecosystem-wide effects and microbial processes. Holobiont systems (a host organism and its associated microorganisms) are studied to assign roles in imparting ecosystem resilience and microbially mediated responses to climate change. Marine microbiomes and biofilms are investigated for their effects on a range of marine ecosystem processes, from benthic community formation, biodegradation, and blue carbon, to corrosion of submerged structures.

ENGINEERING SINGAPORE'S SEAWALLS TO ENHANCE BIODIVERSITY

Stephen Summers[1], Peter A. Todd[2], Philip Liu[3], Tan Koh Siang[4], William Birch[5], Scott Rice[1,6]

Securing experimental marine biodiversity enhancement tiles to an existing seawall in Singapore. *Image credit: Marine Science R&D Programme, Singapore*

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Introduction

It is estimated that more than half of the world's population live in coastal locations (< 100 km from ocean), which will increase with global population growth. Concurrently, with a changing climate, sea levels could rise by up to 80 cm by the end of this century. This has inspired many nations with coastal exposure to reinforce their defences against sea level rise to avert catastrophic impacts on coastal inhabitants. One such defence commonly employed is the seawall, which is usually featureless and constructed of materials that are less amenable to colonisation by marine organisms. Despite their negative impacts on coastal habitats and environments, seawalls are deemed a necessary trade-off to negate the threats posed by rising oceans. Hence, it is prudent to maximise the ecological potential of seawall installations.

For this endeavour, different materials, coatings, and textured tiles were evaluated for their ability to support a healthy and biodiverse marine community, from the micro to the macro scale. The aim was to ultimately convert a hostile engineered structure into a well-functioning living component of a healthy coastal ecosystem, through understanding means by which the primary microbial colonisers impact the settlement of higher organisms.

Main findings

Biodiversity and community dynamics

Given the scale of the manmade seawalls surrounding Singapore, it was important to ascertain the current biological diversity of the coastal areas. Several surveys documented and characterised the physical and biological state of the seawalls and compared these to the natural rocky shore environment. Species richness and community composition of the residents of these two environments were significantly different, confirming the seawalls were indeed impacting the natural coastal communities.

A series of field- and laboratory-based experiments elucidated how the physical and biological dynamics of a seawall environment impact the ecological community overall, for modelling. The predator-prey dynamic was observed, impacts of seawall hydrodynamics were assessed to determine the impact on invertebrates, and the thermal properties of the seawalls were measured to determine how the thermal ecology differed to that of a natural rocky shore.

Upgrading of existing seawalls

This project investigated a series of measures to ecologically enhance an existing seawall. The main focus was the installation of topographically complex plates called "BioBoss" tiles around Singapore's shoreline, which were monitored for community formation compared to established communities. The tiles provided increased topographical complexity for the seawall as well as different materials used for seawall construction. Increased complexity resulted in increased biodiversity, at several measured scales. Bacterial diversity was impacted at the scale of nanometres (surface texture), while larger macroorganisms were susceptible to larger, centimetre-scale complexities. In a larger scale experiment clusters of tiles covering around 15-20% of the seawall resulted in the largest increases in biological species richness, compared to adjacent unmanipulated seawalls.

The impact that biodegradable polymers may have on seawall community settlement was assessed. Biodegradable polymers,

seeded with various polysaccharides, were applied to granite surfaces. The polymers initially developed a layer of extracellular polysaccharide, from bacterial and algal origins, before appearing to degrade. The polymers both masked the chemical signature of the granite but also slowly released the polysaccharides into the water column. An initial effect on microbial biofilm formation was observed but this was diluted over time to become immeasurable within one month. This period, however, could be sufficient to influence the settlement of higher organisms, as microbial primary colonisers direct the settlement of subsequent colonisers, such as algae and sessile invertebrates.

Seawall tile materials

Microbial settlement was assessed on concrete (typical seawall material), two sandstone variants, marble, granite and limestone, to determine the impacts the materials had on initial biofilm formation. Different materials promoted microbial biofilm formation at different rates, yet within four weeks any influence from the underlying material was no longer observable in the biofilm community. This was repeated for invertebrate and algal settlement and, similarly, there was no significant effect of different material types on settlement.

Invertebrate settlement assays

Biodegradable polymers spiked with polysaccharides can enhance invertebrate settlement. Larval settlement was also assessed for the different seawall materials (granite, limestone, marble and sandstones), with calcium-based rocks more likely to promote coral settlement. Specific bacterial groups selectively attracted different invertebrates, demonstrating highly complex invertebrate-microbial interactions, supporting previous studies.

Future directions

This was the first interdisciplinary seawall ecoengineering project to be conducted in a tropical environment, providing valuable insights to this worldwide issue. An international network of collaborators has been established. Future challenges involve upscaling this work, including developing an ecologically appropriate method of eco-engineering that can be delivered at multiple levels, nationally and internationally.

ADAPTATION AND RESILIENCE OF CORAL REEFS IN SINGAPORE

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[5] iThree Institute, University of Technology Sydney, Australia

Introduction

The Central Indo-Pacific that encompasses Singapore is home to the highest diversity of reef species in the world. Despite high maritime and coastal stresses, Singapore hosts a remarkable diversity of corals, with 255 species of reef-building corals. Since the early 1960s, numerous reef habitats have been lost to land reclamation for urban uses, including petrochemical industries and port facilities. The remaining reefs experience relentless chronic stressors, including sediment damage due to land reclamation and seabed dredging, leading to cumulative losses of more than 60% of the original reef area and up to 37% of documented coral species.

The prevailing paradigm for coral reefs dictates that such chronic impacts would shift coral-dominated reefs to degraded rubble covered with seaweeds, but reefs in Singapore continue to support diverse and resilient coral communities. To understand how these species-rich habitats continue to endure, the adaptation and resilience of coral reef organisms were studied, investigating the responses of reef corals and their associated microbiomes to heat and sediment stresses, as well as competition with seaweeds. This project was coupled with research focusing on the genealogical and environmental history of Singapore's reefs over the last few millennia. By extending the historical record of how corals reacted to environmental change, and explicitly testing alternative hypotheses on how corals and their symbionts have responded to environmental stressors in the past and present, the project has begun unravelling how Singapore's reefs have persisted in one of the most urbanised marine environments in the world.

Main findings

Ecological and microbial ecology of coral reefs

Reef corals live in symbiosis with multiple microbial organisms, principally the microalgal endosymbionts known as Symbiodiniaceae, as well as Bacteria, Archaea, Fungi and viruses. These associations are modified or can break down during times of stress. For example, heat stress can drive the expulsion of the algal endosymbionts, resulting in coral bleaching. To investigate responses of the coral holobiont – comprising the coral host, Symbiodiniaceae, and microbiome – to contemporary stresses, comprehensive characterisations were made of these microbial components in Singapore's reef corals. Analyses showed that the Symbiodiniaceae communities were generally low in diversity, which could be a result of the strong selective pressure exerted by the prevalent turbidity.

Bacteria associated with corals and reef sediment were also examined, showing fine-scale spatial and temporal differences in bacterial composition. For example, corals at the lee and windward sides of the Southern Islands host distinct bacterial communities. A yearlong monitoring programme of two coral species on three reefs in Singapore also revealed a temporal shift in the microbiome with differences maintained between sites. A 10-day reciprocal transplantation study of corals between sites further demonstrated that coral microbiomes responded quickly to transplantation – even within a day – and remained dynamic throughout the experiment.

Reefs in Singapore have been impacted by sedimentation. Controlled experiments found that heat elicits extensive responses from both the coral host and endosymbionts, but a combination of heat and sedimentation stressors can have synergistic effects on gene regulation in the coral holobiont, accompanied by photophysiological responses.

The coral microbiome is sensitive to both direct contact and water-mediated interactions with seaweeds, while coral physiology is compromised only when in direct contact. Exposure of coral larvae to increasing concentrations of seaweed exudates generally leads to increased larval mortality and decreased larval settlement rates. Microbial response also varies with the concentration of algal exudate rather than the specific algal species.

This project has uncovered the genetic and ecological connectivity of organisms such as corals, sea anemones, and symbiont communities including Symbiodiniaceae and bacteria. Additional organisms and microbial components need to be characterised for their distribution and genetic lineages to explain connectivity at various levels – from communities, species and populations to microbiomes. This information is necessary for prioritising sites for habitat protection as they may be critically connected genetically and ecologically to affect recovery potential during times of stress, especially as the impacts of heat stress and sea-level rise are set to increase in intensity and frequency.

Coral adaptation and resilience to environmental stress was investigated through experimental manipulation and examination of coral reefs located around the Southern Islands of Singapore. *Image credit: Lindsey Deignan, SCELSE*

Future directions

Understanding such interactions with increasingly sophisticated approaches, as obtained in this study, enables the coral holobiont to be used as a biomonitor of environmental change, providing probable trajectories of coral health in the region. This will provide an effective early-warning system that enables rapid and precise projections of future reef conditions to be established.

The ability to bioengineer the coral holobiont in an informed way provides a mechanism to promote recovery and resilience and hence optimal ecosystem services.

Frontiers in Marine Science (2020) 6:831.

Microbial Ecology (2022) 83:608–618.

INSIGHTS INTO VIRAL-PHYTOPLANKTON DYNAMICS OF SINGAPORE WATERS

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Introduction

Viruses are the most abundant biological entities in the oceans, and they can shape phytoplankton composition by reducing the abundance of certain taxa while, simultaneously, regulating the fluxes and export of nutrients through the food chain (Figure 1). Viruses can cause large phytoplankton blooms to collapse, and the use of viruses as a biological control agent to artificially terminate harmful algal blooms (HABs) in coastal waters has become a focus of investigation. Viral control is especially important for HABs because, due to chemical and mechanical defences, they are generally not subject to heavy grazing pressure by zooplankton.

This project used an integrated analysis of viral and microbial assemblages in a time series spanning more than two years to uncover the basis of the relationship between viruses and their planktonic hosts in Singapore coastal waters, where HABs occur and cause significant economic damage to the aquaculture industry.

Main findings

Both technological and ecological insights have been gained as a result of this research programme. A rapid and precise identification approach for phytoplankton species, specifically targeting HAB species, has been developed using third-generation sequencing technology. The MinION sequencer (Oxford Nanopore) was used to sequence the whole 18S rRNA gene as well as a portion of the 28S rRNA gene. The high error rate associated with Nanopore sequencing technology is a significant disadvantage. To overcome this constraint, a custom bioinformatics workflow was established that uses sequence redundancy to compensate for the inaccuracies. The MinION sequences were subsequently compared to precisely annotated sequences obtained using either Sanger or Illumina sequencing. As a result, this technology can now quickly and precisely identify phytoplankton blooms in Singapore, which occur on an annual basis and serve as a warning sign to fish farmers.

Moreover, the long-read sequences obtained using this method have been used to construct the Long Amplicon Sequence Repository (LASeR), which is accessible at laser.ase.ntu.edu.sg.

At the same time, this project has established the first long-term observatory for the viriome (the collective biome of viral communities) in Singapore coastal waters by collecting monthly samples of phytoplankton and viruses at five different stations, together with the associated oceanographic parameters (temperature, salinity, nutrients, DOM/POM).

The viral population in each sample was identified using a hybrid strategy with two sequencing technologies (Illumina and Nanopore). Thousands of nearly entire viral genomes could be recovered from sequence assembly, revealing that viruses employ a variety of distinct life strategies in response to fluctuations in the availability of nutrients and host organisms. Additionally, the research discovered seasonal dynamics of viral communities, primarily influenced by the monsoon systems. The viral composition was found to be unique to Singapore and the Johor Strait, reflecting the difference in trophic status between the two habitats.

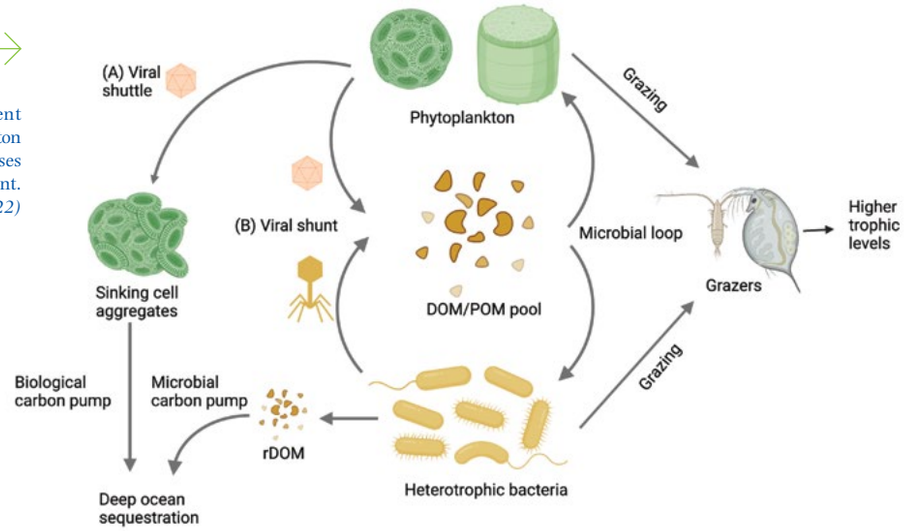

Figure 1: Viral control of nutrient carbon fluxes, phytoplankton populations and processes in the marine environment. *Image credit: Viruses (2022) 14: 702*

Future directions

The opportunity to develop novel methodologies in metagenomic sampling and sequence analysis has expanded our capacity to obtain regular time-series data of microbiome and viriome samples at five locations throughout Singapore. In the future, broadening the scope of this research to include higher resolution time-series and the study of RNA viruses, rather than just DNA viruses, will reveal new important details about the interplay between HABs and their viruses in Singapore. A new solid-phase PCR approach is being developed to provide quantitative insights into the absolute abundances of major RNA virus types as a function of changes in environmental conditions. This will expand the understanding not only of the top-down regulation of planktonic communities by viruses, but also their role in biogeochemical cycling.

Viruses (2022) 14: 702.

PLASTIC DEBRIS: BIOFILM- AND MICROBIOME-BASED SOLUTIONS

Sakcham Bairoliya[1,2], Cao Bin[1,2], Ana I. Catarino[3], Simon Cragg[4], Theodore Henry[5], Jamie Hinks[1], Jonas Koh Zhi Xiang[1,2], Federico M. Lauro[1,6], Fei Xunchang[2], Lim Doa Wei[1], Sabine Matallana-Surget[7], Nur Hazimah Mohamed Nor[6], Stephen Summers[1], Janelle Thompson[1,6], Zin Thida Cho[1,2]

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Introduction

Over the past decade, marine plastics and microplastics have received much media attention; pictures of animals ensnared in plastic litter emotively fuelled this public interest. As the world recovers from COVID-19, such images will likely resurface, but this time with masks, face shields and takeout dining containers littering the shoreline and endangering wildlife. The mundane reality, however, is that most of the marine plastic litter quietly sinks to the seabed where it is conveniently forgotten. Knowing the fate of this plastic litter is important as the environmental impacts of plastic debris are well documented, and if plastics persist in the marine environment, then the impacts too are persistent.

Researchers within SCELSE are working on several aspects of plastic debris. They are monitoring the oceans to provide an accurate estimation of how much plastic is out there and what risks it may pose to both animal life as well as human health. To combat the issue, how the plastic debris degrades is also being actively investigated, both in nature and industrial environments. This aspect of the research aims at stemming the flow of plastic debris reaching the natural environment through optimised industrial processes. Further, the research aims to understand what happens when the plastics initially interact with microorganisms in the coastal areas of Southeast Asia as well as the eventual deposition of the plastic in the benthos sediments. The final component of the research looks to develop new types of plastic that are fit for purpose but are not going to persist in either an industrial waste or natural setting, meaning that the increases in plastic debris we see today can finally be halted and the recovery permitted to start.

Main findings

Where are we finding marine plastic debris?

A census of how much plastic is currently present in the waters in and around Singapore is investigating the sources, impacts and solutions for plastics in Southeast Asian coastal environments. SCELSE's collaborative investigation of plastic debris started in early 2021, developing an integrated field sampling protocol for rapidly collecting microplastics and the surrounding microbial community, adhering to best practices and ensuring high quality and reliable data. The next steps involve collecting microplastic and microbiome samples from Singapore, Indonesia, Thailand and Malaysia, over a two-year period, to understand the spatial and temporal variation in microplastic-associated microbial community composition.

Further insights on the degradation of the smaller plastic particles by microbes and their potential impact as a vector for transport of pathogens will be provided. This will involve state-of-the-art techniques such as acoustofluidics and metaproteomics.

Bespoke sampling setup designed to specifically target the smallest of microplastics. This filtration design can collect high volumes of water and particulate matter, including microplastics, on separate filters. In addition, this will also collect the microbial assemblages associated with these microplastics. *Image credit: Nur Hazimah Mohamed Nor, ASE, NTU*

What happens to the plastic in the ocean?

General organic matter in the ocean breaks down and is biodegraded by microbial enzymatic digestion. However, plastics are typically made from long chain polymers that are more recalcitrant to degradation, hence the increasing volume of plastics in the ocean will eventually sink to the benthos when a density threshold is met, as demonstrated by the international collaboration 'Plastics in marine snow – the fate of nanoplastics'.

Knowing the fate of this plastic litter is a global priority and it is essential that deep sea plastic biodegradation is understood under conditions that are environmentally relevant to the deep sea. Unfortunately, the skills to assess deep sea plastic biodegradation are globally rare. The combination of skills that exist among SCELSE, and collaborators from NTU's Asian School of the Environment (ASE) and School of Civil and Environmental Engineering (CEE) represent a complementary blend that uniquely positions the team to carry out such trailblazing research in the current project 'Deep sea degradation of polymer-packaging litter – a new way to assess new materials'.

How does this impact us?

The simple answer is that microplastics affect humans in our daily life and are not restricted to the marine environment. Each day, millions of plastic fibres are released into the air we breathe (e.g., from furniture and clothing), the food we consume (e.g., from packaging). There is mixed evidence on the ecotoxicology of this volume and type of plastic debris, but a precautionary approach means that we should be monitoring the point sources and plastics that we may encounter. One such source is seafood, filter feeders

Field trials have been initiated to examine the colonisation of a selection of materials (PP, PET, HDPE, oxo - HDPE) in the natural environment. With regular sampling this setup will monitor both the initial biofilm communities but also the persistent biofilm inhabitants. *Image credit: Sakcham Bairoliya, SCELSE*

ingest these particles and can transport the plastics into their tissues and then directly to the human food supply lines. This is the topic of the team's research on the 'Effects of ingested plastic particles on digestive system physiology'. Preliminary findings have shown that microplastics are present in mussels grown in Singapore's coastal aquaculture farms. Further investigations on the composition of the polymer types and size distribution of particles in these organisms will be performed. The potential for these plastics entering the food chain and acting as a vector for disease is currently under investigation.

What is the solution?

Plastic is a great resource and has allowed humans to rapidly develop technologically. Therefore, it is not feasible to phase out polymer-based building practices in the near future. One avenue open to us is biodegradable plastics. These are polymers designed to degrade in both industrial and environmental conditions. One project underway among SCELSE, the School of Civil and Environmental Engineering at Nanyang Technological University, Singapore and the Circular Materials Laboratory at A*STAR, involves laboratory and field tests for environmental degradation of biodegradable plastic packaging, focusing on Southeast Asia, and a team of commercial partners is investigating the biodegradable potential of various plastics films. To achieve this, plastic films exposed to enriched wastewater environments are being examined to monitor the rates of biodegradation. In parallel, the same plastic films are examined under exposure to natural marine conditions to understand biodegradation in the oceans. To date, several promising polymer candidates capable of breaking down and disappearing have been identified. However, more evidence is needed to confirm the ultimate fate of these new plastic films and the role microbial communities are playing.

To further address this role of biofilms in breaking down plastic debris, the enzymatic nature of the biofilms associated with plastics is being investigated. To understand plastic-bacteria interactions in the Southeast Asian marine environments, field incubation studies have been set up at Bendera Bay and Lim Chu Kang. Four types of plastics (PP, PET, HDPE, oxo-HDPE) and glass have been exposed to the natural environment, with sampling conducted at regular intervals to determine the microbial community succession over the experimental period of one year. Novel plastic degrading enzymes and organisms will be identified using high throughput sequencing and classical microbiology techniques.

Marine Pollution Bulletin (2018) 130: 258-267.

MICROBIALLY INFLUENCED CORROSION IN THE DEEP OCEAN

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Introduction

Corrosion refers to the degradation of materials due to the effects of its environment. Metallic materials tend to revert to their natural oxidation states when interacting with the environment; this process is usually very slow but can be accelerated through various abiotic and biotic processes. Microbes can colonise metal structures creating biofilms and by virtue of their metabolic activities, they create microenvironments on the metal surface that may differ remarkably from the surrounding environment and thus either hinder or speed up the corrosion process. The process where microorganisms drive the corrosion is called microbially influenced corrosion (MIC). In the marine environment, where corrosion is prevalent due to chlorides in seawater, more than 20% of the entire cost of corrosion damage can be attributed to the MIC.

In the past, MIC has been studied extensively under ambient pressure, which represents sea surface and shallow seawater conditions. An increase in the exploitation of deep-sea resources has heightened the need for deep-sea infrastructure. Therefore, understanding the performance of materials in the deep sea is becoming more vital. The deep sea has characteristically extreme temperatures, high salinity and high hydrostatic pressure. As an operating environment, the deep sea possesses challenges for infrastructure as installation, inspection and repairs are difficult and expensive. Thus, the corrosion on deep-sea structures and equipment must be thoroughly understood, closely monitored, and modelled to avoid failures but also over-engineered to ensure safe and economic operations. Despite this need, there are only very few reports describing MIC in deep-sea conditions. This programme was initiated and funded to fill this knowledge gap.

Main findings

During this research programme, major advancements in laboratory and field techniques for deep-sea MIC research have been made. This includes the development of a novel high-pressure chemostat with simultaneous real-time monitoring of corrosion rate, resulting in a technology disclosure. This instrument allows for continuous adjustment of the nutrient regimes and removal of end-product metabolites, which is the most accurate simulation of true deep-sea conditions to date.

Laboratory experiments with high hydrostatic pressure reactors have shown that different sulfate-reducing bacteria have the capacity to either increase or protect metals against corrosion and when corrosive, display different types of pitting corrosion.

At the same time, samples from two deep-sea expeditions (sampling depths of 1,000, 1,500, 2,000 and 5,000 metres) (Figure 1) were cultured and enriched for deep-sea microbial communities. These cruises also served as a platform for conducting long-term environmental experiments that analysed corrosion of mild steel mooring chain links deployed in the deep sea 10 years prior. The corrosion form, rate and products were studied and characterised using a combination of metallurgical methods and microscopy (Figure 2). Simultaneously, metagenomic sequencing was used to characterise the taxonomic composition and functional capacity of the highly corrosive biofilm that formed on the surface of the mooring chain links. The high corrosion rate and localised nature of the corrosion (Figure 3) suggested that the biofilm was responsible for a significant portion of the observed corrosion. Microbial taxa involved in sulfur cycling were found to be highly enriched in the mooring chain microbiome and distinct from taxa found in the surrounding sediment, providing the first evidence that deep-sea microorganisms involved in dissimilatory sulfur cycling were accelerating corrosion while scavenging electrons from the metal surface. This study was a significant step forward in understanding the differences in the corrosion process induced by microorganisms in the deep sea compared to surface waters.

Figure 1:
Deep-sea sampling on board a JAMSTEC research vessel r/v *Kairei*. *Image credit: Pauliina Rajala, SCELSE*

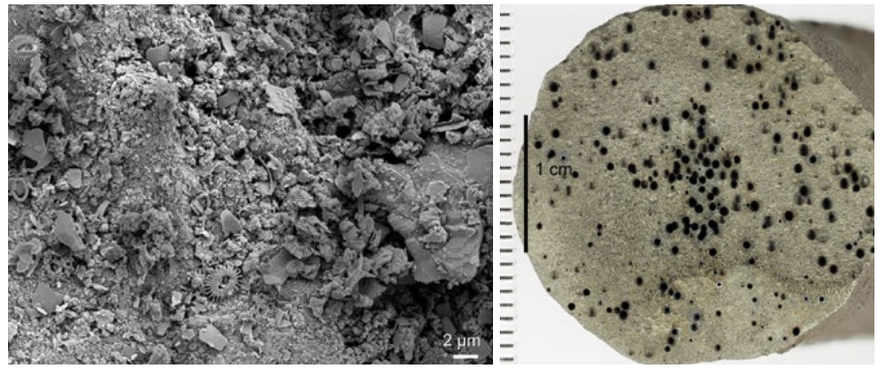

Figure 2:
Corrosion product harbouring diverse community of marine organisms and end of mooring chain link showing intensive localised corrosion.

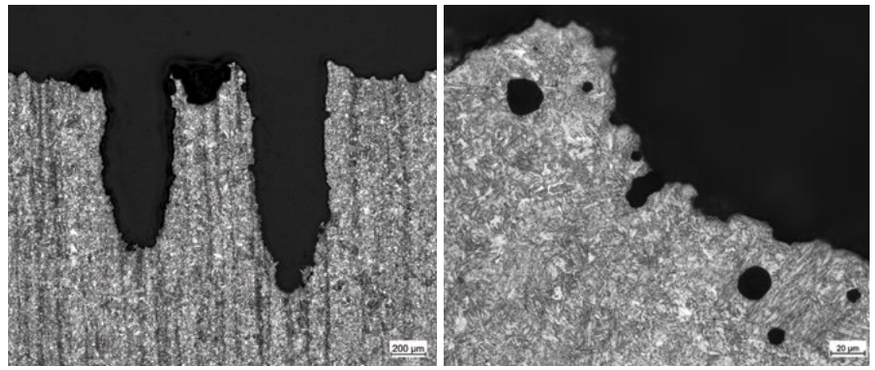

Figure 3:
Cross-sections of etched mooring chain links exposed to deep-sea conditions for 10 years revealing localised corrosion and the microstructure of the steel.

Microbiome (2022) 10: 4.

Journal of the Electrochemical Society (2017) 164: C532-C538.

Future directions

The ongoing MIC research programme will ensure that the expertise, equipment and resources (such as deep-sea cultures) will continue to provide invaluable insights into how microbes influence corrosion in the deep ocean. In addition, the research team has strengthened collaborations with Exxon-Mobil and Keppel Offshore to further support the project and provide direct impact for industrial partners and continues to seek new collaborations with other researchers in the field. The role of microbial communities involved in mediating corrosion will continue to be interrogated to develop corrosion prognosis models and predict metabolic functions performed by the microbial community. These will be used to create hypotheses around specific mechanisms of corrosion, to develop and implement genetic tools to test hypotheses around the roles of specific genes or pathways in corrosion and to exploit the discoveries to improve predictive MIC models and corrosion sensing.

ANTIBIOTIC RESISTANCE GENES IN JOHOR STRAIT MARINE SEDIMENTS

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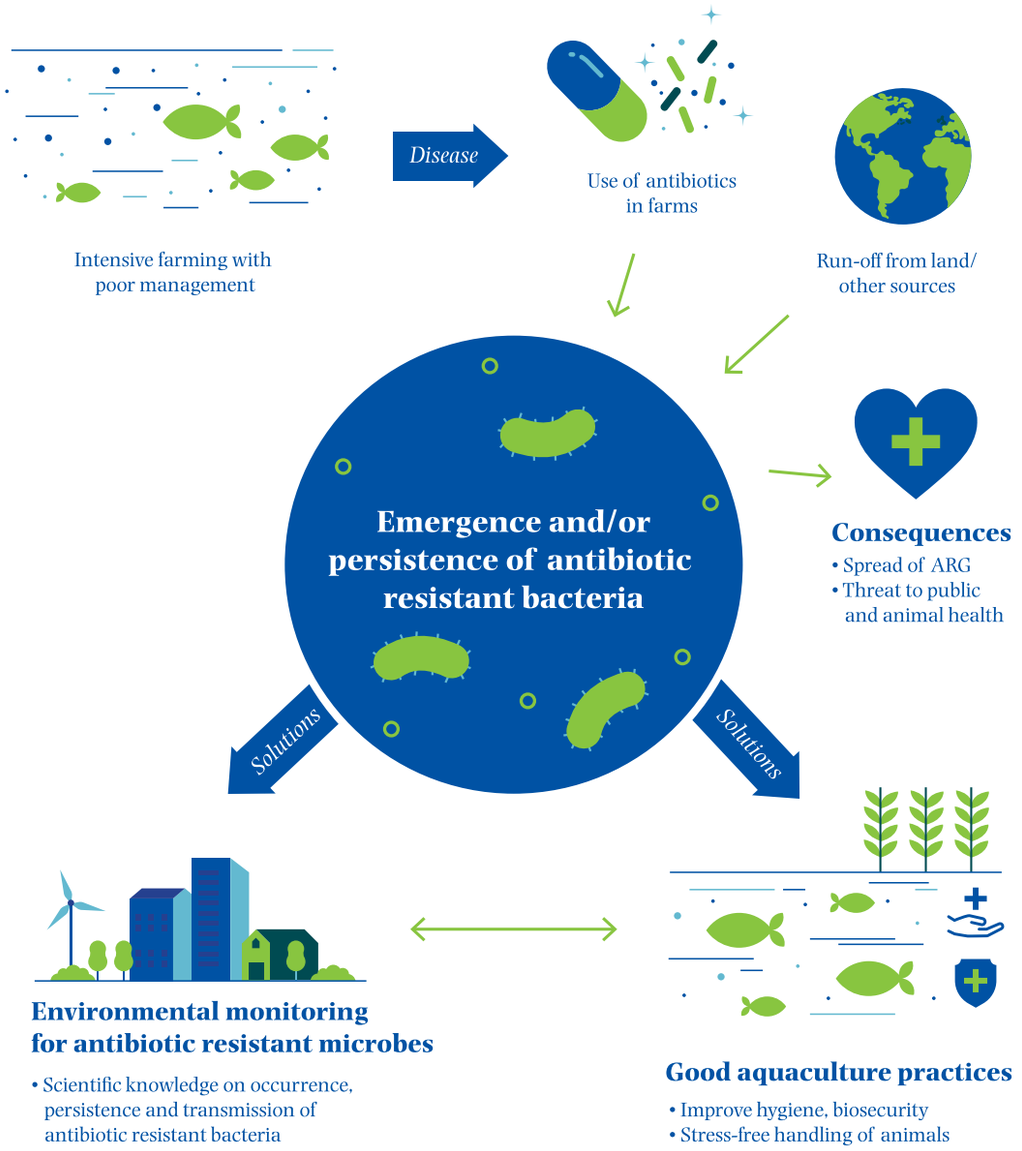
Introduction

The decline in global wild fisheries has fuelled an increasing demand for intensive aquaculture practices. Antibiotics or antimicrobial compounds are still being used in countries with major aquaculture industries. Other sources of antibiotics in the environment include run-off or leaching from livestock farms and municipal waste treatment plants. The presence of antibiotics selects for antibiotic resistant bacteria, and antibiotic resistance is emerging as a global threat to public health. This project investigates the prevalence of antibiotic resistance genes (ARGs) in coastal marine sediments along the Johor Strait in Singapore. A shotgun metagenomic sequencing approach was conducted to screen a wide spectrum of ARGs.

Main findings

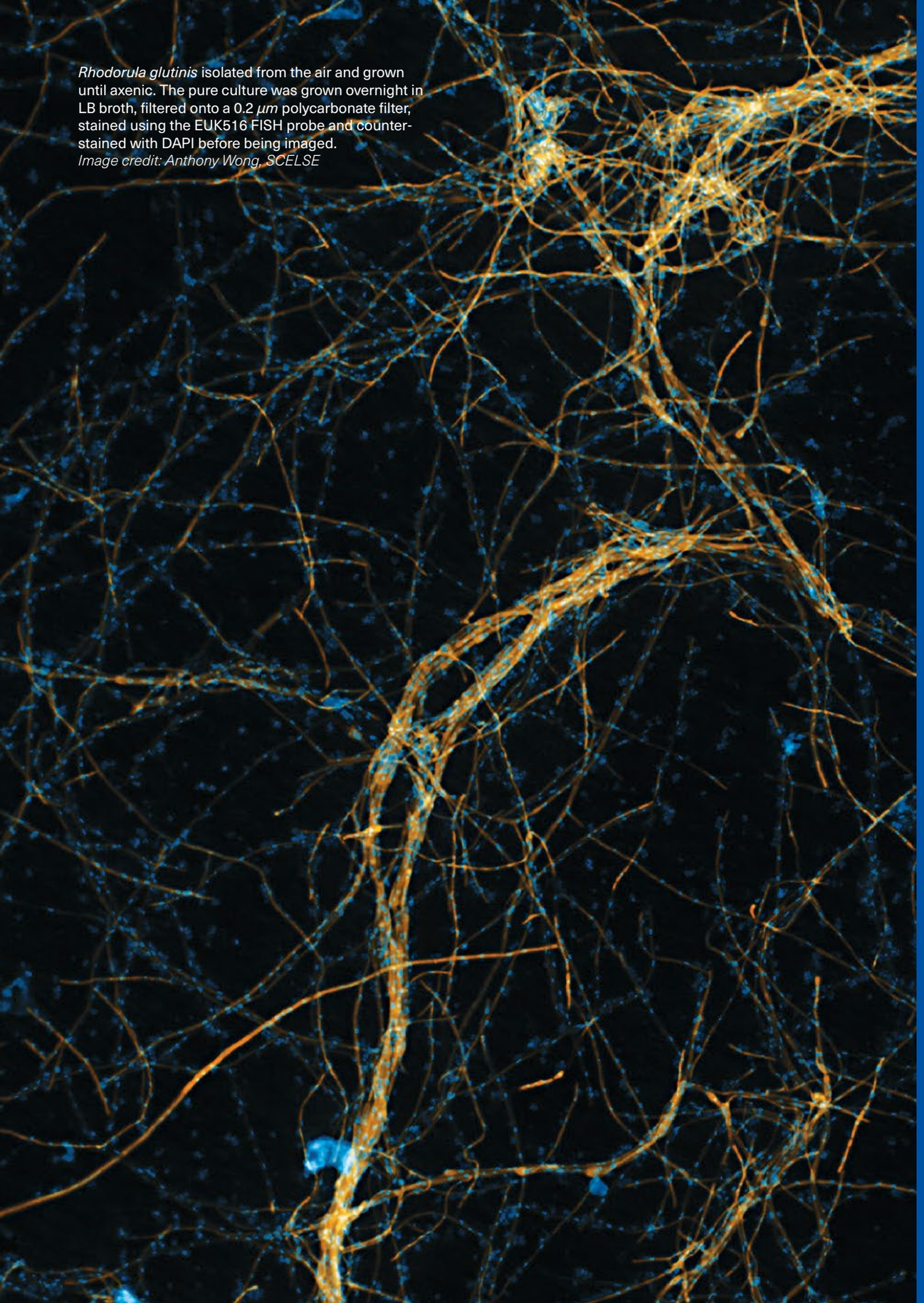
ARGs occured at very low abundance across all sediment samples, with a positive hit rate ranging from 10^{-4} to 10^{-6} %. Time and sampling location within the Johor Strait did not influence the abundance or ARG profiles. Collectively, western Johor Strait contains more ARGs compared with eastern Johor Strait; however, due to their low prevalence and abundance, no statistical analysis was conducted.

Major antibiotic gene classes detected in the western Johor Strait include those that confer resistance to tetracycline, aminoglycoside and lincosamide antibiotics as well as *rpo* and macrolide-efflux genes. Chloramphenicol and macrolide-lincosamide-streptogramin B resistance, and ABC transporter genes were also present, among others. Tetracycline resistance is the most abundant gene class in sediment samples and the genes identified are closely related to *Clostridium perfringens, Enterococcus faecalis, Staphylococcus aureus, Streptococcus suis, Streptococcus pyogenes* and *Gardnerella vaginalis*. This study suggests that antibiotic resistance genes that are closely related to the well-known antibiotic genes are present at very low levels in the Johor Strait sediments and are not elevated at aquaculture sites.



Future directions

Less stringent parameters will be applied to the data to identify potentially novel ARGs and re-evaluate the positive hit rates of ARGs across all samples. Given Singapore's plans to expand coastal aquaculture, routine monitoring of antibiotic resistance genes in coastal sediments will provide an early warning system for the emergence of antibiotic-resistant microorganisms and their resistance genes. This will help safeguard Singapore's marine resources and prevent resistance genes from entering aquaculture facilities and thus the food chain.



Rhodorula glutinis isolated from the air and grown until axenic. The pure culture was grown overnight in LB broth, filtered onto a 0.2 *µm* polycarbonate filter, stained using the EUK516 FISH probe and counter-stained with DAPI before being imaged.
Image credit: Anthony Wong, SCELSE

RESEARCH THEMES

Environmental genomics and surveillance

SCELSE's air microbiome programme encompasses Singapore's urbanised tropical environment, and addresses the universality of bioaerosol community patterns across different geographic regions. As a tropical city-state, Singapore hosts a unique range of natural and artificial climatic conditions, to which airborne microbes respond, and addresses the universality of bioaerosol community patterns across geographic regions.

SCELSE's multidisciplinary approach to investigate Singapore's urban ecosystems addresses bioaerosol community composition and function, air microbiomes and human health, pathogen detection and modelling. 'Omics analysis provides an in-depth understanding of ultra-low biomass environments, with sampling designs informed by aerosol physics and theoretical frameworks based on ecology, public health implications and systems biology.

THE DEVELOPMENT OF GENOMIC TECHNOLOGIES

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Introduction

Capturing the dynamics of biological communities through genomics entails constant challenges regarding biomass availability, DNA extraction quality, and adaptation of established and modern sequencing platforms. To address these challenges, the experimental requirements of a wide array of samples need to be adapted. For example, microbial communities in low biomass systems such as air or surfaces need to be collected in ways that maximise biomass, while maintaining DNA integrity. Conversely, even when biomass is not a limiting factor, established high-throughput protocols often need careful optimisation. Sequencing of diverse insect species in large quantities for phylogenetic and population studies constitutes one such example. The importance of this observation manifests itself in the underrepresentation of entire phyla or even ecosystems in the publicly accessible sequence databases.

To meet these challenges and to improve sequence database content, the Meta-'omics and Microbiomes cluster genomics research team is continuously developing novel techniques for DNA library construction. Specimens that are difficult to sequence are focal points of these developments. As an outcome, the High-throughput Sequencing Facility now sequences ultra-low biomass samples with unprecedented quality and evenness. Likewise, a novel three-pronged approach to next-generation sequencing of insect mitogenomes established quality high-throughput protocols for the taxonomic identification of diverse insect species. The team is also involved in testing new protocols and sequencing techniques for microbial single-cell sequencing.

In addition to protocol development, the High-throughput Sequencing Facility has been at the forefront for implementing the latest sequencing technologies. In this regard, the team works with various manufacturers of sequencing technologies within early access programmes. As an outcome, a diverse array of sequencing platforms is available at SCELSE with an emphasis on precision metagenomic sequencing for accurate species-level identification, as well as on the generation of platinum-grade whole genome assemblies for microbial organisms from a wide range of environments.

Main outcomes

Novel approaches for sequencing of ultra-low biomass samples

The microbial ecology of the hydrosphere and geosphere have been well explored using both culture-dependent and independent approaches. In comparison, one remaining frontier, the atmosphere, is understudied. The key challenge for the exploration of airborne microbial communities is the low biomass density in the atmosphere, as compared to aquatic or terrestrial ecosystems. In the past, this has necessitated long sampling times, e.g., days, weeks or even months. High-volume air sampling, paired with modified and optimised DNA extraction and ultra-low biomass library preparation protocols, have enabled amplification-free sequencing of air samples to become a reality for time intervals as short as hours or even minutes. This accomplishment was made while generating species-level identification using metagenomics sequencing and an in-house analytical pipeline.

In the process, DNA extraction and library preparation protocols were developed that address issues such as small DNA amounts or the presence of inhibiting substances. These are frequently co-extracted with the DNA from environmental sources and ultimately influence the sequencing outcome and sequence data quality. The novel library preparation protocols enable the sequencing of as little as 500 pg DNA, while producing consistent and amplification bias-free results (Figure 1).

To achieve the above goals DNA input amounts needed to be standardised at defined minimum sample concentrations. Consequently, accurate sample quantitation at sub-nanomolar concentrations is a key requirement to ensure comparability across

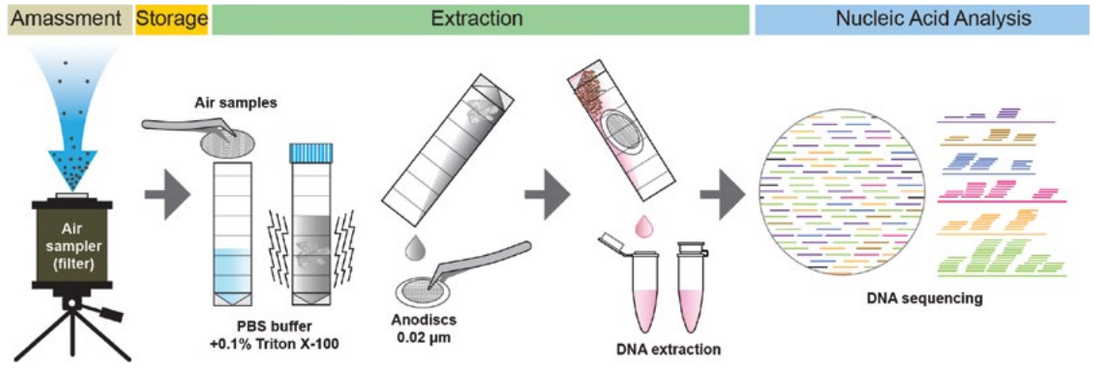

Figure 1: Sampling and analysis pipeline for metagenomic analysis of ultra-low biomass environmental samples.

large sets of samples. This normalisation step enables all ultra-low biomass samples to be uniformly processed. In addition, the ultra-low biomass library preparation protocol has been automated on our fleet of liquid handlers, thus massively increasing sample throughput compared to manual preparation. To date, thousands of ultra-low biomass air samples have been successfully sequenced, equating to more than 7 terabases of sequenced data. In addition, the ultra-low biomass pipeline is also being applied to low biomass samples from other environments such as sputum from patients with respiratory diseases, ocean and drinking water samples, as well as viral samples from surfaces.

Single-cell sequencing

Single-cell genomics is a cultivation-free approach that provides direct access to the genetic information of a cell. The advantage of single-cell genomics over metagenomics is that the genomic content of each individual cell within a given sample is analysed in its entirety, rather than as a fraction in a mixture of many genomes. This simplifies some of the challenges associated with genomic assemblies from metagenomic data sets, and provides a direct link between the genome and any additional cellular DNA content that may originate from mobile genetic elements such as phages or plasmids, which cannot be assigned to a specific taxa with the standard metagenomic sequencing approach.

In collaboration with a research group at Waseda University in Japan, a high-throughput microfluidics-based pipeline has been developed for the direct isolation of microbial single cells from environmental samples. In short, with the use of a custom-build microfluidics device, particles from environmental samples are encapsulated into microdroplets. The encapsulated cells are subsequently lysed before undergoing multiple displacement amplification, library preparation and short-read as well as long-read sequencing, followed by *de novo* genome assembly. To date, hundreds of bacterial and fungal single cells isolated from air in Singapore have been processed and sequenced with the microbial single-cell pipeline.

Unique approach to mitogenomics sequencing of *Diptera* sp.

Molecular evolutionary analyses were historically based on mitochondrial markers consisting of single genes or short sequence intervals, particular in higher organisms with very large genomes. More recently, complete mitochondrial genomes have become commonly used for in-depth phylogenetic and population studies. In particular for vertebrates, generation of complete mitochondrial genomes is well established, including those of extinct species. Sequencing of complete mitochondrial genomes provides higher resolution of between and within species relationships, in addition to improving the characterisation of genome evolution and patterns of substitution rate.

In contrast, the sequencing and assembly of complete mitochondrial genomes of invertebrate species has progressed at a much slower pace. This is especially true for insects, whose mitochondrial genomes have previously been obtained by isolation from whole cell lysates, in combination with restriction fragment or shotgun sequencing. Those initial data sets serve as references for the further development of universal primers. Even today, many invertebrate mitochondrial genomes are still based on primer walking and Sanger sequencing, resulting in an underrepresentation of molecular studies on invertebrate evolution, compared to other animal taxa. Even alternative approaches using large amplicons of several kilobases in length, generated by long-range PCR, are limited to only close relatives.

Using the novel high-throughput mitogenomics approach for insects, 91 mitochondrial genomes were generated from 32 different species of flies from the Schizophora radiation, using whole insects, body parts and ethanol-preserved specimens. This study not only contributed to the comparative mitogenomics and population-based analysis of invertebrates, but has also provided new insights into insect diversity and population history. This approach does not require the whole animal specimen and can be applied to specific body parts. As a consequence, entomology collections may be studied on a molecular level without the destructive sampling of entire specimens.

True flies are insects of the order *Diptera* and encompass one of the most diverse groups of animals on Earth, occupying almost every niche in terrestrial ecosystems. More than 150,000 species have been described that parasitise plants and animals, act as biological and mechanical vectors of diseases, serve as biological control agents, as well as model organisms for science. The clade Schizophora contains the majority of the family level diversity among Dipterans and represents a recent rapid radiation of lineages. The resulting relationships among Schizophora families remain a challenge for fly phylogeny. The rapid radiation in combination with a low extinction rate has led to a diversity that surpasses even the number of all terrestrial species of vertebrates. The scarcity of fossil records and the sparse availability of genetic data make Schizophora an interesting target for large-scale molecular evolutionary analyses. To address these questions regarding diversity and taxonomy, the genomic analysis protocol developed for insects was extended to whole genome analysis by combining traditional long-range amplicon approaches with short-read and long-read whole genome shotgun sequencing of total DNA.

Future directions

The established sequencing protocols will continue to be tested and optimised, while simultaneously new techniques will be developed. This will allow the team to continue offering cutting-edge sequencing services. The adaptation of state-of-the-art technologies, even for the most difficult samples, will address emerging needs in the field of genomics from a wide range of collaborators within or outside of SCELSE. Keeping the existing technology platforms and facilities relevant will require a sustained stream of developments and upgrades. In the course of this process, the latest advancements in sequencing technologies will continue to be evaluated and incorporated in lockstep with the in-house work flows. This strategy has not only kept SCELSE at the forefront of biofilm and microbiome research, but has also allowed for the adaptation and fast reaction times to public health matters, such as the current COVID-19 pandemic (see *Pathogen surveillance and source tracking* section).

npj Biofilms and Microbiomes (2021) 7: 37.

Scientific Reports (2016) 6: 21762.

REFINED CAPACITIES IN BIOINFORMATIC ANALYSIS PIPELINES AND INFRASTRUCTURE

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Introduction

Over the past decade, SCELSE has developed and refined its capacities to successfully sequence and analyse samples taken from technologically challenging environments to an unprecedented level. For bioinformatics analyses, the high sample numbers and large data volume generated per sample can become a significant bottleneck for processing times, with complex bioinformatics pipelines causing processing to take up to months to complete. The cluster's continuous development and testing of assembly and analysis algorithms paired with their keen knowledge of current computational technologies enable a high degree of accuracy and reliability in results generated while achieving timely processing. This development also lends itself to the creation of novel bioinformatic approaches, whereby researchers have applied tools such as machine learning and artificial intelligence to the questions of microbial composition and taxonomic identification. The additional development of platforms for storage and sharing of the sample collection and processing information ensures ease of access to key sampling metadata components across the team. This makes it possible for multiple researchers to investigate a set of samples in various manners, without restricting the well of information needed to draw a deeper understanding of results, which would otherwise not be possible without fully understanding the environment and processing each sample has undertaken.

Main findings

Advancing bioinformatic analysis

Accelerated species-level metagenomicanalysis (dbassign)

SCELSE has developed a method of species-level identification in metagenomic samples for improved resolution of microbiome composition and function. The method is several-fold faster than existing analysis tools, while allowing for taxa identification to the level of species and strains. The method utilises already completed or scaffold-level bacterial genomes available in various public databases hosted by the National Center for Biotechnology Information (NCBI). Sample sequences are aligned to a nucleotide database containing all documented, complete bacterial genome sequences in the NCBI database. The resulting nucleotide-to-nucleotide alignments are filtered for low-quality bases, low-scoring mapping and repeat structures. Once the alignments are relatively free of the confounders, the lowest-common-ancestor (LCA) algorithm is applied to the high-quality alignments. The algorithm will decide if an alignment can be confidently assigned to a species, or if it should be moved to higher-level taxa, in the case of multiple species hits. The method is compatible with the MEGAN visualisation tool used to view taxonomic classification and for further data exploration.

In addition to this technique's implementation on a general CPU-based computing server, the method is being tested and retrofitted onto field-programmable-gate-array (FPGA) technology. The most resource-intensive part of the technique is the short-read alignment against the reference database. Offloading this task to an FPGA-card will accelerate this step by up to 20-fold.

Increased precision and processing time of microbial air samples

To investigate and profile the microbial composition of the air environment, optimised metagenomics analysis techniques were employed, utilising a high-performance computing (HPC) system on thousands of bioaerosol samples. The first part of the project employed a metagenomics pipeline consisting of a sequence quality control tool, a nucleotide-protein aligner (RAPSearch) and a metagenomics classifier (MEGAN). This version of the pipeline was implemented in a 2,200 CPU-cores HPC cluster. Towards the end of the programme, a final revision of the analysis pipeline led to the incorporation of an improved nucleotide-protein aligner (Kaiju) and the updated pipeline was implemented in a 13,000 CPU-cores HPC cluster. The evolution of the analysis pipeline was necessary to produce a reasonable analysis turnaround time in the face of increasing sequence length and depth, and importantly, an exponentially increasing number of protein reference sequences. To provide context, over the programme's five-year period the content of the NCBI-nr protein database tripled, resulting in significantly increased processing times requiring higher computational resources. By using the new metagenomic pipeline in combination with the upgraded HPC system, the analysis time of an average turnaround of 3 to 5 months for a single batch of samples was reduced to less than a day.

Machine learning/AI

Modelling factors driving taxonomic distribution using Bayesian networks

To gain a better understanding of how environmental factors influence microbial species, Bayesian probabilistic graphical models (Bayesian networks) were applied to our temporal variation of air microbiome data sets (see *Environmental surveillance of airborne microbial communities* section). This has enabled relationships of causal interactions among factors and taxonomical groups to be documented. In addition, the resulting model represents a powerful tool to analyse, in terms of probabilities, how environmental changes affect the behaviour of the microbes.

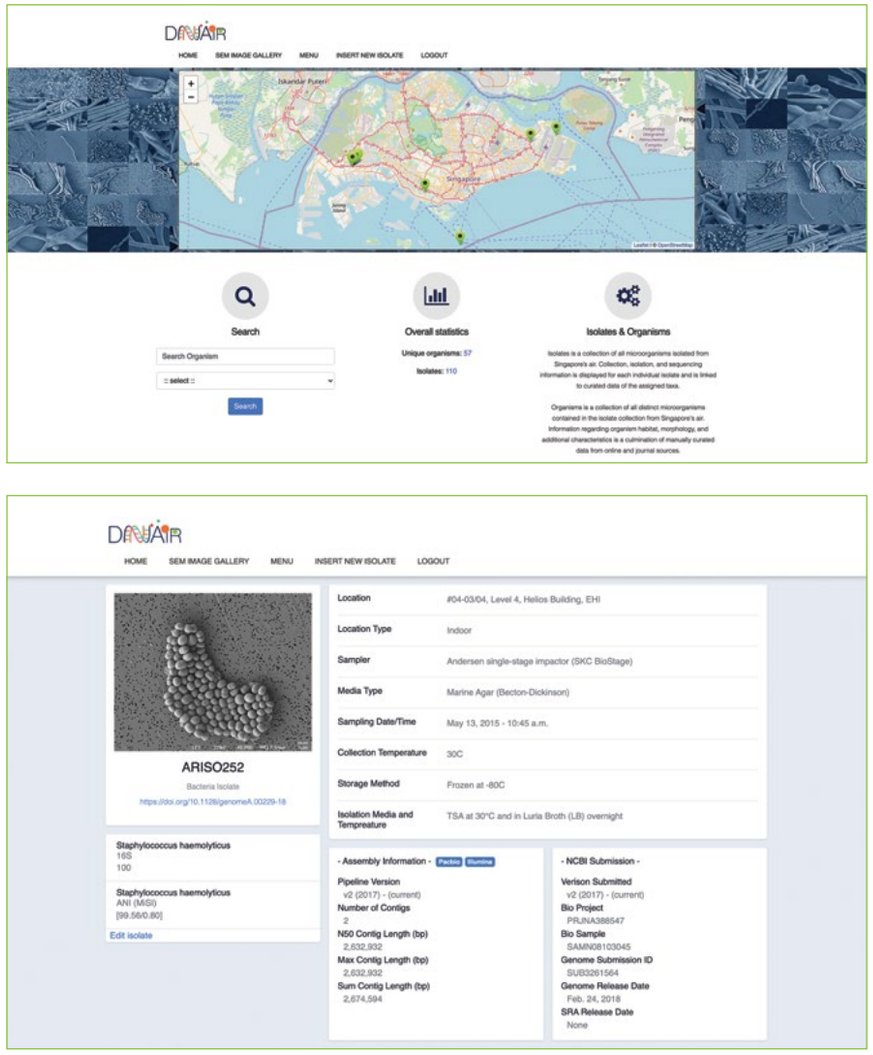

Figure 1:
DNAir web interface

Investigation of the transcriptional dynamics of viral infection in the ocean – support vector machines (SVM)

In addition to the application of standard data analysis procedures, a machine-learning approach to classify small DNA fragments (down to 100 bp length) based on their origin was developed, tested and implemented. This method was applied to separate host-like sequences from viral-like sequences. Due to the high similarity of host and viral sequences, this task would have been unfeasible using traditional approaches based on alignment against DNA databases.

As a test case, this method was used to investigate the transcriptional dynamics of viral infection in the ocean, using data extracted from a previously published metatranscriptome profile of a naturally occurring oceanic bacterial assemblage sampled Lagrangially over three days. The approach uncovered the existence of light-dark oscillations in the expression patterns of auxiliary metabolic genes in cyanophages that follow the harmonic diel transcription of both oxygenic photoautotrophic and heterotrophic members of the community.

Metadata storage and access

DNAir – Airborne isolate whole genome database

The DNAir database is a microorganism-centric collection of isolates obtained and cultured from Singapore's air, stored with corresponding information on isolate-collection methods, sequencing metadata and SEM images. Organisms thought to be unique to air have been submitted to NCBI for review and their subsequent genome submission information recorded. DNAir aims to reveal the nature of the organisms obtained from air by allowing users to view isolate morphology, and classification by multiple taxonomic identification methods, along with manual curation of each organism's known ecology. The current web application has 110 bacterial genomes with 57 distinctly identified organisms (Figure 1). The continually expanding isolation collection is complemented by collaborative input from ecologists to reliably classify and annotate the remaining collection.

MetaLIMS – A simple open-source laboratory information management system for small metagenomic labs

MetaLIMS is used in tandem with DNAir and was built as an in-house sample metadata database for sample collection, DNA extraction and sequencing submission information for all samples collected for the air microbiome project. This information can then be migrated to DNAir as continuous isolate-collection samples are generated for a more concerted investigation of organisms from air. The centralised repository of environmental sampling parameters as well as sample processing metadata allows this information to be easily accessible to all members within the team, to enable a clear understanding of subsequent bioinformatic results.

Future directions

As public reference databases continue to grow each year, the time and complexity of organism identification and analysis also increase. This, along with ongoing advancements made in this field of research, fuels the continual drive and curiosity to develop workflows and bioinformatic applications. Therefore, the team will continue to advance its own facilities as well as integrate and share algorithms and technologies in-house and among the broader community.

ENVIRONMENTAL SURVEILLANCE OF AIRBORNE MICROBIAL COMMUNITIES

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Introduction

The atmosphere is a challenging environment for microbial ecosystem research compared to its terrestrial and aquatic counterparts, owing to its gaseous nature that results in extremely low biomass densities, which test the limits of existing molecular analysis. Using novel sampling and analysis strategies, SCELSE's air microbiome team has studied airborne bioaerosols, describing community composition, and temporal and spatial dynamics. Thus far, the variability in community structure, driven by factors such as time of day, temperature, humidity and precipitation, has been described at high resolution. The possibility of the atmosphere representing an airborne ecosystem, where cells are not merely dispersed, but rather exist in a metabolically active state is being further investigated. To this end, a uniform sampling/experimental design was applied, to ensure both environmental sampling and analysis pipelines followed a validated and reproducible standard operating protocol. The resulting metagenomic data analysis pipeline provides a robust and comprehensive description of air microbiome dynamics. In addition, for the first time, environmental surveys combined comprehensive biological data sets with matching physico-chemical and meteorological parameters, thereby allowing the impact of meteorological and climatic conditions of airborne microbial communities to be assessed.

Main findings

a. Airborne microbes follow a precise diel cycle

The atmosphere is the most underexplored microorganism-harbouring ecosystem due to the technological challenges inherent in the sampling of an ecosystem of global dimensions, that also contains ultra-low biomass. The Meta-'omics and Microbiome team's advancements in biomass collection, DNA extraction, library preparation and sequencing techniques have enabled a metagenomics time course study of unprecedented temporal and taxonomic resolution, examining the time scales at which airborne microbial community composition fluctuates throughout a day, between days and across months. The microbial composition of tropical air in Singapore was assessed by collecting samples 12 times per day, for two hours per time point, over five consecutive days. This sampling regime was repeated in three-month intervals, resulting in four independent time series over 13 months, covering the wet and dry seasons with opposing wind directions. The resulting 795 air metagenome samples, from 265 time points, generated a total of 2.27 tera base pairs – the equivalent to about 760 human genomes – which were analysed for community composition and dynamics.

This study demonstrated for the first time that the microorganisms in air follow a precise diel cycle, with larger variation in microbial diversity observed within a single day, than was observed on a day-to-day or month-to-month basis.

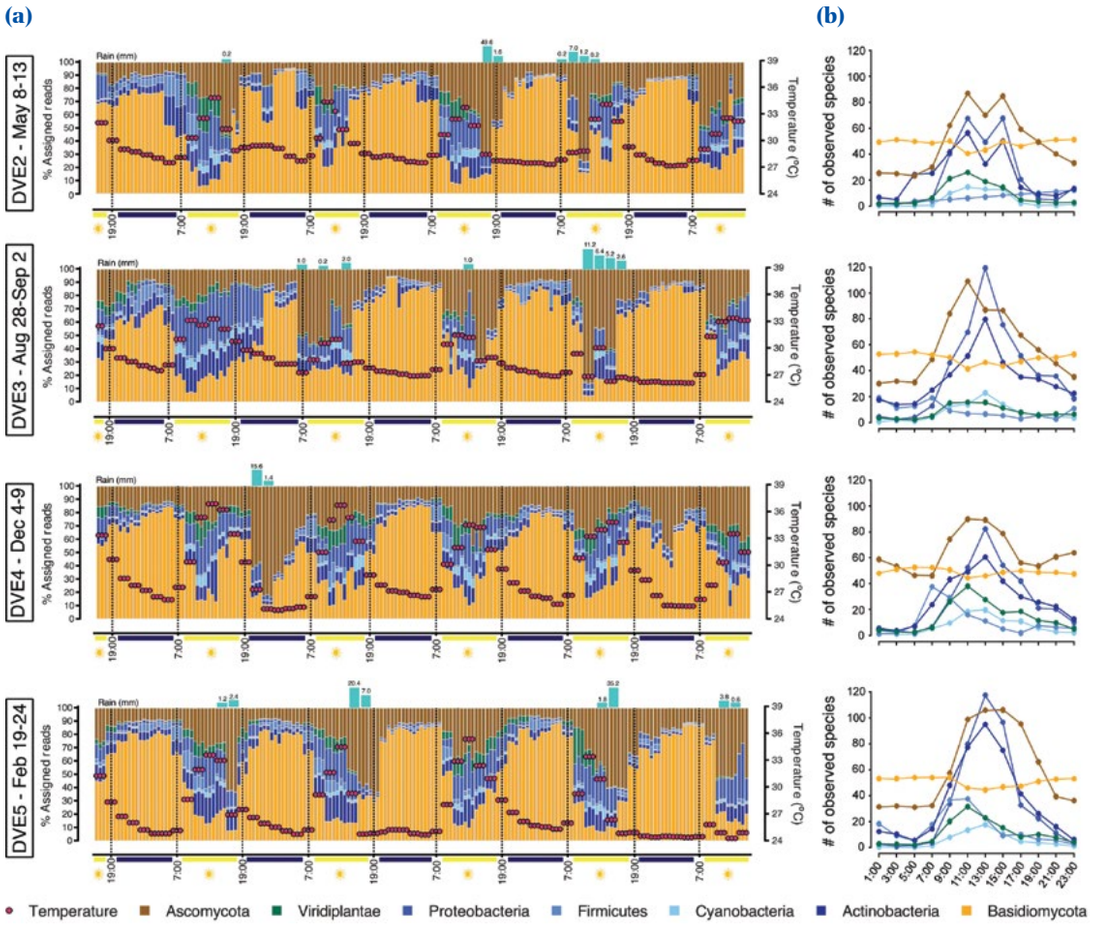

Figure 1:
Taxonomical breakdown and temporal resolution of the air microbial communities. (a) Relative abundance of seven taxonomic groups of organisms in air. Rain events with recorded amounts of precipitation (blue bars) and temperature distribution (red buttons) are denoted for each time series. The timescale is indicated (bottom), as well as day and night samples denoted by sun and moon symbols. (b) Observed number of species by taxonomical groups in each of the four-day variation sampling (DVE). The graph depicts the change in species abundance per group, indicating the robustness of the Basidiomycota community versus strong daily variation of the community composition for the other six phyla.

These within-day observations showed significant day-night differences, with various microbial groups increasing up to 10-fold at midday or during rain events, while others dominated during the night. Temperature was one of the key driving factors for the observed diel dynamics.

Surprisingly, the study revealed larger microbial diversity in air samples compared to those collected in aquatic, terrestrial or human host environments (Figure 2). Contrary to other planetary ecosystems,

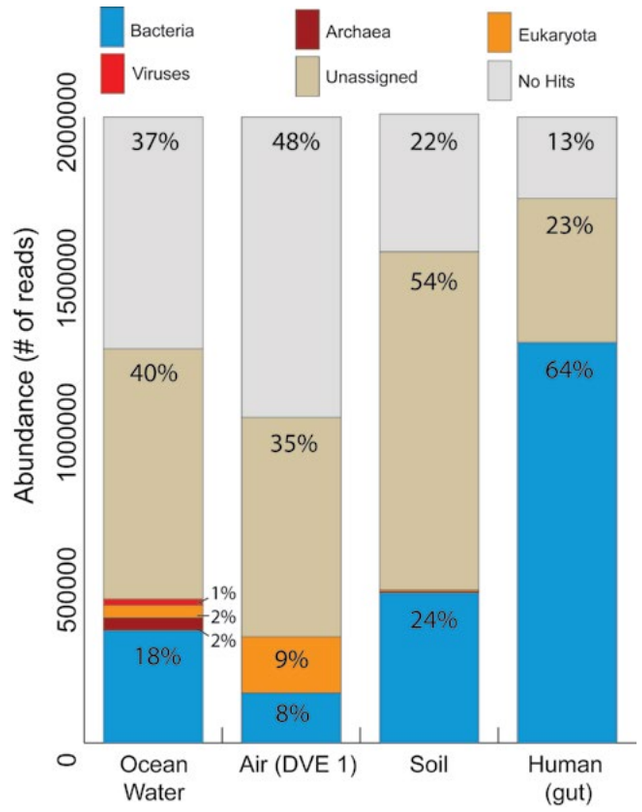

Figure 2:
Taxonomic structure of microbial communities from aquatic, atmospheric, terrestrial and human gut environments. (a) Relative abundances of identified microbial taxa are shown as DNA read counts of normalised metagenomics data.

which are dominated by prokaryotes, the tropical air samples are dominated by eukaryotic phyla such as Basidiomycota and Ascomycota fungi. As human individuals are inhaling up to one million microbial cells per day, the role these microorganisms play for the human host warrants further investigation. In particular, the strong correlation of specific fungal and bacterial species with different environmental parameters make them suitable biomarkers for investigating their role in the built environment.

b. Seasonal impact on the air microbiome

The taxonomical composition of the air microbiome across seasons in the temporal climate of Western Siberia was studied using the above mentioned analytical pipelines to provide a comparison to the tropical system. The environmental surveys covered a temperature range from 26°C to -22°C. At the lower temperature extreme during winter, the airborne biomass decreased up to 170-fold. This was largely due to a strong decrease in fungal microorganisms during periods of low temperature. In general, the summer air microbiome was similar to those observed in parallel studies in Europe and also the tropics with a mix of Basidio- and Ascomycota fungi, as well as a wide range of bacterial taxa. In winter, the atmospheric biomass was dominated by mostly bacterial and Ascomycota taxa. The difference in temperature between seasons correlated with a cessation in the diel cycle of airborne microbial communities during winter, in similar ways as those observed for the higher air layers in our vertical stratification experiments described next page (section C).

Figure 3:
Association of airborne microorganisms with different altitudes in the lower troposphere. Images taken of field sampling using (a) meteorological tower (MT) and (b) research aircraft (RA). Taxa cloud graphs plotting the mean relative abundance of the species identified (c) in the MT sampling at ground level (x axis) and in the 200-metre tower-top air layer (y axis) and (d) in the RA sampling on the ground level (x axis) and in the 1,000- to 3,500-metre air layer (y axis). Only species significantly diverging between ground and high altitude are plotted ($P < 0.05$, manyglm method).

c. Vertical and spatial variation of the air microbiome

Intrigued by the earlier findings that the airborne microorganisms in the tropics follow a precise diel cycle, the dynamics and variability of airborne microbial communities associated with different layers of the vertical air column were investigated. One impact of the previous diel cycle sampling was the establishment of refined methods for sequencing low biomass. This laid the groundwork for designing sampling regimes exploring the boundaries and organisation of vertical air microbiomes that display even lower biomass densities.

The first vertical air sampling study was carried out at various levels of a 49-storey residential urban high-rise building (156 m asl) in Singapore, and at the base (1,360 m asl) and summit (2,502 m asl) of an Alpine mountain in Switzerland. Initial results suggested very little change in the air microbiome composition at these varying elevations. However, it was concluded that the large solid faces of the building and mountain altered the natural airflow, mixing the air layers from different levels and consequently transferring airborne microorganism from lower to higher altitudes and vice versa.

An innovative sampling methodology was devised that would not restrict air flow and could truly capture the air microbiome composition of different air layers in the vertical air column. This vertical testing array was established in Germany and comprised a 200 m meteorological tower (Karlsruhe Institute of Technology, Institute of Meteorology and Climate Research, Karlsruhe, Germany) and a research aircraft (Technische Universität Braunschweig, Institute of Flight Guidance, Braunschweig, Germany). Synchronised measurements of meteorological parameters and microbial biomass were taken from ground level up an altitude of 3,500 m. The temporal and spatial resolution of this study demonstrated that the diel cycle of airborne organisms is a ground-based phenomenon and is absent at heights more than 1,000 m. This finding led to the discovery that temperature, not sunlight or humidity, is the single most important parameter that influences the composition of airborne microbial communities. The finding resulted in the insight that rising global temperatures will alter the existing air microbiome and lead to a much wider dispersal of airborne microorganisms. Besides the stratification of airborne biomass in the atmosphere above the mixing layer height, this study also showed for the first time that the atmosphere acts as a sink in the sky for microorganisms. As a result, higher air layers aggregate a large microbial diversity, which eventually reaches a global range of dispersal. This insight explains the observation of highly similar microbial taxa in aquatic and terrestrial ecosystems around the globe. The temperature-driven nature of dispersal can affect the distribution of airborne plant pathogens and potentially impact agricultural crops, placing additional threats on global food security.

In addition to demonstrating airborne biomass dynamics across atmospheric boundary layers, the analysis provides a working model for airborne microbial communities as key drivers for the biological organisation of the planetary air ecosystem. The analysis further demonstrated that at least 50% of identified airborne microbial taxa (n = ~10,000) are associated with either ground-level or higher altitude locations. This finding allows for a better understanding of the dispersal patterns of specific microbial taxa in the vertical air column. One such group is the radio-tolerant bacteria that withstand ionising radiation, desiccation, UV radiation, or oxidising agents. Air layers above 1,000 m contained up to 20-times higher concentrations of this diverse group of bacteria. Ionising radiation from the sun and outer space is hypothesised to result in the evolution of radioactive tolerance in these bacterial phyla. Interestingly, bacteria living on terrestrial surfaces are not exposed to such levels of radiation and also do not exhibit radio-tolerant traits. The observation of the association of certain groups of bacteria at greater heights with radio-tolerance shows the potential for physiological traits to have developed over very long periods of time, as these species have adapted to survive in this harsh environment over millions of years. These observations will therefore pave the way for studying the evolutionary principles such as radio-tolerance at higher layers of the earth's atmosphere.

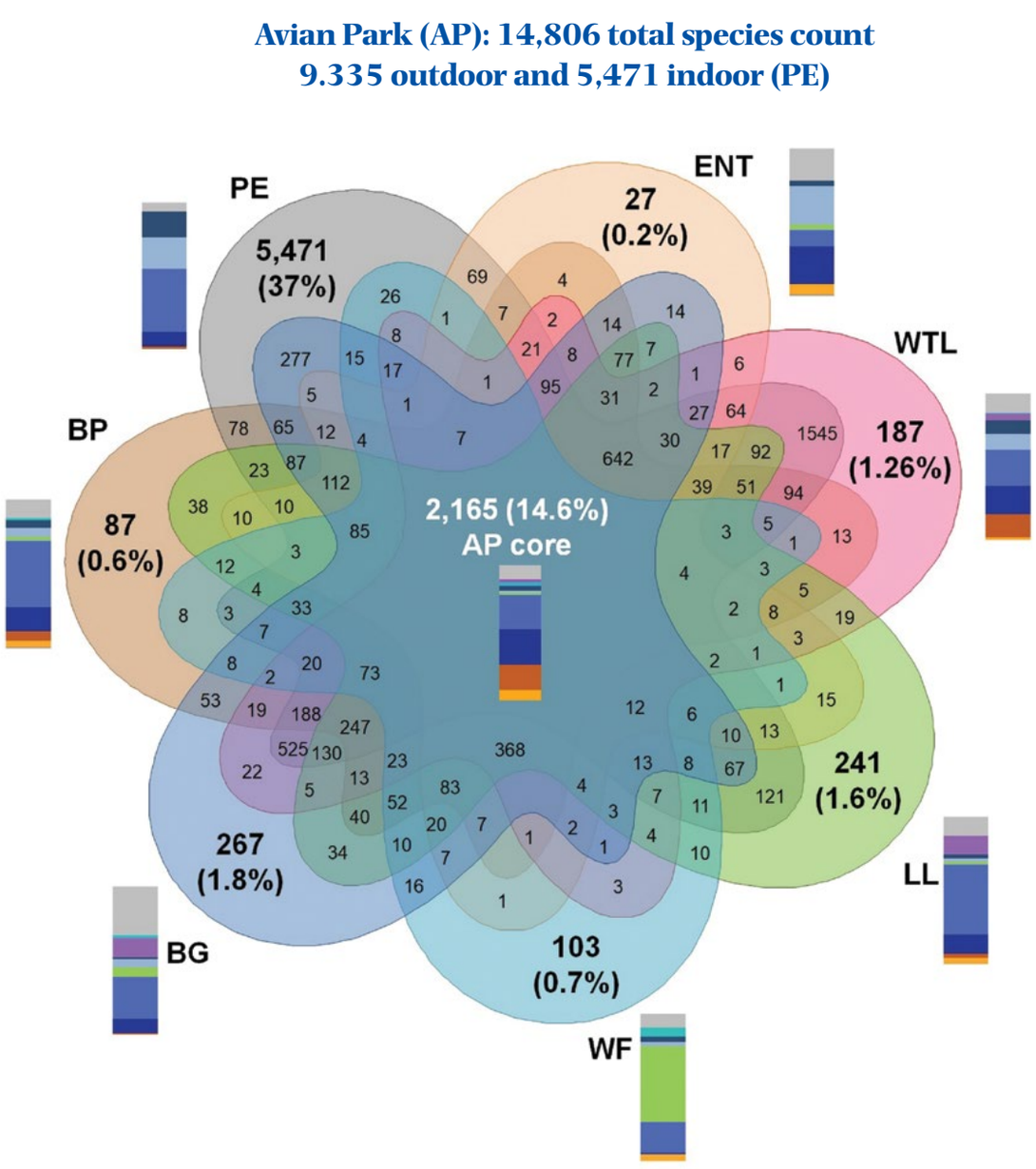

Figure 4:
Local and remote sources for the air microbiome. Species-level analyses. PE – Penguin Enclosure, ENT – Entrance, WTL – Wetlands, LL – Lory Loft, WF – Waterfall, BG – Bridge, BP – Birds of Play (children's playground). Venn-diagram of species co-occurring in seven microhabitats of the avian park (AP). The core species identifiable across all the microhabitats are indicated in white. Bars indicate the relative composition (in counts of species) of the corresponding location-specific microbial communities.

d. Short-range contributions of local sources to ambient air

In this study, the contributions of local sources to ambient air within a 160,000 m^2 tropical avian park were assessed. Air samples from seven locations situated 160 m–400 m apart were sequenced and analysed. Each site represented distinct microhabitats, defined by either characteristic land features, such as lakes or waterfalls, or had a high density of specific bird species. The air samples from each location showed characteristic air microbiomes, defined by the abundance and richness of airborne microbial community members. Outdoor microhabitats contained up to 18.6% location-specific taxa, while a third of the air microbiome was shared between sites. The remaining microbial taxa were identified as the ambient air background of urban tropical Singapore, established using a reference data set from a location 2 km away. This data was collected during a year-round sampling campaign in course of the diel cycle studies. Only 3.9% of species were specific to the urban site located on a building within the Nanyang Technological University Singapore campus. This study integrates the observations from the diel cycle and vertical stratification projects, as it reiterates the day-time dependence of the air microbiome, as well as the fact that air masses below the boundary layer height are highly admixed and therefore form the ambient air background of a metropolitan area. The study further demonstrated the pronounced differences between indoor and outdoor locations, as shown for the penguin enclosure sampling site at the bird park. For this setting, no fungal airborne microorganisms were found, which strongly contrasts with the vegetation of the outdoor settings. As a result, sites with animal and human host microbiomes were likely to be dominated by bacterial bioaerosols, while an abundance of fungal taxa was observed for tropical settings where wood-rotting fungi belonging to the Basidiomycota phylum are most frequent. The qualitative and quantitative assessment of local sources of airborne biomass against the ambient air background therefore allows for the identification of local source contributions even in naturally ventilated outdoor settings. Our analyses therefore suggest that even within a small-scale area one can accurately identify contributions from local sources to the ambient air background, despite the prevailing mixing of air masses caused by atmospheric turbulence.

Future directions

Important avenues for further work will require investigation into the extent to which the diel cycle of airborne microorganisms is a global phenomenon, and how these natural dynamics are impacting the distribution of airborne biomass around the world. Further, it is important to understand how the large fluctuations in airborne microbial communities between day and night are affecting the wellbeing of human populations, in particular respiratory patients suffering from asthma, COPD and bronchiectasis.

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HOST-MICROBIOME DYNAMICS

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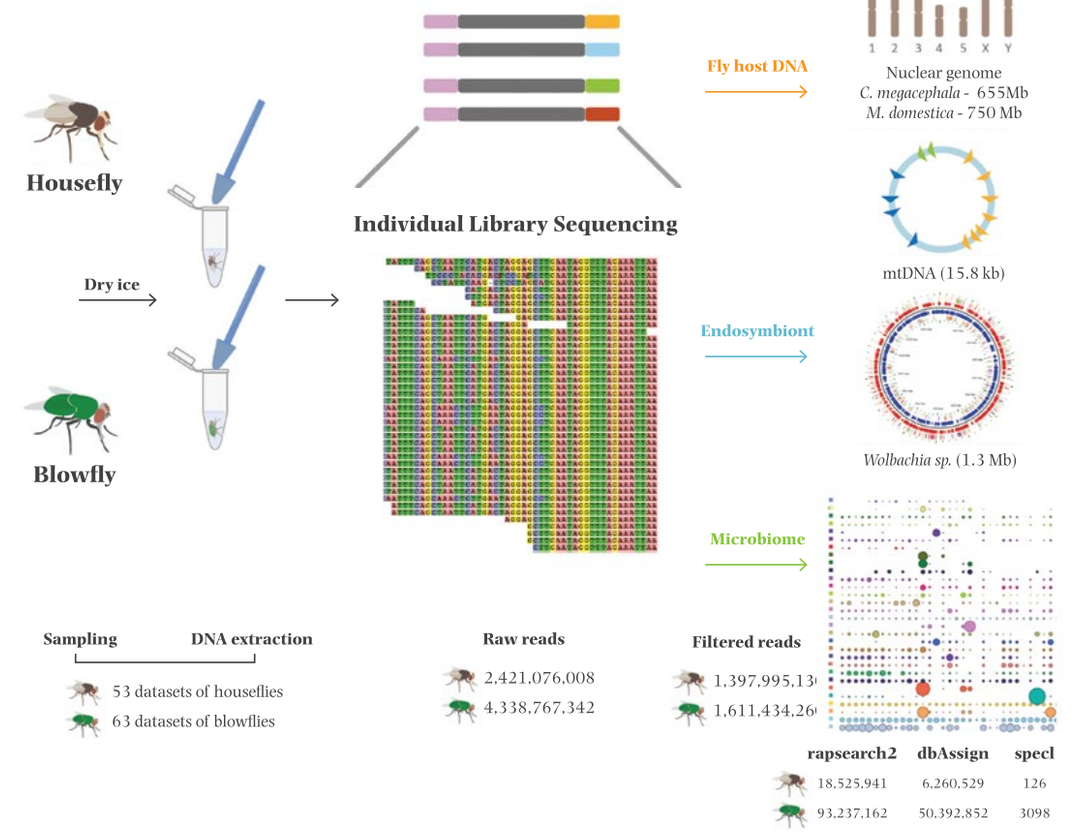
Introduction

The interactions of higher organisms and their specific microbial communities are studied within the holobiont concept, as the combination of host and microbiome is increasingly being recognised as discrete ecological units. In particular, the physiological effects of the microbial metabolism is coming into focus when studying ecosystem function and host wellbeing.

One such example includes insects such as mosquitoes and flies. They can potentially transmit opportunistic pathogens between human hosts, causing nosocomial outbreaks as vectors for both viruses and bacteria. Understanding insects as a holobiont identifies the true agents of emerging opportunistic pathogens instead of labelling the insect host as a vectors of disease. The microbial-based concept enables new biological strategies for control of disease spread, thereby minimising ecological damage, as insects, particularly flies (*Diptera*), play important roles as pollinators and biodegraders in nature.

Alongside the increasing importance of knowledge of the insect microbiome, research into mammalian host-microbiome interactions show growing evidence suggesting a link between the human microbiomes with a variety of chronic diseases, not exclusively gastrointestinal-related. Studies have shed light on microbiota interactions with the host through a multitude of pathways. Increasing knowledge of the impact of the host microbiomes on humans necessitates the extension of research into rodent models, which would not otherwise be permissive in humans. SCELSE is meeting this challenge by designing animal model experiments and conducting metagenomics analysis of faeces, sputum and bodily tissues to establish mechanisms and causality of the interaction of the microbiome with both, the insect and the mammalian host.

Figure 1:
Summary of sampling data sets, data generation and analyses. Blowflies and houseflies were collected in individual vials and immediately placed on dry ice until DNA extraction. Samples were individually sequenced in a multiplexed run, generating 6,759,843,350 reads for both fly species. The blowfly draft genome generated in this study and the housefly reference genome (RefSeq number GCF_000371365.1) were used as filters to remove host-related reads. The final metagenomic data set included 3,009,429,390 reads for 116 flies. Reads were processed with three different bioinformatics methods and assigned to bacterial taxa using the rapsearch2 algorithm against the NR database (v. April 2015), the dbAssign in-house script (https://github.com/aakrosh/dbAssign) against a database with 5,614 complete and chromosome-level assembled microbial genomes (v. April 2016) and a BWA approach against specI clusters.



Main findings

Insect microbiome

Flies and mosquitoes are important vectors of diseases in urban centres around the world. However, studies of insects as carriers of disease have solely focused on their roles as biological vectors. This project is surveying insects in their role as mechanical vectors and characterising their associated microbial communities.

The microbiomes of 116 individual flies (blowflies and houseflies) from varying habitats on three continents were

collected and subjected to high-coverage, whole genome sequencing. This allowed for genomic and metagenomic analyses of the host and its associated microbiome at the species level (Figure 1). A stochastic distribution of human pathogens, such as *Helicobacter pylori,* was detected, thereby demonstrating the potential of flies as proxies for environmental and public health surveillance. A major part of the blowfly microbiome is composed of the endosymbiont *Wolbachia* sp., but the presence of pathogen-like microorganisms was also observed, associated with urinary tract infections, diarrhoea, pneumonia and nosocomial infections. To improve the metagenomics analysis of the microbiomes, the blowfly genome was completely sequenced using PacBio and Illumina HiSeq instrumentation. The total size of the blowfly genome is estimated to be 687.9 Mb and the assembly consists of 5,896 scaffolds with an N50 of 317 Kb. The blowfly genome generated in this project is one of the most complete insect genomes sequenced to date.

Methods developed for these models are now being applied for the *Aedes aegypti* and *Aedes albopictus* mosquito species that act as vectors of dengue, chikungunya and Zika viruses in Singapore and other tropical locations.

A metagenomic study of the microbial communities associated with *A. aegypti* utilised 60 female mosquitoes and 10 mosquito larvae (4th instar). In addition to genomic data, transcriptome data was generated and analysed to assess relevant differences in the expression patterns of bacterial taxa *in vivo* (live host) and *in vitro* (bacterial cultivars). Besides *A. aegypti*, the microbiome of the tiger mosquito, *A. albopictus*, is also being analysed. A total of 12 *A. albopictus* adults, collected at different locations in Singapore, showed a strongly reduced microbial diversity compared to *A. aegypti*, the main dengue virus vector. These findings may elucidate the differences in vector competence in Singapore and help direct future strategies for controlling vectors of the dengue virus.

Rodent microbiome

Rodents provide robust and tractable models to study the mutual relationship between animal host and faecal microbiomes. Many scientific questions about the human gut microbiome require intervention and experimental manipulation that in many cases are not admissible in humans. SCELSE is meeting this challenge by designing animal models/experiments and conducting metagenomics analysis of faeces, sputum and bodily tissues to establish mechanisms and causality of the interaction of the microbiome with the animal host.

The projects addressed four overarching themes. (1) Viability and longevity of long-term gut microbiome transplants; (2) impact of a high fat diet on the mammalian gut microbiome; (3) impact of different sugar diets on the mammalian gut microbiome; and (4) impact of bile acid production on the mammalian gut microbiome.

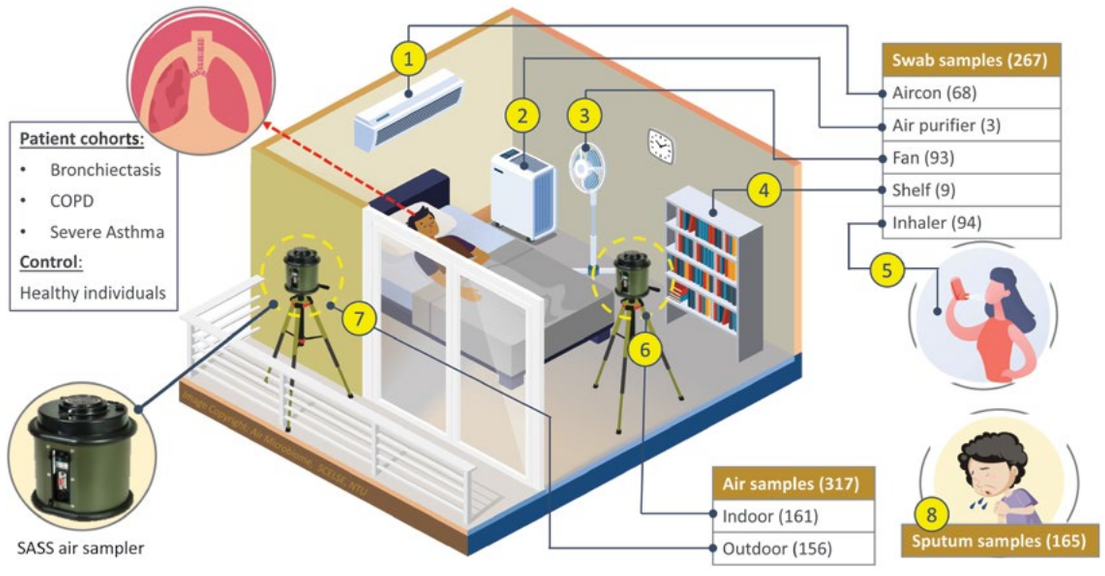
(1) Stools from both juvenile and mature mice were transplanted into juvenile mice to investigate the effects on the recipient microbial community. After a two-week settling period, DNA and RNA from stool samples were sequenced. Overall, stool transplants transformed the recipient's gut microbiome to that of the donor's gut microbial community, and the transformation persisted even after a prolonged period of time. These results provide additional insights into the development of viable faecal microbial transplant therapy in human cohorts.

(2) The microbiome studies also investigated the effect of different diets on the gut microbiome, using mice as a model, and the subsequent effect of reversal to baseline diet. After nine weeks of experimental diets, DNA from the stools was sequenced. All subjects' diets were subsequently reversed to 'normal' chow for nine weeks, then stool DNA was again sequenced. *Akkermansia* and *Alistipes* bacteria appear to be a gut microbiome signature for high fat and sugar diets. In analysed liver transcriptomes of the subject mice, methylation patterns suggested a lingering effect on the mouse liver, even after the diet was reverted back to 'normal'. Overall, this study provides insight into the effect of changing diet and how it may leave permanent effects on the host's body and physiology.

(3) The effect of stress and environment on the gut microbiota was studied using the rat caecum. The study involved four rat treatment groups: control, stress-treated, control in an enriched environment and stress-treated in an enriched environment. Each treatment consisted of eight rats and DNA from their stools was sequenced. This study demonstrated that *Lactobacillaceae* bacteria were the major components of community shifts in stress-treated rats in an enriched environment, while *Lachnospiraceae* bacteria affected microbial community assemblage patterns in stress-treated rats in normal environments.

(4) To explore the consequence of the CYP8B1 bile acid production gene on mouse gut microbiota, stools from two cohorts were investigated (wild-type and CYP8B-gene knockout mice). The microbiomes of the wild-type cohort harboured more genes related to general metabolism processes than the knockout cohort. In contrast, the knockout cohort

Figure 2: Home and respiratory project sampling collection.



possessed more genes related to flavone and flavonol biosynthesis. This study provided insights into the impact of the CYP8B1 gene knockout on the bile metabolism and the mouse gut microbiome community structure. Presently, the mechanism that drives the observed metabolic differences remains unclear.

Human microbiome

The complexity of the microbiomes associated with Singaporean environments will likely impact human microbiomes and these will in turn influence pathogenesis and morbidity.

This theme is extensively studied by SCELSE and here divided into seven key projects. (1) Home and respiratory microbial composition of patients with respiratory disease; (2) relationship between gut microbiome and human cardio-phenotype cohort; (3) gut-microbiome interaction in Parkinson's disease; (4) gut microbiomes, human ageing and lifestyle; (5) metagenomic analysis of arterial plaque; (6) infants with skin allergies (Growing Up in Singapore Towards healthy Outcomes – GUSTO); and (7) probiotic supplement effect on infant cohort.

(1) Home and respiratory microbial composition of patients with respiratory disease: In ongoing collaboration with LKCMedicine, NTU, the microbial component of samples taken from respiratory disease patients was analysed to discern microbial signatures unique to each disease. Sputum DNA was sequenced from five cohorts: non-diagnosed/control, bronchiectasis, chronic obstructive pulmonary disease (COPD), severe asthma with fungal sensitisation (SAFS), and bronchoalveolar lavage (BAL) from COPD patients. This study was paired with overnight indoor and outdoor air and surface sampling of patient homes conducted by SCELSE's air microbiome team to ascertain possible environmental triggers and contributors to disease severity (Figure 2). Ultra-deep metagenomic shotgun sequencing was used to evaluate airway resistomes and relate them to host and environmental microbiomes. This approach uncovered a core airway resistome dominated by macrolide resistance linked to the host microbiome. It also presents the possibility that inhaler devices may act as a surrogate of the host airway microbiome and remain a key resistance reservoir. Advances in this study's paired understanding of respiratory disease microbiomes with patients' home environments will also help to inform the patients on possible changes to their lifestyle or home that can improve their condition.

(2) Relationship between gut microbiome and human cardio-phenotype cohort: The association between alterations in cardiovascular stiffness and distinct differences in gut bacterial profiles were investigated comparing two cohorts (normal vs abnormal) cardiovascular stiffness phenotypes (stiff). Stool samples were sequenced for microbial analyses, as well as dietary habits. An elevated abundance of *Bacteroides* and *Paraprevotella* was observed in the 'stiff' cohort, while *Clostridioides*, *Prevotella timonensis* and *Peptostreptococcaceae* bacteria showed elevated abundances in the 'normal' cohort. Both the *Burkholderia* and *Clostridium* bacteria were more prevalent in subjects with meat-heavy diets. This preliminary data provides a foundation for future studies to examine the role of diet, and/or interventions, on altering gut bacteria with a view to ameliorate cardiovascular stiffness and improve cardiovascular structure and function.

(3) Gut-microbiome interaction in Parkinson's disease: The gut microbiome profile differences between Parkinson's disease patients and healthy individuals were investigated using Singapore-based Parkinson's disease subjects, in collaboration with the National Neuroscience Institute. Patient's data were collected and stool sample DNA sequenced, allowing for metagenomics results to be compared between the two groups. The Parkinson's cohort presented a significant reduction of *Lachnospiraceae* bacteria and increased abundance of *Bacteroidaceae* bacteria compared to the healthy group. In addition, the Parkinson's cohort's microbiome displayed significantly more gene capacity for the following functions: sugar utilisation, lactose and galactose uptake, glycolysis and gluconeogenesis, mannose metabolism, and maltose/maltodextrin utilisation. This study provides preliminary data to demonstrate the relationship between the gut and brain condition of Parkinson's disease patients.

(4) Human ageing and lifestyle: The study's overarching hypothesis is that humans who exercise have a distinct trajectory of microbes, which differs to those living a more sedentary life. This ongoing project will profile 400 human individuals in the coming years. The gut microbiome profile distinctions between people of different activity were assessed using an age-based cohort, categorised by their daily lifestyles (active versus sedentary). Stool sample DNA was sequenced and data collected from all subjects, including answers to health-related questionnaires. The sedentary cohort presented an elevated abundance of Gamma-proteobacteria, while several Firmicutes and Bacteroides species were more prevalent in the active cohort. Long-term objectives include identifying a 'healthy' ageing microbiome that can be used as a template for the design of future intervention strategies targeting the gut microbiome by means of food intervention and micronutrient supplementation.

(5) Metagenomic analysis of arterial plaques: Atherosclerosis is considered a chronic disease of the arterial wall that can lead to obstruction of peripheral arteries, congestive heart failure, heart attack and stroke in humans. Some recent studies have established the presence of bacteria in atherosclerotic plaque samples and suggested their possible contribution to the development of cardiovascular disease. To investigate the bacterial community composition in atherosclerotic plaques, human samples derived from patients who underwent endarterectomy due to recent transient cerebral ischaemia or stroke were compared to a control group of patients who all died from causes not related to cardiovascular disease. The data suggest that a wide range of microbial agents is present in atherosclerotic plaques, with an intriguing new observation that these biofilm communities displayed differences between symptomatic and asymptomatic plaques, as judged from the taxonomic profiles in these two groups of patients. Several prominent species prevalent in the plaque tissue include *Lactobacillus rhamnosus*, *Neisseria polysaccharea*, *Helicobacter pylori* and *Acidovorax* spp.

(6) Infants with skin allergies (Growing Up in Singapore Towards healthy Outcomes – GUSTO study): This study investigates the possible link between an infant's early gut microbiome profile and their susceptibility towards skin allergies in later life. A pool of infants with eczema allergy and non-eczema was selected, with sampling conducted at ages of three weeks, three months, six months and 12 months, in collaboration with the National University Hospital System. Several bacterial genera were significantly elevated in the eczema cohort, such as *Veillonella*, *Streptococcus* and *Enterococcus*. From the functional perspective, the eczema cohort's microbiomes possess a higher gene capacity for lipid, glycan and fatty acid metabolism.

The gut microbiome profile was distinct in the eczema infants. This data can be used to elucidate possible links between allergies and gut microbiome profiles, which can inform possible probiotic therapies for infants with severe skin allergy.

(7) Probiotic supplement effect on infant cohort: With the aims of investigating the effect of probiotic supplements on caesarean and vaginally delivered infants in Singapore, stool samples were sequenced and analysed. Danone Nutricia Research Singapore (DNR) provided the sample cohorts comprising the mother and either vaginally delivered infants (VD), or caesarean-delivered infants (CS). In the study, the infants were mixed fed. Subjects from each group of infants born by elective C-section (synbiotic, prebiotics and control) received the study product corresponding to their allocated group in addition to breastfeeding. The group of vaginally born infants (reference group) was also mixed fed and a control formula was provided to ascertain whether observed differences between study arms and the reference group resulted from mode of delivery and nutritional interventions.

DNA was sequenced from stools of the mother and infants collected within 3–5 days of delivery. The microbiomes of the mother cohorts were distinctly more diverse compared to those of the infant cohorts' microbiomes, with a high-profile distinction between VD and CS-delivered infant gut microbiomes. In general, the gut microbiomes of CS infants have significantly less capacity to metabolise breast milk. This could inform on the need of *Bifidobacterium* supplementation from alternative sources to allow better breast milk metabolism in CS infants.

Future directions

Host microbiome studies using different 'omics approaches are still evolving, but will continue to provide important insights into systems-level research. Future efforts will be directed towards obtaining an even greater level of in-depth understanding of the complex host-microbiota interactions. These will take current projects beyond the primary prokaryotic components of the microbiome and will increasingly require population-level research also for the host systems. Furthermore, there is a need for expanding analytical pipelines to also include fungal and viral taxa.

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Gut Microbes (2020) 12:e1801964-1801962.

BMC Microbiology (2021) 21:191.

CULTUR-OMICS DATA SETS FOR SINGAPORE ECOSYSTEMS

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Introduction

Microorganisms in Singapore and other tropical ecosystems are vastly underrepresented in globally available DNA sequence databases, despite a significant increase in such sequence information over the past decade (>100,000 fungal organisms; >400,000 bacteria). Over the past 15 years, advances in high-throughput sequencing technologies have largely reduced the cost of DNA sequencing, while increasing the length of a DNA sequence being deciphered. Together with novel assembly algorithms, this allows for the generation of complete microbial genomes at a much faster rate. Further, the dissection of complex microbial community structure achieved through complete genome analysis of microorganisms cultivated *en masse* complements the metagenomics approach that analyses the total DNA content of a sample without prior cultivation. While metagenomic analyses avoid cultivation biases, they are restricted by relatively low microbial representation in public DNA databases. The combination of metagenomics and cultivar sequencing enables comprehensive environmental datasets to be generated.

One such example is the airborne microbial community in Singapore, for which taxonomic identification was successful for less than 30% of the generated DNA reads. As the vast majority of this community is uncharacterised and hence not represented in reference databases, this project sought to generate high-quality whole-genome assemblages from hundreds of bacterial and fungal isolates.

Main findings

Reverting back to classical microbiological isolation procedures and combining them with recent advances in molecular and analytical technologies enable high-throughput cultivation, whole genome sequencing and assembly workflows, a novel field termed 'culture-omics'. Particular challenges for this project involved low success rates for cultivation, as low as 1% of the total diversity encountered. To overcome this restriction, more than 20 different media types, as well as widely varying cultivation parameters were employed. The adjusted culture methods captured the large taxonomic diversity of fungi, in particular the Basidiomycota. The genomes of fungal taxa that remained uncultivatable were attained by direct-sequencing of wild-grown fruiting bodies or other biomass. Optimised nucleic acid extraction methods were developed to obtain high-quality, intact DNA from an array of different cell types for a combination of long-read and short-read sequencing. The *de novo* assembled fungal genomes were further validated to ensure the quality of the automated assembly pipeline for these very large genome sizes. In combination with metagenome-based taxonomic analysis, the cultivation efforts are focused on achieving equal representation as the phyla and taxa as encountered in the metagenomic datasets. This culture-omics approach has yielded high-quality 'platinum-grade' whole-genome assemblies of bacterial and fungal genomes specific to the Singapore environment.

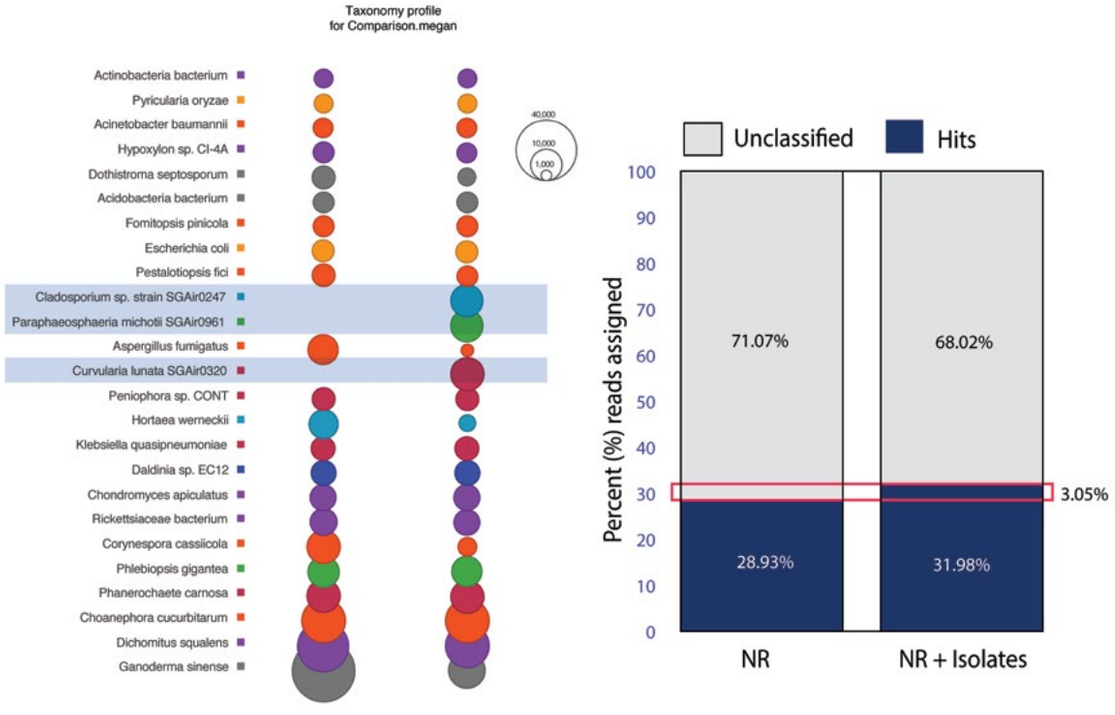

Figure 1: Location-specific reference genomes improve resolution of metagenomic analysis.

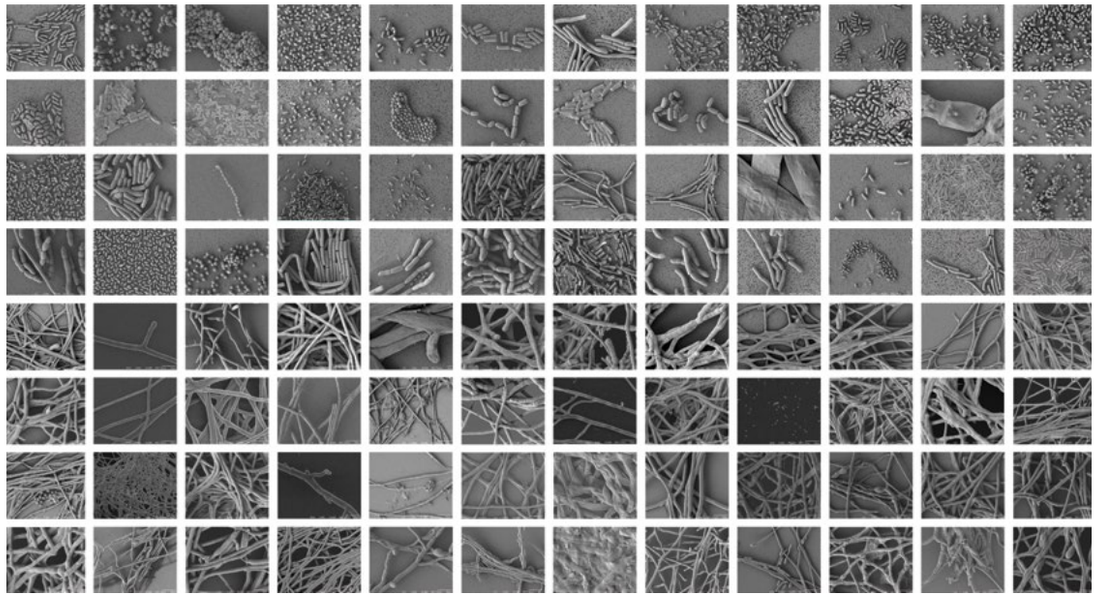

Figure 2: Scanning electron micrographs of bacterial and fungal air isolates.

The addition of reference genomes to SCELSE's metagenomic query sets underlines the importance of these cultivation efforts as species-level analysis resolves top-ranking taxa to hits among locally identified organisms, which previously remained unidentified (Figure 1). The culture-omics approach was further validated through the additional resolution of taxa in internationally collected air samples, which show an increase of >20% of identifiable fungal microorganisms. Extensive cultivation and sequencing efforts are accompanied by scanning electron microscopy-based imaging of the isolated cultivars, allowing for phenotypic features to be assessed (Figure 2). The combination of genomic and imaging data allows us to elucidate genotype-phenotype correlations that further our understanding of these organisms' role in their native environments.

The resulting collection now supports research efforts not only for air microbiome studies, but also serves as an important reference for microbiome research of all ecosystems and environments.

Proceedings of the National Academy of Sciences of the United States of America (2019) 116(46): 23299–23308.

Gut Pathogens (2021) 13(1): 6.

Mycopathologia (2020) 185(3): 591–594.

Gut Pathogens (2020) 12: 12.

+ 25 publications in *Microbiology Resource Announcements.*

Future directions

In collaboration with experts in both bacterial and fungal ecology and taxonomy, the functional content of newly sequenced genomes is being analysed. These studies are being followed up by laboratory experiments that test the predicted physiological capabilities and metabolic reactions, which may result in translational outcomes.

GENOMIC FORENSICS

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Introduction

Genomics plays a key part in understanding the potential causes of disease or pathogen contamination in food supplies. The signatures of microbes and their communities act like a fingerprint, thereby helping to identify the origins and modes of transmission. In some instances, the identification of pathogenic strains of specific microorganisms requires the sequencing of the whole genomes of a microorganism. The genome-wide analysis enables a strain-level identification, which positively identifies sources of microbial contamination by cross referencing with public genome databases of known foodborne disease agents recovered from suspected sources. Identified disease agents might not act independently, but in concert with other organisms in a specific environment. As such, in addition to whole genomes, metagenomic sequencing can be used to inform us of co-occurrences within microbial communities. These taxonomic patterns can serve as an early warning signal for unusual growth patterns in monitored food supplies. By pairing the above genomic tools with the knowledge of microbial ecology, SCELSE's expertise has been made available to the public health sector, and contributes to informing public health policies.

Main findings

Food quality and safety – foodborne microbial communities

In collaboration with the NTU Food Technology Centre (NAFTEC), SCELSE applied genomics and metagenomics for food quality and safety assessment. This collaboration established a Singapore-based database of frequently encountered microbial food pathogens, and provided a rapid diagnostic tool for the identification of potential sources. The approach has already been deployed at a large scale in the United States of America and resulted in a new programme headed by the FDA in which food processing companies are providing access to their products and manufacturing sites for monitoring. This system resulted in a much reduced time frame for identifying general sources or even implicating entire industries in the instances of severe food poisoning epidemics. The information obtained can be used to track the source of the pathogen contamination in certain food batches, thereby limiting the economic damage resulting from nationwide recalls.

The Singapore study assembled complete genomes of bacterial pathogens commonly found in food contamination outbreaks. Ninety-six *Salmonella* bacterial isolates were sequenced using Illumina instrumentation to build complete genome assemblies, looking further into the genetic make-up of specific isolate to investigate the genome's DNA methylation pattern.

Forty-six of the 96 *Salmonella* isolates belonged to *Salmonella enteritidis* species. *S. enteritidis* is the predominant cause of foodborne infections with clinical symptoms such as diarrhoea, fever and abdominal cramps within 12 to 72 hours of exposure. Furthermore, this species possesses plasmids containing either beta-lactam or tetracycline antibiotic resistance genes, with implications for the rise of antibiotic-resistance foodborne pathogens.

Investigation of morality in painted terrapin turtles (*Batagur borneonensis*)

SCELSE aided the Singapore Zoo in investigating the cause of death in several painted terrapins (*Batagur borneonensis*) turtles. Metagenomic analysis of liver and heart samples revealed signatures of *Clostridium* strains in the terrapin's liver tissue. As many as 300 sequencing reads perfectly aligned to *Clostridium botulinum* plasmids containing the C2 Toxin gene. *C. botulinum* is a bacterium ubiquitous in nature, often found in soils and water. Specific pathogenic strains display a severe toxicity in human intestinal infections, causing food poisoning and wound infection. While the C2 toxin is not cause for *C. botulinum's* hallmark neurotoxicity, it has been shown to be cytopathic for many different cell types, causing an increase in intestinal secretion, vascular permeability and cell haemorrhaging. As an outcome, SCELSE's investigation led to the implementation of protocols that prevented the *C. botulinum* infection from spreading further and also informed on the required disinfection procedures for the turtle habitats.

Flies as proxies for microbial communities in the environment

Members of *Diptera* taxa (flies) act as mechanical vectors, making contact with various body parts and spreading potentially infectious disease. In general, it was assumed that flies transmit microorganisms by regurgitation or excretion. Individual houseflies and blowflies (n = 116) were

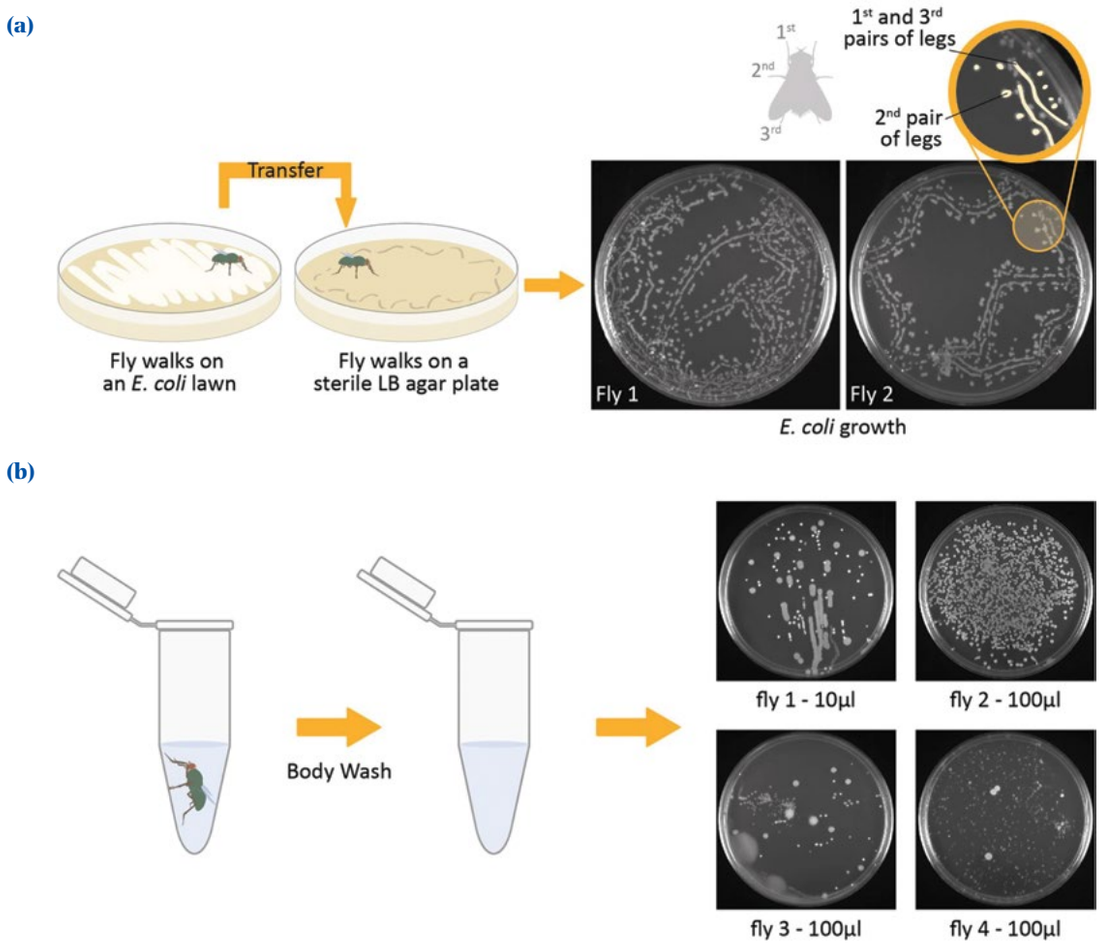

Figure 1:
Microbial transport by a mechanical vector. (a) Blowflies were exposed to a petri dish with an *E. coli* lawn and then allowed to walk on a fresh, sterile agar plate. The path of the fly walking on the agar can be seen as footprints after incubation. The line of growth indicates that dispersal of bacteria by the blowfly occurs mainly via the legs. The track pattern matched the arrangement of the three pairs of legs, with the first and the third pair resulting in nearly continuous, linear bacterial growth, and the second pair of legs generating separate circular colonies on the outside of the lines. In a few instances, it was possible to observe bacterial growth between the lines of growth probably from mouthparts. (b) Growth experiments to evaluate the viability of bacteria on the outer body surface of blowflies. Flies were sampled in urban environments near a food court and washed with PBS sterile buffer for 10 minutes. The buffer was then spread in LB agar plates and incubated at 37°C overnight. Up to 30,000 CFUs were estimated from body washes, showing that bacteria can be transported on the exterior surfaces of flies in a viable state that they can be cultivated.

sampled from different habitats on three continents and subjected to high-coverage, whole-genome shotgun sequencing, identifying specific host-associated microbiomes for both fly species. The two fly species, however, also showed an overlapping core microbiome. In an anatomical study, legs and wings were identified to harbour a high diversity of microbes and are an important route for microbial dispersion. In detail, the feet of the flies consistently released viable bacteria at every step they took on an agar surface (Figure 1). This demonstrates the vector capacity of flies to transport viable, cultivatable bacteria from one place to another in an airborne manner. This mechanism accounts for the majority of the fly's pathogenic potential, as other body parts such as head, thorax and abdomen showed significantly less microbial content. Further evidence of bacterial viability on the outer body of a fly was verified with bacterial growth from sterile buffer used for washing the surface of individual flies, collected in urban environments. The washes yielded up to 30,0000 colony forming units (CFUs) per fly.

The environmental sequencing approach here detected a stochastic distribution of human pathogens, such as *Helicobacter pylori*, further demonstrating the potential of flies as proxies for not only environmental, but also public health surveillance. The precision of our taxonomic identification of bacterial species led to the conclusion that carrion flies are a proxy for the environment from which a significant part of their microbiome is acquired. It highlights the importance of the surveillance of fly microbiomes, especially in densely populated areas. If included in public health surveillance programmes, it will be possible to predict and prevent routes of transmission of microbes and potential pathogens mediated by these vectors.

Gut Pathogens (2018) 10:20.

Scientific Reports (2017) 7: 16324.

Future directions

SCELSE will continue to collaborate with institutions in Singapore to help uncover the signatures of microbes in their communities to aid in both an understanding of the causal influence of illness and contamination as well as helping to safeguard public health by affecting positive change in public health policy.

Pseudomonas aeruginosa biofilms surrounded by mouse corneal immune cells at day-2 infection.
Image credit: Joey Yam Kuok Hoong, SCELSE

RESEARCH THEMES

Host-microbe interactions

(for human health)

All higher organisms live in intimate association with microbes. The interactions are primarily positive or neutral, with microbes imparting protection from pathogens or providing nutrients to the hosts, which in turn provide favourable niches for residing biofilms. However, microbes are also the causative agents of disease in a host and, as biofilms, are responsible for acute and chronic infections.

SCELSE's host microbiome (holobiont) interactions research covers several host organisms, from plants and invertebrates in aquatic and terrestrial systems to humans, to understanding the broad scope of interactions, and complex associations between hosts and their microbiomes. This knowledge provides insights into the healthy functioning and wellbeing of the host organisms, with broader implications for ecosystem health.

The response of an organism to competition and environmental change involves both the host as well as its microbiome. As such, holobiont systems can be manipulated to promote resilience, such as increased tolerance of temperature stress in corals, or modifying gut or skin microbiomes for host health and pathogen deterrence. Understanding microbiomes as a whole and as component parts is imperative to ensuring a healthy host.

HOST-PATHOGEN INTERACTIONS DURING BIOFILM-ASSOCIATED INFECTION

Overview

Invading microorganisms must overcome a wide range of host defence factors to cause disease. These include inherent environmental factors and host-directed antimicrobial responses. Biofilm formation represents a key pathogenesis mechanism that enables bacterial colonisation and persistence within the host in a variety of disease settings, including urinary tract infection, wound infection and heart infection (endocarditis). Such biofilms are frequently resistant to antibiotics and may create a reservoir for both chronic reinfection and the selection and transmission of antibiotic resistance. Examination of bacterial-host interactions in the context of both *in vivo* and *in vitro* models of infectious disease allows for the identification of key bacterial virulence factors, and the mechanisms by which these factors may modulate immune system clearance. Better understanding of these complex, interdependent host-pathogen interactions seeks to pave the way for the development of novel therapeutics for chronic, biofilm-associated infectious disease.

Urinary tract infection

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Introduction

Urinary tract infections (UTIs) are one of the most common worldwide bacterial infections. They occur when pathogens present in the faeces ascend the urethra into the bladder, colonise and trigger a host response, or are introduced as catheter-associated urinary tract infections (CAUTI). UTIs are caused by a range of pathogens, most commonly by (uropathogenic) *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis* and *Staphylococcus saprophyticus*, and are frequently biofilm-associated and resistant to conventional antimicrobials. The global disease burden of UTIs has been estimated to be about 150 million cases per year and greater than $6 billion in direct healthcare costs. The rise of multidrug resistance (MDR) among uropathogens threatens to further increase this health and economic burden.

E. faecalis appears to evade immune clearance during UTI infection, and there is an urgent need to understand the molecular mechanisms of pathogenesis and host evasion underlying *E. faecalis* monospecies UTIs as well as mixed-species UTIs caused by *E. faecalis* and other *uropathogens*. SCELSE research focuses on understanding how pathogenic biofilms survive the immune response elicited during CAUTI, which may lead to novel treatment targets and approaches.

Main findings

Catheterisation was found to initiate increased expression of genes associated with defence responses and cellular migration, with ensuing rapid and sustained innate immune cell infiltration into the bladder. This persistent sterile inflammatory response renders the host hypersensitive to *E. faecalis* and uropathogenic *E. coli* (UPEC) infection. Colonisation was achieved using a 100-fold lower inoculum than for infection of an undamaged urothelium. Interestingly, while catheterisation created vulnerability for Gram-negative UPEC infection, colonisation by the Gram-positive uropathogen *E. faecalis* was reduced.

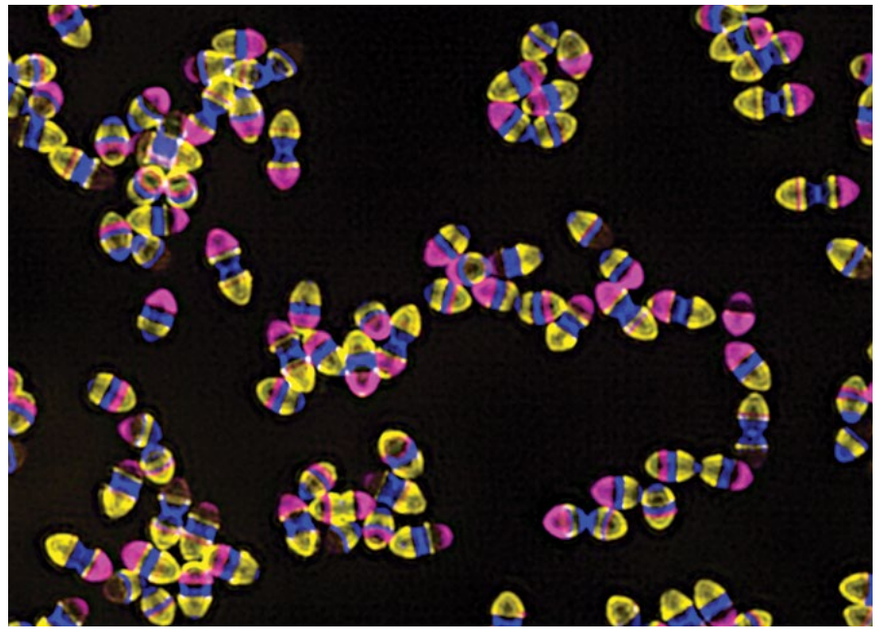

Figure 1: Examining the dynamics of *E. faecalis* cell wall formation. *E. faecalis* cell walls labelled using three different coloured dyes, applied at different times. Scale bar: 5 µm.

Hence, a single prophylactic antibiotic treatment, administered concurrently with catheterisation, protects against UPEC and *E. faecalis* bladder and catheter colonisation as late as six hours after catheter implantation. Thus, this study has revealed a simple, safe and immediately employable intervention to prevent CAUTI, with the potential to decrease one of the most-costly hospital-incurred, biofilm-associated infections.

Examining in detail the host response to *E. faecalis* infection *in vivo*, SCELSE researchers found that *E. faecalis* prevented proinflammatory (NF-κB) signalling in key cells of the innate immune system (macrophages) in the presence of immune-stimulants, as well as during polymicrobial infection with *E. coli*. *E. faecalis* and *E. coli* co-infection in the CAUTI mouse model described above resulted in a suppressed upregulation of macrophage proinflammatory genes in the bladder compared to *E. coli* infection alone. Moreover, co-inoculation of *E. faecalis* with a commensal strain of *E. coli* into catheterised bladders significantly enhanced *E. coli* CAUTI. Taken together, these results support the concept that *E. faecalis* suppression of NF-κB-driven responses in macrophages promotes polymicrobial CAUTI pathogenesis, especially when co-infected with less virulent or commensal *E. coli* strains.

E. faecalis resists the onslaught of innate immune defences such as antimicrobial peptides (AMPs) by limiting their interaction with discrete focal targets on the bacteria via membrane lipid modifications. Virulence factor secretion and assembly were shown to occur at specific locations in *E. faecalis*. Given the necessity of the general secretion pathway in bacteria and the contribution of virulence factors to disease progression, the focal points that coordinate these processes are attractive antimicrobial targets. Indeed, cationic human β-defensin AMPs were shown to interact with *E. faecalis* at discrete septal foci, and this exposure disrupts sites of localised virulence factor assembly. Modification of anionic lipids by a multiple peptide resistance factor (MprF), a protein that confers AMP resistance by electrostatic repulsion, renders *E. faecalis* more resistant to killing by defensins and less susceptible to focal targeting by the AMPs. These data suggest a paradigm in which focal targeting by antimicrobial peptides is linked to their killing efficiency and to disruption of virulence factor assembly.

Future directions

In an effort to better understand and ultimately treat *E. faecalis* biofilm-associated infections such as CAUTI, ongoing research seeks to elucidate the *E. faecalis* factors that specifically suppress the host immune response, as well as the host cellular pathways. Moreover, the research is also aimed at understanding the molecular mechanisms by which *E. faecalis* coordinates focal virulence factor assembly. This work employs a model virulence factor, the Ebp pilus, which is essential for biofilm formation and CAUTI.

Proceedings of the National Academy of Sciences USA (2013) 110: 20,230–20,235.

PLOS ONE (2014) 9: e97798.

PLOS ONE (2017) 12: e0175886.

JCI Insight (2016) 1: e88178.

Infection and Immunity (2017) 85: e00378–17.

Chronic wound infection

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Introduction

Chronic wound infection represents a major public health issue both globally and in Singapore. The healthcare cost of non-healing chronic wounds has recently been estimated at $25 billion annually in the United States. Patients with underlying health conditions such as diabetes and obesity are at heightened risk of wound infection. *E. faecalis* is one of the most commonly identified pathogens isolated from wound infections, including diabetic foot ulcers (DFU). Biofilm formation drastically increases *E. faecalis* virulence and persistence, providing protection against external treatments and immune system clearance, generating a reservoir for chronic reinfection, and promoting antibiotic resistance.

The microbiology of chronic wounds is complex and tends to be polymicrobial and biofilm-associated, further complicating treatment approaches. *In vivo* models of biofilm-infected cutaneous wounds are used to elucidate mechanisms contributing to biofilm formation, persistence, potential synergistic interactions among bacteria and how microbes influence host repair mechanisms. Alongside this, *in vitro* examination of *E. faecalis* interactions with various host cells (immune and skin) provides insights into bacterial subversion of the immune system. Both provide potential avenues for novel therapies for chronic wound infection.

Main findings

SCELSE researchers used a mouse wound excisional model to characterise the infection dynamics of *E. faecalis* and show that infected wounds result in two different states, depending on the initial inoculum. Low-dose inocula are associated with short-term, low-titre colonisation, whereas high-dose inocula are associated with acute bacterial replication, biofilm formation, and long-term persistence (up to seven days). High-dose infection and persistence are also associated with immune cell infiltration, suppression of specific inflammatory cytokines and delayed wound healing. During high-dose infection, a specific bacterial factor – the multiple peptide resistance factor (MprF) – contributes to *E. faecalis* fitness by resisting immune clearance. This work suggested that both immune modulation and resistance contribute to persistent, non-healing wounds.

Further, viable *E. faecalis* was present within both immune and non-immune cells at the wound site up to five days after infection, raising the prospect that

intracellular persistence contributes to chronic *E. faecalis* infection. *In vitro* keratinocyte and macrophage infection models were used to demonstrate that *E. faecalis* becomes internalised and a subpopulation of bacteria can survive, and even replicate intracellularly. Bacteria persisted up to 24 hours in single-membrane-bound compartments, with no apparent fusion with the lysosome, suggesting that *E. faecalis* blocks endosomal maturation. Indeed, intracellular *E. faecalis* infection results in varied intracellular trafficking, including a marked reduction of key endosomal trafficking proteins. Further, intracellular *E. faecalis* derived from infected keratinocytes were significantly more efficient in re-infecting new keratinocytes. Together, these data suggest that intracellular proliferation of *E. faecalis* may contribute to its persistence in the face of a robust immune response, providing a reservoir of bacteria primed for subsequent reinfection.

E. faecalis co-exists with *E. coli* and other pathogens in wound infections, but mechanisms that govern polymicrobial colonisation and pathogenesis are poorly defined. During infection, bacteria must overcome multiple host defences, including nutrient iron limitation, to persist and cause disease. The contribution of *E. faecalis* to mixed-species infection when iron availability is restricted was investigated, showing that *E. faecalis* significantly enhances *E. coli* biofilm growth and survival *in vitro* and *in vivo* by exporting the amino acid L-ornithine. This metabolic cue facilitates *E. coli* biosynthesis of specific iron-acquisition molecular machinery, allowing *E. coli* growth and biofilm formation in iron-limiting conditions that would otherwise restrict its growth. Thus, *E. faecalis* modulates its local environment by contributing growth-promoting cues that allow co-infecting organisms to overcome iron limitation, promoting polymicrobial infection.

Future directions

Ongoing research seeks to examine in further detail *E. faecalis* interactions with macrophages, as well as first-responder immune cells neutrophils. A diabetic mouse model will examine chronic wound infection. For polymicrobial infections, research is continuing to further understand how ornithine cues upregulate iron acquisition in *E. coli*, as well has how iron-limitation and iron-excess differentially signal *E. faecalis* to promote biofilm formation. Further, *E. faecalis* mixed-species wound infection involving *Pseudomonas aeruginosa* and *Staphylococcus aureus* is being investigated, to identify the mechanisms for synergistic and antagonistic interactions between the organisms during biofilm formation.

Cell Host and Microbe (2016) 20: 493–503.

PLOS Pathogens (2022) 18 (4): e1010434.

Interactions of enterococcal biofilms and neutrophils on heart valves

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Introduction

Infective endocarditis is a biofilm-related infection of the heart valves causing almost 50 thousand deaths per year globally. Predisposing conditions such as valve degenerative disease, congenital valve anomalies, prosthetic valves and other cardiac implants, and intravenous drug use make heart valves and surrounding tissue more susceptible to infection. Bacteria entering the bloodstream can attach to the damaged heart valves and grow as a biofilm, leading to the formation of vegetation, a mass of platelets, fibrin, inflammatory cells and bacteria. Although inflammatory cells are present in high numbers in vegetations, they cannot clear the infection. Treatment of infective endocarditis relies on combination antibiotic therapy administered intravenously for up to six weeks. However, declining efficacy of antimicrobials in treating biofilm infections leads to infected tissue needing to be removed and valves replaced in 50% of patients, resulting in a 42% five-year mortality. Thus, there is a need for alternative, less invasive treatments to decrease short- and long-term mortality and reduce the healthcare burden.

Main findings

The limited immune response in infective endocarditis suggests that key effectors of innate immunity are either disabled or ineffective. Delineating the mechanistic details of the interactions between immune cells and bacteria will uncover new targets for treatment. These interactions were studied using rats with aortic valve lesions infected by *Enterococcus faecalis*. This pathogen successfully attached to the valves and formed microcolonies within six hours, whereas host cell infiltration at the site of the infection remained low. In contrast, valves were heavily infiltrated by immune cells at 72 hours of infection. Flow cytometry showed that neutrophils constituted about 80% of immune cells in vegetations, characterised by an increase of the RP-1 surface antigen in the presence of bacteria, indicating that neutrophils were activated.

Neutrophils have three main antimicrobial functions: degranulation, phagocytosis and neutrophil extracellular trap release (NETosis). Immunostaining of heart valve vegetations revealed large biofilm microcolonies surrounded by large numbers of neutrophils. The nuclei of neutrophils had lost their typical horseshoe shape, a sign of chromatin decondensation. In support of this finding, histone H3, the protein that helps in DNA packing, was modified in a way that promotes the unpacking of DNA. Both observations are typical hallmarks

of NETosis. Despite phagocytosis being a primary function of neutrophils, engulfment of *E. faecalis* was rare *in vivo*.

To investigate what drives the neutrophil response towards NETosis in the presence of an *E. faecalis* biofilm, neutrophils were isolated from human and rat blood. Surprisingly, both rat and human neutrophils were able to engulf *E. faecalis in vitro*, regardless of bacteria being planktonic or biofilm-based. Only a low percentage of neutrophils exhibited hallmarks of NETosis. Addition of platelets to biofilms *in vitro*, one of the main components of vegetations and a well-described trigger for NETosis did not shift the neutrophil response towards NETosis. Similarly, when the biofilm was opsonised with human or rat plasma, the neutrophil response remained unchanged.

Taken together, these results suggested that other factors, such as the inflammatory milieu at the site of the infection or bacterial factors specifically expressed in the body, might account for triggering NETosis during infection.

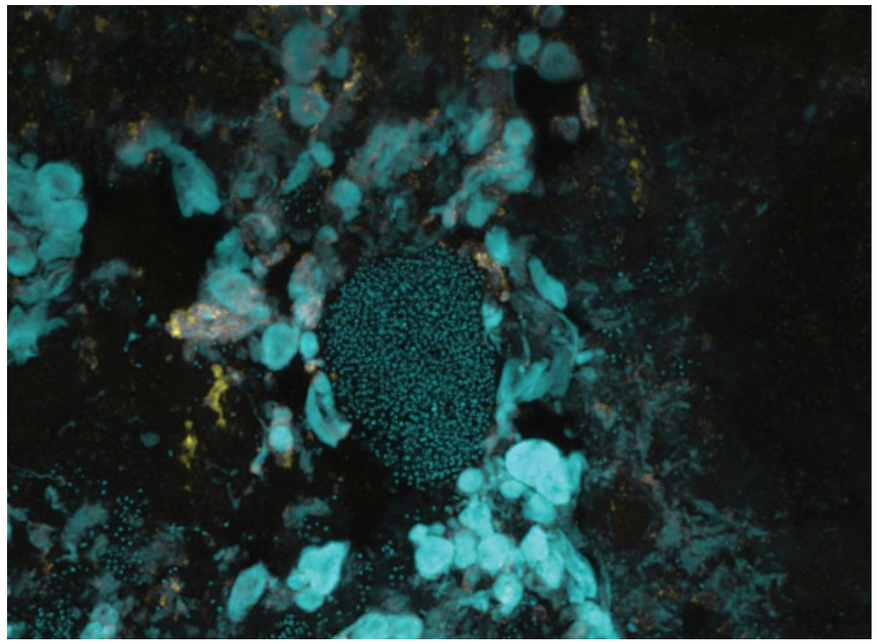

Figure 2: Neutrophils surround an *E. faecalis* biofilm during infective endocarditis. Image depicts a Z-stack projection of heart valves isolated from 72 hour *E. faecalis* infected rats, stained for DNA (blue) and citrullinated histone H3 (yellow).

Future directions

Research is ongoing on the mechanisms behind the neutrophil response towards NETosis, instead of phagocytosis in infective endocarditis, and whether NETs contribute to the containment of the infection. Future studies will focus on how the inflammatory cytokine milieu and bacterial virulence factors modulate the neutrophil response, and characterise the neutrophil response in human heart valves from patients with infective endocarditis. A better understanding of host-biofilm interactions in infective endocarditis will provide fundamental insights into the mechanism by which host responses could be modulated to fight infection.

INDOOR AIR MICROBIOMES AND RESPIRATORY DISEASE

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Introduction

While treatments for chronic respiratory disease such as bronchiectasis focus on pharmacologic interventions to treat respiratory symptoms and periodic exacerbations, little attention is paid to the contribution of environmental factors, particularly in the home setting to the course, consequences and severity of disease. Changes to the respiratory microbiome are hypothesised to be a critical output of this gene-environment interaction. A key unknown is the relationship between the microbiome in the air we breathe and the microbiome that lives and thrives within the human airway.

Nature Medicine (2021) 27: 688–699.

American Journal of Respiratory and Critical Care Medicine (2020) 202: 433–447.

European Respiratory Journal (2020) 56: 4934.

European Respiratory Journal (2020) 56: 203.

European Respiratory Journal (2020) 56: 2000418.

European Respiratory Journal (2019) 54: OA5141.

Main findings

This proof-of-concept study brought together the interdisciplinary expertise of the NTU Integrated Medical, Biological and Environmental Life Sciences (NIMBELS) partners from LKCMedicine in chronic airways disease and airway microbiome sequencing along with SCELSE's expertise in pioneering methodologies for metagenomic sequencing of microbial components of the air. The team assessed the precise relationships between air microbial composition in a patient's home to that of their lung and established the role of the 'personalised exposome' on disease course and consequence. Decoding such relationships allows for the identification of potentially modifiable environmental factors that may alter disease course or improve outcomes, which in turn will instigate a paradigm shift in the field of chronic respiratory disease. Working with clinical collaborators, the team recruited patients with bronchiectasis, COPD and severe asthma to analyse their sputum, blood and home environment (air and environmental microbiome samples). The data collected were compared to data from healthy individuals without chronic lung disease as well as cohabitants from the patients' homes (non-disease environmental controls). Using the rich data-sets, the team developed novel analytical frameworks for an integrated assessment of 'microbiomics' in the air, respiratory airways and environment for personalised clinical application, assessment and prediction ('intergrative microbiomics').

GUT MICROBIOME FOR HEALTH AND WELLBEING

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Introduction

The human and animal gut harbours a rich and very complex microbial community referred to as the microbiome. This microbial community develops sequentially from birth, and a decline in diversity is reported with ageing. These microbes play a significant role in the health of the host and impacts most organs and responses in the body, including immunological, metabolic and neurological. An unfavourable shift in the composition of the microbiome is referred to as dysbiosis. SCELSE's gut microbiome programme focuses on studying the composition and function of these microbes and developing strategies for modulating the gut microbiome for improved human health. Since the microbiome is developing from birth, and linked with poor health in the elderly, our research has focused on these stages. The new-born component studied pre-term low weight babies, as these are more pre-disposed to stresses that have a negative impact on gut microbiome development and health of the infant. With the elderly, a multi-omics approach studied elderly Singaporeans to identify biomarkers of ageing linked to the gut and oral microbiome. An *ex vivo* model evaluated potential functional foods, e.g., probiotics and prebiotics, for the capacity to modulate the microbiome. These technologies are also being applied in aquaculture to provide protection against pathogens as well as to improve growth and feed conversion.

Main findings

For the pre-term baby cohort, the administration of a single *Bifidobacterium* strain or a mixture of three strains was effective in reducing dysbiosis (higher Bifidobacteria and lower Gammaproteobacteria) and increasing levels of desirable metabolites (short chain fatty acids, e.g., propionate and butyrate). During hospitalisation, neonates with congenital gastrointestinal surgical conditions develop gut dysbiosis with low levels of Bifidobacteria and increased abundance of *Staphylococcus*. They also have low levels of short chain fatty acids compared to healthy infants.

With the elderly, the composition and function of the gut and oral microbiome when using metagenomic and metabolomic analyses revealed age-related changes in both microbes and metabolites. These changes correlated with those detected in blood and urine showing altered amino acid and fatty acid metabolism, suggesting that regulation of these pathways contributes to the ageing process. Overall, highly correlated multi-omics signatures that discriminate between elderly and young participants were identified.

To identify functional foods that can modulate the microbiome of the elderly, a laboratory *ex vivo* gut model confirmed the benefits of various dietary fibres, pre- and pro-biotic preparations. This model has also demonstrated the benefits of microbiome regulation with fermented fibre and bioactive rich foods within the programme to develop functional foods for the elderly, including 3D-printed foods.

The concept of gut microbiome modulation is applicable to other hosts and is being applied in aquaculture and poultry farming (see *Food production and microbiomes* section). The focus of this work is to use probiotics and feed ingredients to develop a microbiome that enhances fish health, protects against pathogens and improves feed conversions. To date, pathogen inhibition in laboratory studies has been achieved.

Future directions

The gut model will be used to identify the ingredients and supplements that are most effective at modulating the gut microbiome for health benefits as well as regulating metabolic pathways and indicators of poor health, e.g., dysbiosis. Specialist foods are being developed that contain the vital functional foods and ingredients in innovative delivery systems including 3D-printed foods and encapsulation technologies. Future studies will include elderly people as well as poultry and various aquaculture systems.

GUT-MICROBE LINKED SIGNALLING PATHWAY AND NEUROGENIC EFFECTS

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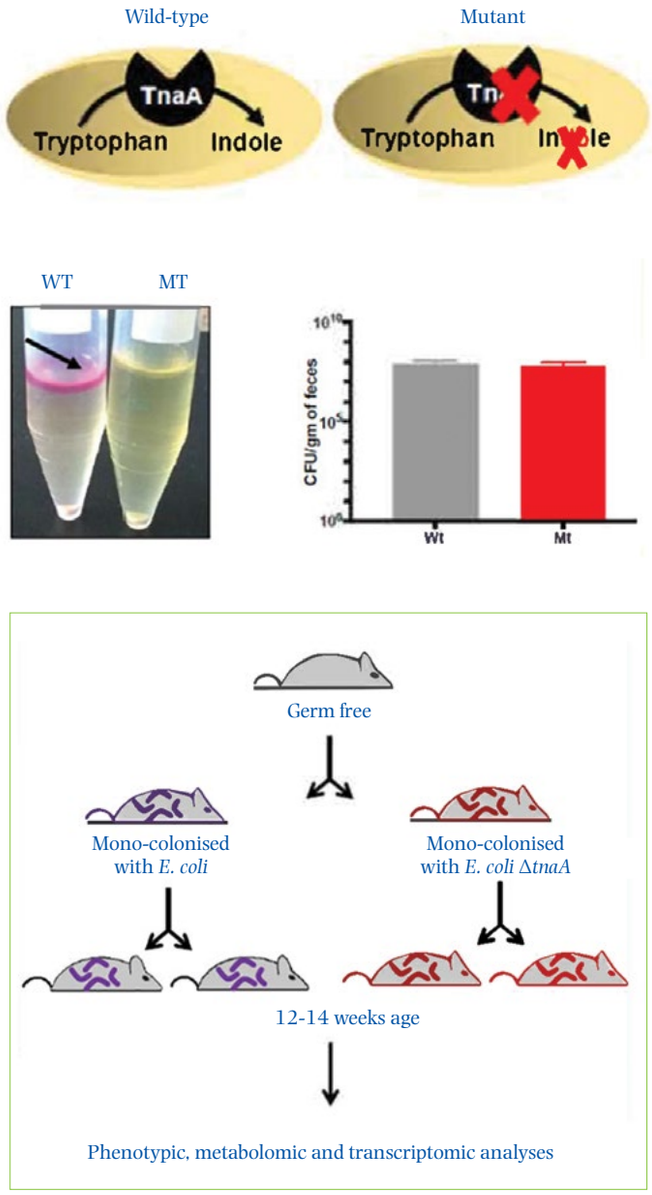
Introduction

Gut microorganisms and their metabolites are critical components for host physiology behaviour, immunity and metabolism. How this is achieved remains largely unclear. To better understand the interplay between microorganisms and the host, the metabolism of tryptophan, an essential amino acid that is metabolised both by eukaryotes and gut microorganisms, was investigated. Gut microbes break down tryptophan into several metabolites that act as ligands for the aryl-hydrocarbon-receptor (AhR) and its signalling pathway. The approach taken enabled the mammalian genome to remain unaffected, and hence only the absence or presence of indole generated by gut microbes in the body was monitored.

To examine the systemic effects on organ function in rodents, germ-free (GF) mice were colonised with wild-type (indole-producing) and tryptophanase-encoding *tnaA* gene-knockout (Δ*tnaA*) mutant (indole-non-producing) *Escherichia coli* (Figure 1).



Acknowledgement for *E. coli* strains:
Prof. Thomas K. Wood, Pennsylvania State University

Scientific Reports (2016) 6: 23820.

Main findings

Preliminary results show a reduction in body weight for many different organs in *E. coli* MT mice.

In a second study, indoles elicited neurogenic effects in the adult mouse hippocampus. Neurogenesis is reduced in GF mice mono-colonised with a single-gene *tnaA* knockout (KO) mutant *E. coli* unable to produce indole. External administration of systemic indole rescues the phenotype and increases adult neurogenesis in the dentate gyrus (DG) of recipient mice. In contrast, neurogenesis is not induced by indole in aryl-hydrocarbon-receptor KO ($AhR^{-/-}$) mice.

Neural progenitor cells (NPCs) exposed to indole exit the cell cycle, terminally differentiate, and mature into neurons that display longer and more branched neurites. The indole-AhR mediated signalling pathway elevated the expression of *β-catenin*, *Neurog2* and *VEGF α* genes, thus identifying a molecular pathway connecting the gut microbe-linked indole-AhR signalling pathway with regulation of adult neurogenesis in the adult hippocampus. The data have implications for understanding mechanisms of brain ageing and for next-generation therapeutic opportunities.

Figure 1:
Model system using germ-free (GF) mice as recipient. Young GF mice were colonised with wild-type (indole-producing) and *tnaA*-mutant (indole-non-producing) *E. coli* (referred as WT and MT mice) by oral gavage. Photo shows Kovac indole tests on WT and MT *E. coli*, with the absence of the pink-colour indole ring in the MT sample.

L'Oréal-SCELSE JOINT RESEARCH INITIATIVE

Viduthalai Rasheedkhan Regina[1], Samantha Yiling Quah[1], Sreelakshmi Cheruvalli[1], Sabrina Ang[1], Kwok Weihao[1], Hashmath Fatimah Binte Jamal Mohamed[1], Sarah Kay[1], Chong Yik Yan[1], Kelvin Kai Wei Lee[1], Chew Yean Ming[1], Adelicia Yong Ling Li[1], Liu Xianghui[1], Saw Nay Min Min Thaw[1], Vedula Krishna[2], Nasrine Bourokba[2], Anjali Jhingan[3], Steven Thng Tien Guan[3], Sylvie Cupferman[4], Luc Aguilar[5], Rohan Williams[1], Tarun Chopra[2], Scott A. Rice[1,6]

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Introduction

The skin microbiome, which is the collection of microorganisms that colonise human skin, plays a key role in maintaining skin health and function. Some of the microbiome members produce short chain fatty acids that help maintain the acidic nature of the skin and secrete antimicrobial compounds that inhibit the growth of pathogenic bacteria. These also interact with keratinocytes (cells in the outermost layer of the skin) through the human immune system's toll-like receptors and induce expression of antimicrobial peptides, thereby modulating the skin's immune response. Several clinical skin conditions, such as seborrheic dermatitis, dandruff, folliculitis and psoriasis, are associated with an imbalance in the relative abundance of the key members of the skin microbiome. This highlights that healthy skin requires the appropriate balance of its microbiome members. However, it is not clear if it is the host factors, host-microbiome interactions, environmental factors or competition within the microbiome that control the balance. Therefore, it is important to understand the key drivers that modulate the relative microbial abundances within the microbiome. This knowledge will assist in developing strategies to modulate the skin microbiome favouring skin health. The traditional approach is to study individual organisms isolated from healthy and non-healthy skin. This approach, however, is insufficient to describe how a community of multiple species of organisms, such as the skin microbiome, which interact with each other as well as with the host, can achieve a stable, non-pathogenic community. Therefore, it is vital to develop mixed-species community systems to study and describe the interactions of such complex systems.

The L'Oréal-SCELSE joint laboratory employs two different approaches. (1) A top-down approach to survey the skin/scalp microbiome of Asian populations by conduction clinical studies, and (2) a bottom-up approach to study the interactions of the individual microbial members by developing a simple and defined biofilm community of skin microbiome members.

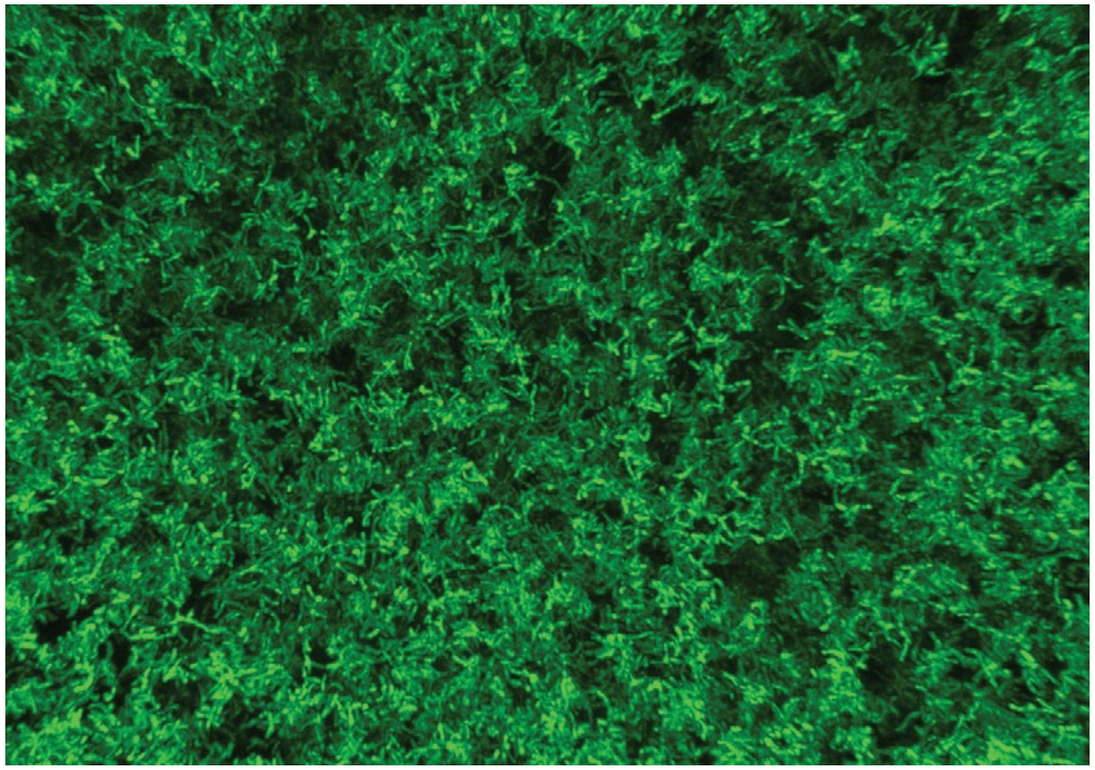
Main findings

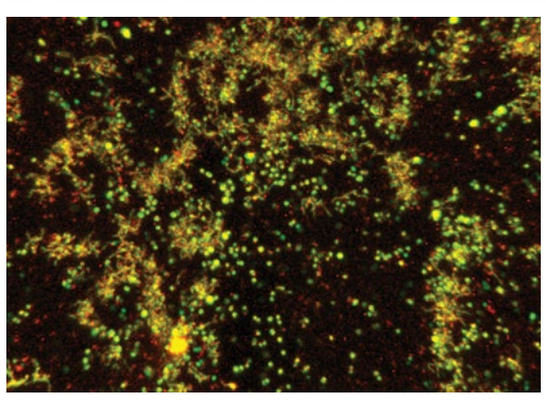
Microbiome and metabolome of healthy and diseased scalp and the hair follicles

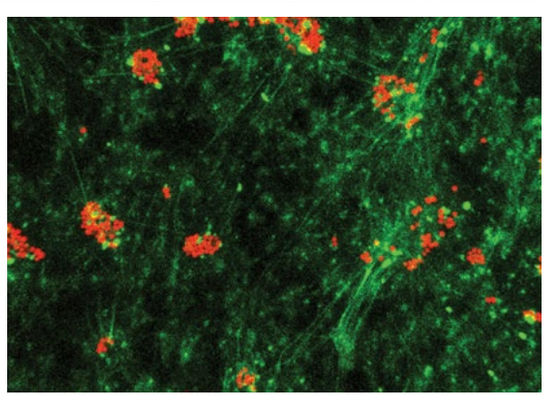
The community diversity of non-healthy skin is significantly different from the healthy skin microbiome (defined as community dysbiosis). This study focuses on the microbiome and the metabolome of the scalp and hair follicles from healthy as well as dandruff/seborrheic dermatitis (D/SD) volunteers, to understand how similarities or differences in microbial community composition and their metabolic products of healthy and D/SD follicles. Whole-genome sequencing was conducted for the total DNA extracted from a small number of swabs and hair follicles from both healthy and D/SD volunteers. Preliminary analysis showed that the scalp microbiome is more diverse than that of the follicles and that the abundant microbial genera in both the scalp and hair follicles are *Cutibacterium*, *Staphylococcus* and *Malassezia*. Further, the relative proportions of these microbial members are significantly different between the healthy and the diseased samples. Metabolites extracted from the scalp swabs and hair follicles were analysed using LC-MS. The metabolomes of the healthy and the diseased scalp are significantly different from each other. Detailed analysis of the metabolome is underway to identify metabolic features and individual metabolites that are specific for health or diseased scalp, which can be used for developing diagnostic as well as therapeutic applications.

An *in vitro* mixed-species skin microbial community model system

DNA sequencing of the skin microbiome has shown that the bacteria, *Cutibacterium* acnes and *Staphylococcus epidermidis*, and fungi such as *Malassezia restricta* constitute most of the skin microbiome. A strong correlation exists between the balance in the relative abundance of these microbes and skin health. Based on the above knowledge, these microbes







Confocal laser scanning microscopy images of single- and multi-species biofilm of skin microbes.

were selected to establish a co-culture, multi-species community model to study the interactions among the three microorganisms. With SCELSE's expertise in establishing and working with mixed-species communities, a robust, reproducible *in-vitro* community model with these organisms was developed and optimised. A generic media for culturing microbes was modified to accommodate optimal growth of all three microorganisms in the model system. This is an important milestone since the role of *M. restricta* in healthy skin is widely recognised but a co-culture model that includes *M. restricta* with the bacteria *C. acnes* and *S. epidermidis* was not available due to the challenge of growing the fungus in the same nutrient media that optimally grow bacteria. In the modified medium, the growth characteristics of both bacteria and the fungus were studied, along with their ability to form biofilms as single-species and multi-species cultures. Furthermore, oligonucleotide probes with unique fluorophores to label the microbe were developed, based on fluorescent *in-situ* hybridisation (FISH). This enables microbial quantification of the microbes and can track their relative abundance over time within multi-species community. With this model system, and the tools, it is possible to study the interaction among the three microorganisms.

Microbial interactions in the model community

Microorganisms in a mixed-species community co-operate as well as compete with each other for common resources. Such interactions shape the community structure and stability of the microbiome. To understand the types of interaction within the community, the three skin microbes were cultured as both single-species and multi-species, and their biofilms studied. The amount of biofilm produced by mixed-species community is higher than that of single-species populations, strongly suggesting co-operative interaction of the microbes. Furthermore, cross-feeding of cell-free supernatants (CFS), harvested during exponential as well as stationary growth phase, showed that the bacteria and fungi have both co-operative and competitive interactions, depending on their growth phases. The above observations strongly indicate that there is certainly cross-talk among the microbes in the community and that they modulate each other's relative abundance depending on their metabolic state.

Future directions

Screening for biofilm dispersal agents

Alternatives for antibiotic interventions for scalp and skin disorders are being investigated. The search is to find compounds that will not kill the microbes but maintain them at an appropriate level. To this end, the co-culture model system is being used to screen for compounds that can disperse biofilms formed by skin microbes. Several compounds known to induce biofilm dispersion in other systems have been tested, with a DNA-degrading enzyme that disperses the biofilm identified. Further screening involves SCELSE proprietary compounds and cosmetic raw materials from L'Oréal to establish their ability to modulate the relative abundance of the members of the model skin microbiome towards skin health.

Understanding root-soil microbiome interactions to foster enhanced plant reslience and productivity.
Image credit: Shruti Pavagadhi, SCELSE

RESEARCH THEMES

Microbiomes in food production

Singapore has identified food security and safety as key areas of national priority, establishing a goal of achieving sufficient locally grown food to meet one-third of the nutrition needs of Singapore by 2030 (30 by 30 goals). The production of sufficient food to meet the nutritional needs of the world's growing population is further challenged by the effects of climate change on rainfall patterns, droughts, floods and the degradation of soil quality. SCELSE is investigating the means by which biofilms and microbiomes can help to meet these goals through their intimate involvement in plant, human and livestock health, alternative sources of nutrition, and valorisation of waste streams.

A SIGNALLING SYSTEM FOR PLANT-ASSOCIATED BENEFICIAL BIOFILMS

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Introduction

A large percentage of microbes in nature reside in the biofilm mode of life, where they are attached to surfaces and are embedded in self-secreted polymeric matrices, in contrast to their free-floating or planktonic lifestyle. In vegetated soils, the plant root–associated microbes are nearly 100-fold higher in abundance than in soils lacking the host influence. This is mainly because plants release up to 40% of photosynthetically fixed carbon in the form of root exudates and volatile organic compounds (VOCs), thus creating a diverse chemical milieu around the roots to the region extending a few millimetres from the root surface, known as the rhizosphere.

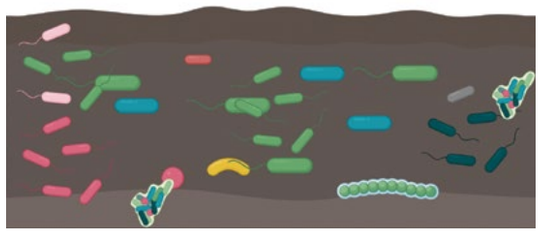
Some exudate constituents, such as polysaccharides, fumaric acid and citric acid can also promote biofilm formation in soilborne *Bacillus* strains that, in turn, provide beneficial functions including biocontrol, bioremediation, nitrogen fixation and stress tolerance. While much is known about the beneficial properties of several exudate constituents in soluble form, very little is known about the role of plant root VOCs (rVOCs) in assembling biofilms in soil microbiota, which may extend the beneficial sphere of host influence below ground to the scale of centimetres.

Main findings

This project has led to several fundamental discoveries and innovations in methodologies to study the below- ground chemical interactions between plants and microbes.

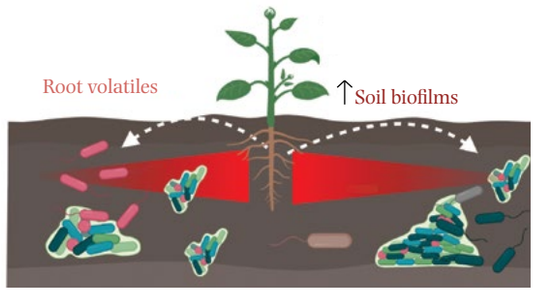
A novel airflow system was developed to direct plant root VOCs towards complex soil microbial community. These rVOCs, specifically oxylipins, promote biofilms in the soil microbiome and are widely distributed across the plant kingdom, from mosses to higher plants, including commercially important crop plants, tomato and rice. Interestingly, despite being beyond the immediate rhizosphere, the resultant biofilms, in turn, promoted plant growth by 25–30%.

As the oxylipin pathway is evolutionarily conserved, this phenomenon serves as an example of how inter-kingdom signalling drives co-evolution of plant-microbe interactions for mutual benefits.



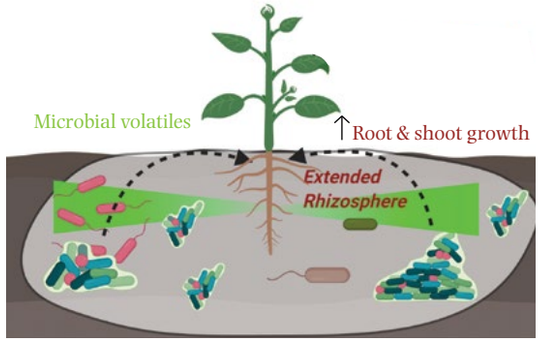
1

Complex soil microbiota exists in both planktonic (free-floating) and bioflim (surface-attached) mode of life depending on the environment



2

As a plant starts growing, specific root volatiles travel long distances and promote biofilm formation in microbes



3

As the microbes stabilise in the biofilms, they return the favour by releasing volatiles that promote plant growth. The rhizosphere effect is extended beyond the classical definition of millimetres away from the roots

Future directions

Based on these findings, nature-based solutions for regenerative and climate resilient agriculture will be developed in both open and closed formats, such as indoor or greenhouse farming. This discovery will play a role in tackling the challenge of increasing crop productivity, yet simultaneously reducing the environmental impact. Two major classes of agri-solutions will be developed: (1) a novel class of agrochemicals that promote soil beneficial biofilms; and (2) ecology-inspired beneficial microbial consortia that are responsive to crop signals or their derivatives and provide services of enhanced crop growth and climate resilience.

Plant and Cell Physiology (2021) 62: 248–261.

ENHANCING AQUACULTURE PRODUCTIVITY

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Introduction

Singapore is striving to produce 30% of its food locally by 2030. However, currently more than 90% of food in Singapore is imported. One major source of protein in the region is fish, and Singapore catches or farms only 10% of the fish consumed in the island state. So to reach the '30 by 30' goals, there needs to be a substantial improvement in aquaculture yields across Singapore. SCELSE is working closely with industry partners to develop these new technologies and ensure that Singapore's food security is enhanced.

By far the most expedient type of aquaculture is the use of sea pens. These allow natural seawater to flow through the pens, bringing fresh and clean water, while expelling poor and soiled water. They are inexpensive and easy to establish yet they are vulnerable to the natural variability of the ocean and the current changing climate. For this reason some aquaculture facilities are retiring sea pen usage in favour of closed system recirculatory systems that are not as susceptible to natural variation in the ocean or climate.

Unfortunately the costs and logistics of large scale aquaculture using closed systems is a limiting factor for many farmers. Despite the benefits of a controlled environment, there are still risks of disease and poor water quality that affect food conversion ratios or even entire harvest feasibilities.

Ongoing research

To combat disease risks, SCELSE is working with an industry partner to develop tools and technologies to monitor the microbial health of closed aquaria. The use of next generation sequencing technologies to observe the health of the system as well as the natural nitrogen removal processes will ensure that the closed systems are healthier and the stocks are not under stress, which can impede the yields. In addition, more natural closed systems are being investigated, in which some of the water purification and nitrogen removal is performed by different trophic groups within the closed system. This approach requires a dedicated effort in aseptic techniques to ensure biosecurity of the stock flora and fauna, while also relying heavily on emulating natural environments in which multiple trophic phases exist in one system. Ultimately monitoring these communities may yield more efficient aquaculture systems, lowering over-stress of stocks and enhancing production. By combining the microbiome data with other data collected in the aquaculture systems (i.e., temperature, fish mass, N_2 levels), machine learning is being employed to proactively monitor and identify stress in fish. This means that any mitigation measures can be applied earlier to lower the risks to stock yields.

ALTERNATIVE NUTRITION SOURCES FROM INSECTS AND MICROALGAE

Production of protein hydrolysate from black soldier fly

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Introduction

Urban populations consume the majority of protein produced globally but have unsustainable, linear food systems, from production to consumption to disposal, resulting in significant nutrient losses. The industrial rearing of insects is a promising strategy for converting otherwise lost nutrients back into protein and fertiliser, particularly to supplement local food production. Insects also have a high feed-conversion efficiency, a lower footprint and less greenhouse gas emissions compared to conventional livestock. Further, the protein content is higher or comparable to conventional food groups. The black soldier fly (BSF), *Hermetia illucens*, is a candidate for industrial rearing. It has a superior feed conversion ratio compared to other edible insects and can convert and recover nutrients from a vast variety of organic streams to protein, oil and chitin. Despite the evident advantages from both sustainable and nutritional standpoints, negative perceptions associated with the appearance present a major barrier to its widespread adoption as food. One effective way to overcome consumer acceptance challenges is to develop refined insect-based protein ingredients that can be readily included in familiar foods. This would require extensive understanding of the functional and nutritional properties of the insect constituents for formulation and development. Microbial fermentation is being used to enhance the nutritional content of the protein constituents of BSF larvae, to optimise nutritional properties for the human diet, by altering the amino acid composition and bioavailability.

Future directions

The impact of the protein hydrolysates/fermented end products have to be studied for their impact on human health. This is being evaluated using an *ex vivo* model system whereby biomarkers produced by the gut microbiome that is linked to health will be examined using metabolomics.

Microalgal-based nutrients

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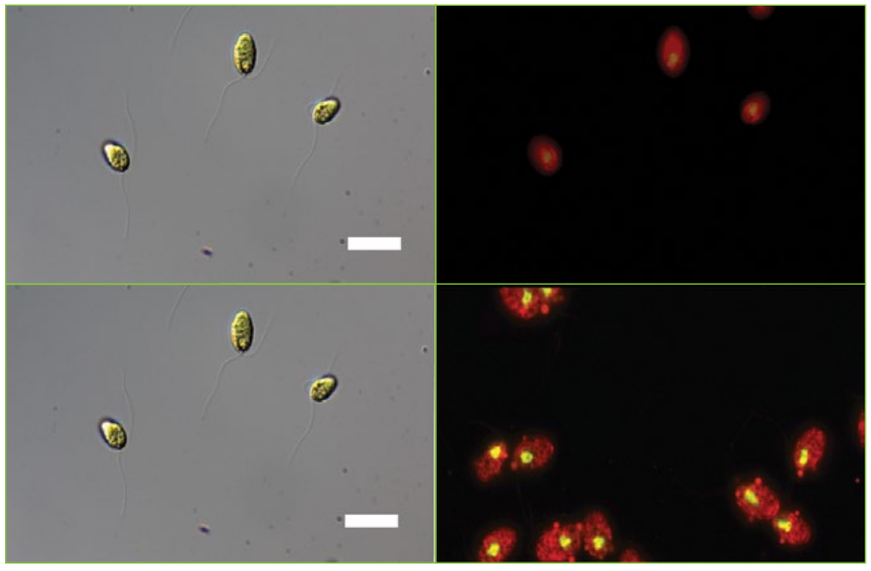
Introduction

In a recently commenced project, SCELSE researchers are maximising the protein and (healthy) fat content of algae by altering their microbiomes. The interdisciplinary team is building various systems to grow microbial biomass from available local water sources such as small farms and food industry. A bacterium that stimulates healthy fat metabolism in algae with no additional inputs has been identified and the team is now identifying bacteria that perform a similar role in protein biosynthesis. The ultimate goal is developing technologies that enhance algal nutritive value for use as stock feed for aquaculture and as a stepping stone towards producing algal noodles.



Bacterial stimulation of microalgal lipid vesicle production. In the bottom two images, a microalga has been grown with a bacterium that stimulates the microalgae to overproduce and excrete lipid vesicles when compared to the microalgae grown alone in the top panels. Cells are viewed through light microscopy and epifluorescence where nile red stains lipids. The red lipid vesicles are more abundant and extracellular in the bottom right where the bacteria and microalgae are co-cutlured. *Image credit: Rebecca Case and Catherine Bannon*

Phytochemistry (2022) 95:113052.

THE ONE HEALTH POULTRY HUB

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Introduction

Supported by the Global Challenges Research Fund (GCRF) of UK Research and Innovation (UKRI), the interdisciplinary One Health Poultry Hub addresses the need to meet rising demand for poultry meat and eggs in developing countries, while minimising risk to international public health. It was launched in February 2019 and investigates *how* and *why* intensification of poultry production increases the risk of infectious disease, and will identify high-risk behaviours, process and environments. The hub is led by the Royal Veterinary College (RVC), London, and comprises 27 partners across nine countries in Asia, Australia, Europe and the UK, with NTU being one of the partners. SCELSE is involved in the detection of pathogens and antimicrobial residues, and development of intervention strategies. The hub's outcomes will have wide regional and global relevance. The project is conducted in the framework of One Health, integrating poultry production and human health, and will transform technology to ensure safer, sustainable poultry production and thereby safer food.

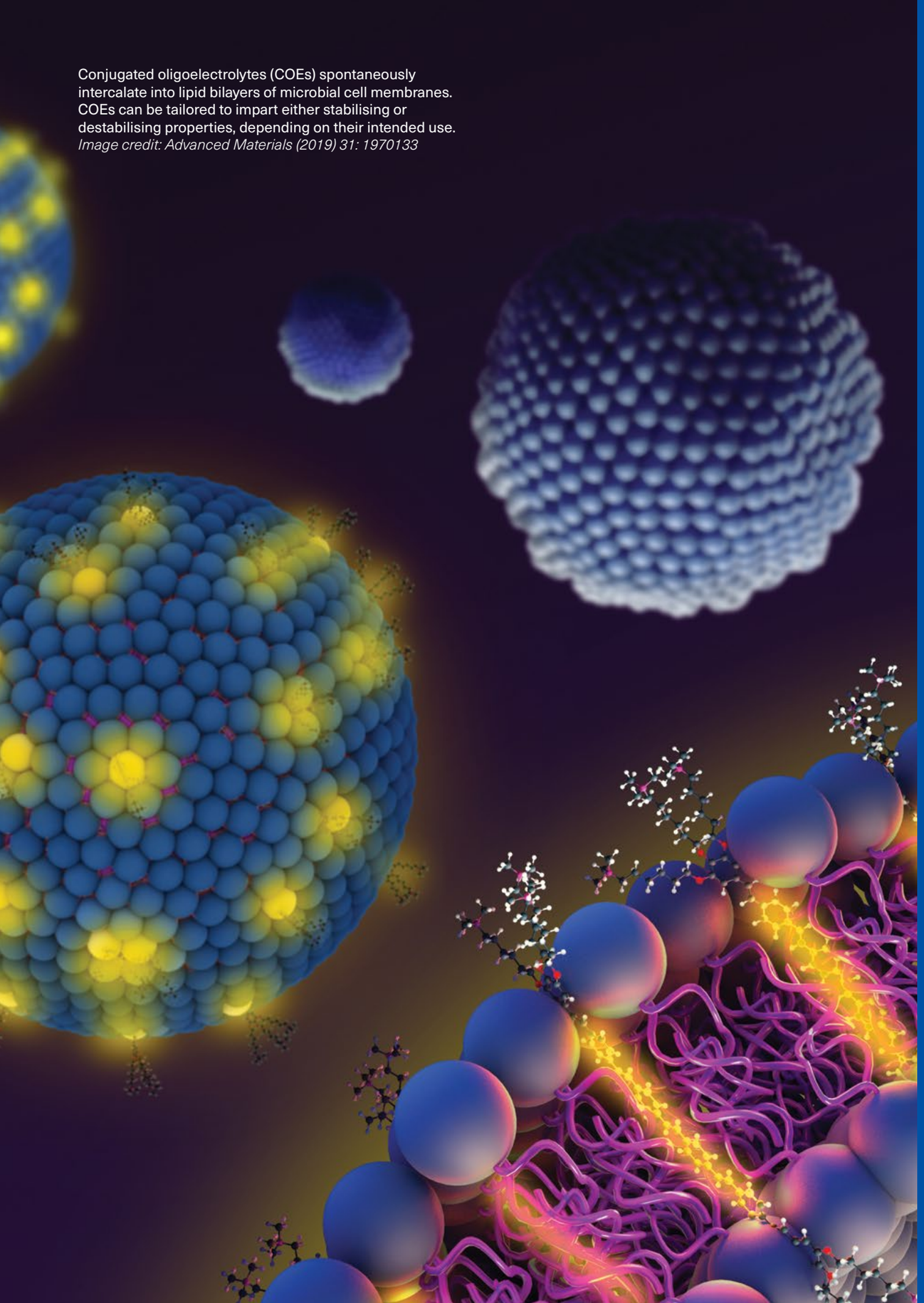
The One Health concepts were developed internationally as a response to increasing levels of foodborne diseases from animals – such as *Salmonella* and *Campylobacter* from poultry. Several developed countries have initiated One Health research and regulatory systems and successfully reversed the increasing trend of these foodborne diseases. The now-recognised major threat of antibiotic resistance in foodborne bacteria also represents a problem for potential One Health solutions.

Main findings

Chicken meat samples from partner countries were tested for antimicrobial residues using a validated detection system developed at SCELSE in collaboration with the EU reference laboratory (ANSES, France) and RVC partners. To date, antimicrobial residues have been detected and correlated with site data, with antimicrobial residues confirmed in meat to be consumed, indicating recent antimicrobial exposure. Feathers from the same animals are being analysed, by the project's UK team, to reflect less-recent antimicrobial usage.

Future directions

Samples from three of the four countries in the hub are in the pipeline to be analysed. *Salmonella* and *E. coli* isolates from chickens and humans will be analysed using whole genome sequencing. The findings will be added to the data across this One Health study to provide an impact on the industry and future regulations.



Conjugated oligoelectrolytes (COEs) spontaneously intercalate into lipid bilayers of microbial cell membranes. COEs can be tailored to impart either stabilising or destabilising properties, depending on their intended use.
Image credit: Advanced Materials (2019) 31: 1970133

RESEARCH THEMES

Bioprocessing and circular economy

Understanding biofilm biology is central for establishing and optimising bioprocesses mediated by microorganisms. This research theme covers a range of specific and broader applications. The bioprocesses can be mediated by very specific alterations that target either the biofilm matrix or cells within the matrix. Alternatively, system-wide manipulations can be implemented to achieve desired bioprocessing outcomes. SCELSE is developing technologies for maximising waste-to-feed circular economy applications, biofilms for biotechnological applications, microbial solvent production, and probiotic and bioactive delivery systems.

STABILISING MICROBIAL MEMBRANES FOR ENHANCED BIOPROCESSING

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Introduction

Liquid biofuels are compatible with the current energy infrastructure, positioning microbial butanol production as an important source of renewable energy. Butanol is an alternative biofuel that can be produced via microbial fermentation but has a poor economic proposition due to its low production yield and titre. Mechanistically, butanol readily partitions itself into the phospholipid bilayer of the membrane and increases the disorder in the hydrophobic tails of the phospholipids. The disordering effect causes the membrane to deviate from optimum fluidity, inhibiting other metabolic functions, which is detrimental to both cell survival and bioproduction. Consequently, the fermentative production of butanol is economically limited by the poor tolerance of the microbes to butanol. Hence, strategies that enable tolerance to extracellular butanol stresses are thus required to preserve biomass in microbial production bioprocesses.

SCELSE has focused on two such strategies that stabilise microbial membranes against butanol fluidisation through the action of membrane insertion molecules delivered as chemical additives: (i) conjugaged oligoelectrolytes; and (ii) carotenoids.

(i) Conjugated oligoelectrolytes (COEs) comprise a hydrophobic conjugated backbone and terminal polar ionic pendants (Figure 1) that can spontaneously intercalate into lipid bilayers. The length of the oligo-phenylenevinylene backbone regulates the effect on lipid bilayers; short COEs typically lead to membrane disruption, possibly because of a dimension mismatch between the COEs and lipid bilayers. Conversely, longer COEs act to stabilise the bilayer, perhaps mimicking natural compounds like hopanoids or non-canonical tetraether lipids. This work was the first demonstration of a materials-based approach to engineer butanol tolerance, as opposed to conventional genetic engineering. The project seeks to rationally design a new elongated COE, S6 (Figure 1), that comprise six benzene rings in its backbone, to understand the extent to which elongation of the COE backbone translates into predictable improvement of membrane tolerance against butanol.

(ii) Carotenoids are naturally occurring compounds that are widely found in thylakoid membranes and the human retina. It is postulated that they exist in these membranes to modulate the effect of heat-induced fluidisation. Polar carotenoids are characterised by the presence of hydroxyl, keto- or epoxide functionalities on the ionone rings that allows the perpendicular orientation of the molecules with respect to the membrane surface (Figure 2). Hence, they act as rivets to increase the rigidity of the lipid bilayer. Conceptually, membranes that comprise polar carotenoids should be less prone to fluidisation under butanol stress. This project seeks to explore the supplementation of carotenoids as chemical additives to microbial cell cultures as an environmentally friendly, materials-based approach to biological strain improvement in microbial biofuel production.

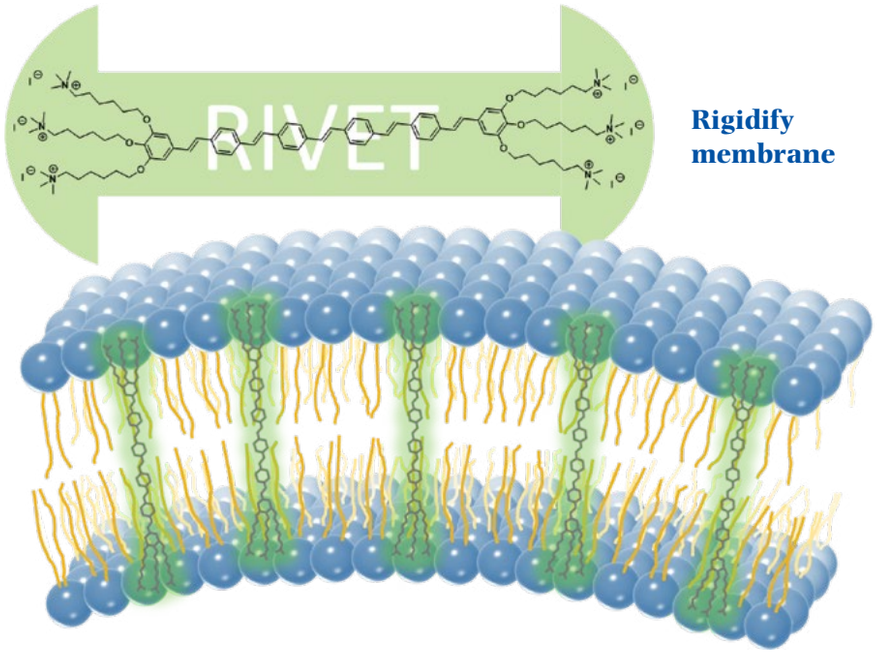

Figure 1: COEs that are longer than the dimensions of the lipid bilayer act as rivets to rigidify the membrane when they insert themselves between the phospholipids. COE S6 shown here has an additional phenylenevinylene repeat unit that is expected to favour greater membrane stability, while the six positively charged side chains (terminal groups) were designed to increase electrostatic attractions, thereby promoting membrane intercalation.

Main findings

(i) Elongated conjugated oligoelectrolyte increases microbial membrane stability against butanol.

The ability of rationally-designed elongated COE S6 to maintain lipid bilayer stability in *E. coli* upon butanol exposure was demonstrated using liposomes. S6 maintained the perfect circular liposome geometry, which was lost in untreated liposomes exposed to butanol. S6-treated *E. coli* K12 demonstrated significant biomass increase and higher growth rates, compared to the untreated cells, when exposed to 1.8% (v/v) butanol. Untreated cells typically fail to divide but form filaments and exhibit signs of membrane pitting on the cell surface when grown in 0.9% (v/v) butanol (sub-inhibitory concentration). Cells treated with the COE S6, however, had a lower tendency to form filaments, suggesting that the cells exhibit stress resistance to butanol exposure. S6-treated cells also maintained the rod-like morphology with smooth surfaces, even under exposure to sub-inhibitory butanol levels. The control *E. coli* K12 cells released a butanol concentration-dependent LPS, indicating the loss of outer membrane integrity. By contrast, COE-S6-treated cells exhibit 50% less LPS release at 1% (v/v) butanol exposure, suggesting that S6 preserves outer membrane structure in addition to the lipid bilayer.

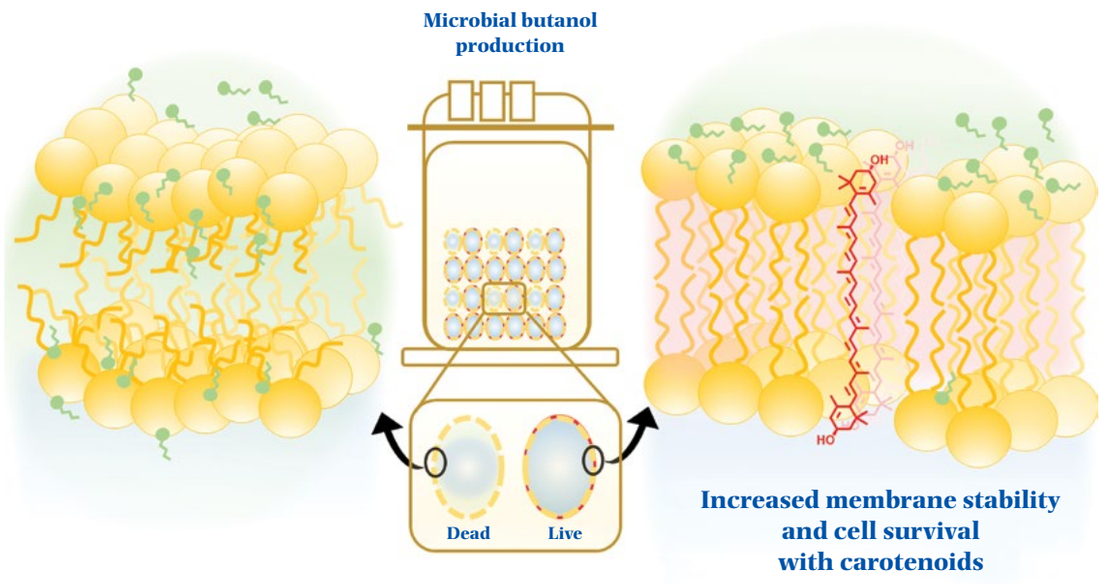

Figure 2:
The spontaneous intercalation of carotenoids into microbial membranes increases membrane stability upon butanol exposure by reducing the fluidisation effect and membrane permeability of butanol.

This work demonstrates the multifunctional protective capacities of the COE S6, which is simple to use and can be extended to potential applications in fermentation engineering for biofuel production. Employing synthetic molecules or materials to direct, modify or curtail microbial behaviour is a novel way to address biological limitations in microbial biotechnologies. Such interventions may open opportunities in energy production, biocatalysis, biosensors, and biosynthesis applications. Nonetheless, tailoring microorganisms through the materials approach can be challenging, especially for cell surface engineering, which may require integration of several components in a multiple-step process. Hence, a single-step, self-assembly guided approach such as using COEs to improve the properties of microorganisms for specific tasks is highly desirable.

(ii) Naturally occurring carotenoids stabilise microbial membranes against butanol fluidisation

As an additional means of improving butanol tolerance in *E. coli*, two polar carotenoids, lutein (LUT) and zeaxanthin (ZEA), were explored as molecular rivets to mitigate membrane fluidisation by solvents. Both carotenoids intercalated spontaneously into liposomes that contained bacterial lipids; phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). Both LUT and ZEA formed carotenoid-rich nanodomains in liposomes, at molar ratios of 1:9 relative to phospholipids (10 mol%). Carotenoids further reduced the fluidisation effect by 62%, in liposomes that were challenged with 3.5% (v/v) butanol. Additionally, membrane penetration of butanol was 38% lower in the presence of high carotenoid content, compared to the untreated control, while butanol-induced membrane damage decreased by up to 30%. Further, *E. coli* treated with both LUT and ZEA achieved a two-log increase in cell viability upon acute butanol exposure of 3.5% (v/v), compared to untreated cells. This is the first time carotenoids have been used to fortify cellular membranes and reduce biomass loss due to butanol, thereby demonstrating a potential biotechnological application for carotenoids to improve the economics of microbial butanol production.

Future directions

SCELSE will continue to develop and optimise COE molecules to improve the properties of microorganisms to facilitate specific tasks. This proof-of-concept work demonstrates that carotenoids stabilise membrane against butanol-induced membrane fluidisation. One advantage of carotenoids as chemical additives is their natural abundance, where they may be enriched in plant-based food wastes that are often utilised as feedstocks for biobutanol production.

Environmental Science: Nano (2021) 8:328.

Advanced Materials (2019) 31:1808021.

IDENTIFYING CHANGES IN MEMBRANE PROTEIN CONTENT IN BIOALCOHOL

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Introduction

Bioalcohol is a promising renewable biofuel that can be produced by microbial fermentation, however, widespread commercial production is inhibited by the toxicity of alcohols to the microorganisms producing them and resultant low yields. Understanding the response of bacterial cells to alcohol will help in the genetic engineering of microorganisms with increased alcohol tolerance. The bacterial membrane is the primary target of alcohol, and membrane fluidity is largely mediated through changes in membrane protein content. Therefore, it is of interest to understand the specific changes in membrane proteins in response to bioalcohols to improve its production.

Main findings

The protein content of the *Escherichia coli* inner and outer membranes changed significantly in the presence of alcohols. Various protein subunits of the inner membrane associated with the transport sugars, oligopeptides and amino acids into the cytosol were increased. The increased transportation of energy producing metabolites into the cell may help to overcome the shortage of cellular energy caused by alcohol stress. The TolC-associated multidrug efflux pump spans the inner and outer membranes of *E. coli* and facilitates the efflux of toxic compounds from the cytosol out of the bacterial cell. In the presence of alcohol, various inner membrane protein subunits of the efflux pump were increased expression.

The changes in the protein expression of *E. coli* in the presence of alcohol suggests the outer membrane is weakened, with compromised integrity and function. Most outer membrane β-barrel proteins showed reduced expression in the presence of alcohol. Various inner and outer membrane proteins involved in outer membrane biogenesis show reduced expression. These include proteins for maintaining outer membrane integrity, lipid asymmetry and functionality.

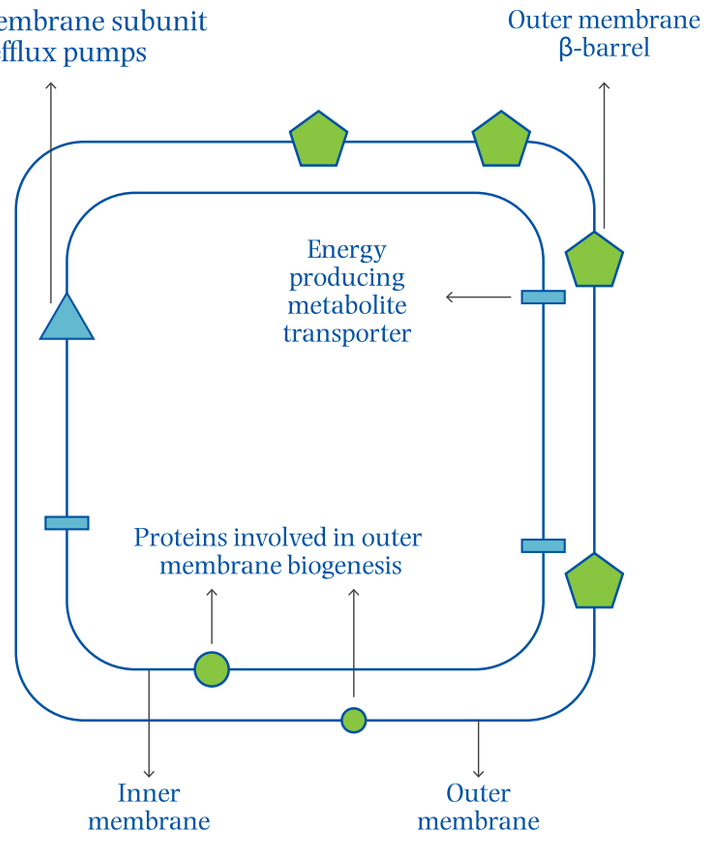

Figure 1: Changes in the expression of various groups of membrane proteins under alcohol stress. Green denotes proteins showing lower expression in the presence of alcohol, while blue denotes higher expression.

Future directions

The current study identified membrane proteins expression in the presence of alcohols. As the membrane is the first region of the bacterial cell to encounter the fluidising effects of alcohol. It is beneficial to understand the regulatory pathways that lead to chances in gene expression in the presence of alcohol. This information would further elucidate the effect of alcohol on the bacterial outer membrane and how the adverse effect of alcohol can be mitigated through rational engineering. Future studies will examine the regulatory pathways that lead to changes in the expression of major outer membrane proteins in the presence of alcohol, and maintenance of membrane fluidity and integrity.

GRAM-TYPING USING CONJUGATED OLIGOELECTROLYTES

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Introduction

Classification of bacteria by Gram-type remains important for clinicians to guide the appropriate use of antibiotics. Gram-negative bacteria have a characteristic, asymmetric outer membrane with lipopolysaccharide (LPS) in the outer leaflet, which acts as a molecular sieve to protect the cell from chemical stress, and a thin peptidoglycan layer between the outer and inner membrane. On the other hand, Gram-positive bacteria are characterised by only a single symmetric, phospholipid membrane and the cell is surrounded by a thick layer of peptidoglycan. Classical Gram-staining typically relies on crystal violet that specifically stains the peptidoglycan cell wall. While Gram-staining with crystal violet has been long established, fluorescence-based probes have been on the rise such as fluorescence *in situ* hybridisation (FISH). While this method differentiates Gram status, it does not allow real-time inspection of live samples. Hence, this project seeks to develop a novel fluorescent probe that are simple to use without cell fixation or pre-staining steps, while demonstrating selectivity between the Gram-positive and Gram-negative bacteria.

Main findings

This work presents a Gram-type selective cellular membrane staining method that takes advantage of the optical and self-assembling properties of the conjugated oligoelectrolyte, COE-S6. This molecule inserts itself into the cellular membrane of Gram-positive bacteria spontaneously. Individual cells can be easily observed by the 'halo-like' patterns that delineate the membrane boundary. COE-S6 partitions less effectively into the cellular membranes of Gram-negative bacteria, most likely because it is impeded by the LPS barrier on the outer membrane. COE-S6 is considered a true Gram stain, where the distinction between Gram-positive and Gram-negative organisms is the presence of an outer membrane that contains LPS. This is opposed to the case of crystal violet which relies on the thickness of the peptidoglycan content between the two Gram-types. For complete visualisation of the two bacterial communities, COE-S6 works well in conjunction with a commercially available and nonspecific dyes, to recognise bacterial Gram-type *in situ* within mixed cultures. This paired dye system is also of practical relevance to Gram-type characterisation in biofilms (Figure 1). Moreover, COE-S6 can simply and quickly differentiate between Gram-positive and Gram-negative bacteria by measuring the enhanced fluorescence intensity after excitation using a conventional fluorometer or by the naked eye by excitation using a UV lamp. Importantly, COE-S6 is a biocompatible dye that does not inhibit the growth of bacterial cells.

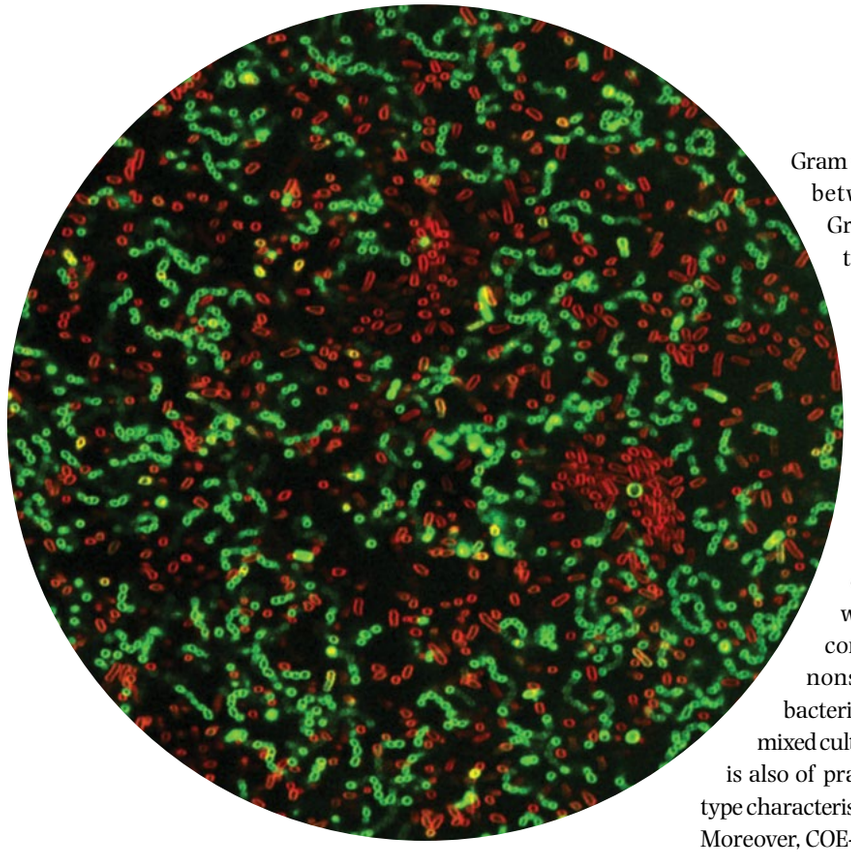

Figure 1:
Confocal micrograph of an *E. faecalis* (Gram-positive) and *E. coli* (Gram-negative) dual species biofilm, showing clear differentiation between the two Gram-types. In green: clear staining of the Gram-positive cocci arranged in chains. In red: nonspecific staining of both Gram-types.

Future directions

Methods that are simple to use and require application of a dye mixture with no fixation or other pre-treatment requirements is highly desirable, especially in clinical settings where the volume of samples for detection is high. Further, dyes should also not inhibit bacterial growth, which will open the opportunity to monitor the behaviour of living cells *in situ*. Developing novel dyes like COE-S6 will enhance the arsenal of tools for clinical microbiologists to make quicker assessments.

Advanced Functional Materials (2020), 30: 2004068.

CONTROLLING BIOFILM THICKNESS FOR BIOTECH APPLICATIONS

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Introduction

The chemical industry is one of the most important economic pillars in Singapore. Over the past few decades, biocatalysis is one of several 'green chemistry' approaches that have been developed as an alternative to conventional chemical synthesis of high value products. One key limitation of this alternative is the sensitivity of biocatalysts to physicochemical stresses such as extreme pH, high temperatures and harsh conditions associated with the use of organic solvents. Biofilms have potential applications in biocatalysis due to the production of extracellular polymeric substances that confer a high tolerance to various stresses and perturbations. A key parameter that affects the performance of biofilm-based biocatalysts is biofilm thickness. Thin biofilms are unable to provide sufficient catalytic activities, while overly thick biofilms contain an unproductive layer of biomass at the bottom due to the poor diffusion of solutes (e.g., substrates and nutrients). Unfortunately, predicting and controlling biofilm thickness, and hence performance, via adjusting physicochemical parameters are highly challenging to the interplay between dynamic biofilm signalling networks and the heterogenous microenvironments in biofilms. Therefore, in this project, the objective was to engineer light-controllable biofilms for efficient and robust biocatalysis through a novel approach based on the role of cyclic diguanosine-5'-monophosphate (c-di-GMP) signalling in the biofilm lifestyle.

Main findings

Efficient control of biofilm thickness was achieved by constructing a near-infrared (NIR; 660 nm) light-responsive c-di-GMP module. The module consists of a light-responsive c-di-GMP synthase, BphS, and biliverdin synthase, BphO. BphS is an engineered bacteriophytochrome that consists of a N-terminal photoreceptor

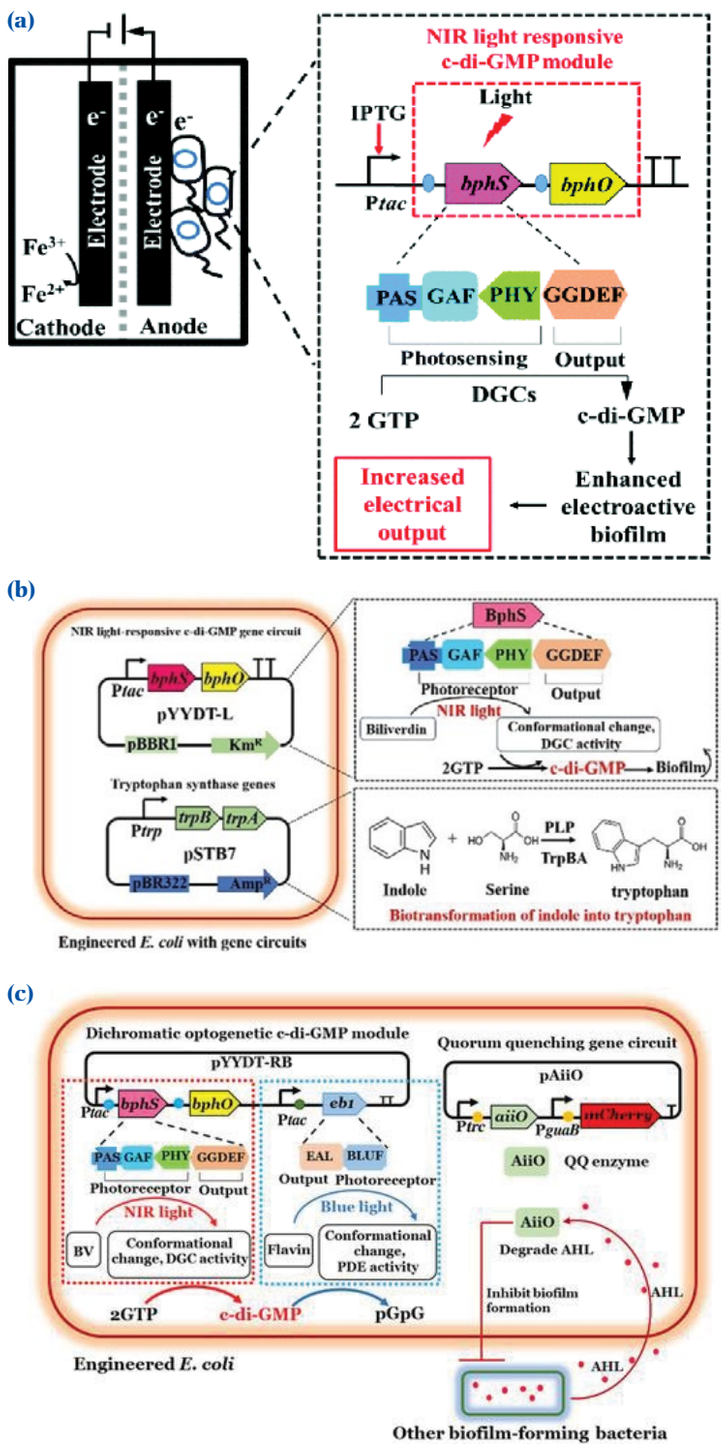

Figure 1:
An overview of the light-responsive c-di-GMP control module and its applications. (a) NIR-light-responsive c-di-GMP module for the increased electrical output of electroactive biofilms. (b) Engineered *E. coli* strain for the enhanced biocatalytic conversion of indole to tryptophan. (c) Engineered *E. coli* strain for the dichromatic control of a dynamic quorum quenching biofilm.

module (PAS-GAF-PHY domain) that binds to the light-absorbing chromophore biliverdin. Attached to this photoreceptor module is a C-terminal diguanylate cyclase (DGC; GGDEF domain). Upon exposure to NIR light, conformational changes to the biliverdin-bound photoreceptor module are efficiently transduced to the GGDEF domain, leading to the activation of DGC activity of BphS. Subsequently, c-di-GMP is synthesised, leading to enhanced biofilm formation. As a proof of concept, this module was adopted for *Shewanella oneidensis*. A light responsive electroactive biofilm with increased electrical output resulting from increased biofilm formation on the anodes of the microbial cell was also achieved.

The ability of the constructed c-di-GMP module to enhance the performance of a biofilm-based biocatalyst was subsequently demonstrated. By introducing the NIR light-responsive module into *E. coli* along with a plasmid that encodes a tryptophan synthase, TrpBA, a biofilm-based biocatalyst was engineered to catalyse the biotransformation of indole to tryptophan and sense NIR light (Figure 1b). This engineered catalytic biofilm overcame the inhibition of indole on biofilm formation by *E. coli* and reduced the reverse reaction from tryptophan to indole. By applying the engineered biofilm to catalyse the biotransformation of indole into tryptophan in submerged biofilm reactors, exposure to NIR light successfully enhanced biofilm formation.

This study demonstrates the feasibility of using light to modulate biofilm formation to increase the catalytic biofilm's tolerance to external stressors and improve its performance for chemical production. Subsequently, a bi-directionally controllable, quorum quenching biofilm was achieved by introducing a NIR light-responsive DGC and blue (465 nm) light-activated phosphodiesterase (PDE; 465 nm) into *E. coli*. To enable the production of a quorum quenching (QQ) enzyme that degrades N-acyl homoserine lactones (AHL)-based quorum sensing (QS) signals, the gene I was introduced into the *E. coli* strain (Figure 1c). The engineered light-controllable *E. coli* strain was then grown on water purification membranes and successfully inhibited biofouling caused by a model biofouling organism.

Future directions

This project established a novel strategy for controlling biofilm dynamics in biofilm-mediated bioprocesses, understood to be the first report on the construction and application of a light-responsive synthetic c-di-GMP module for controlling biofilm dynamics using light. Furthermore, dichromatic control of a bacterial biofilm capable of adjusting its thickness under alternate exposure to NIR and blue lights was achieved by placing a c-di-GMP synthase and hydrolase under optogenetic control. Since c-di-GMP is a universal biofilm regulator, the approaches developed here will be investigated for other bacteria and applied to the design of several controllable biofilm-enabled applications. The findings of this project can potentially be incorporated into industrial processes benefiting the chemical industry in Singapore and across the globe.

Biology of the Cell (2013) 105: 59–72.

ACS Synthetic Biology (2014) 3: 802-810.

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ChemSusChem (2019) 12: 5142-5148.

Science Advances (2018) 4: eaau1459.

FROM FOOD-PROCESSING WASTEWATER TO AQUACULTURE FEED

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Introduction

Seafood, derived from both wild-catch and aquaculture, is the largest animal protein industry in the world, with the latter experiencing faster growth than any other animal protein sector over the past two decades. There is demand for protein ingredients that maintain feed performance, benefit aquaculture health and stabilise supply during industry expansion. Protein can be produced through the cultivation of various microbes, and is generally referred to as single cell protein (SCP) or microbial protein. SCP produced from food-processing wastewater (FPWW) is a promising source of protein for sustainable animal feed. Collectively, FPWW possesses attractive characteristics for SCP production, such as a continuous global year-round production of process water rich in dissolved C, N and P compounds. These wastewaters are also regarded to be free of pathogens and other hazardous contaminants, and can support microbial growth in bioreactors. Thus, obtaining SCP from FPWW for aquaculture feed is an option with great potential.

SCELSE's research aims to develop a microbially-based integrated sustainability approach in the food industry through advanced resource recycling of food-processing wastewaters, to produce high-quality aquaculture feed. Currently, there are about 125 fish farms in Singapore producing close to five thousand tonnes of fish per year, or 10% of local fish consumption. However, most of their aquaculture feed is imported and derived such from traditional sources. The soy and beer industries are two important

sectors in the food industry in Singapore. For such food-processing industries, additional economic value can be created through advanced resource recycling of their nutrient-rich wastewater streams. This research is aligned with the '30 by 30' vision, whereby 30% of Singapore's nutritional needs will be produced locally by 2030, up from less than 10% today. It also contributes towards achieving the United Nations Sustainable Development Goals (SDG) for food security, and sustainable management of water and sanitation.

The project involves four industrial partners that provide food-processing wastewater from the soya bean processing, beer brewing, and seafood processing industries, as well as a collaboration with a global partner specialising in animal feed nutritional additives.

Main findings

SCELSE has demonstrated that food-processing wastewater can be converted directly into microbial protein that meets animal feed requirements by using a mixed culture-based bioconversion approach. This was the first study to produce SCP by mixed-community fermentation starting directly with FWW. Further, this work pioneered a deep chemical and microbial characterisation approach of FWW, including metagenome-assembled genomes and isolated strains to assess genetic potential. For example, soya bean processing wastewater was used to harness its chemical/microbial characteristics to operate 22 bioreactors of 4 L working volume in five separate studies (30–90 day duration). Biomass was successfully grown and cell protein measured for reactors started with and without inoculum. Amino acid content contributed 50% of the biomass, including all essential amino acids for fish feed. Protein measurement methods and the protein yield per N-content in soya bean processing wastewater and time were also optimised. Furthermore, a preliminary fish trial with reactor-grown microbial biomass showed that sea bass fry fed on a 50:50 diet of single cell protein and fishmeal were statistically indistinguishable (in terms of length and weight) from fish on control diet (Figure 1). Furthermore, preliminary cost-benefit and life cycle environmental impact analyses showed the economic feasibility and sustainability potential of SCP production.

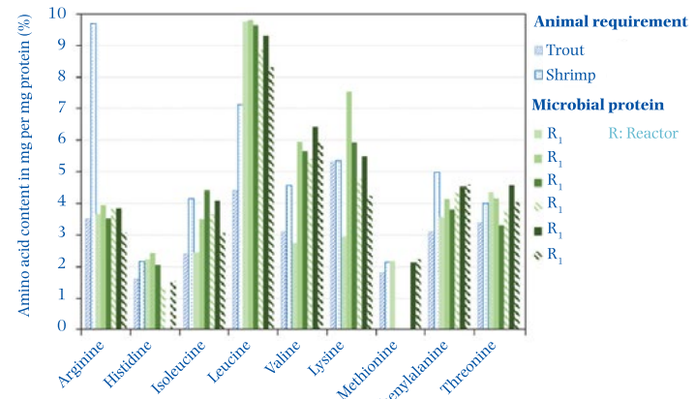
Food-processing wastewater (FWW)
- No sterilisation
- Fewer contaminants

Bioreactor fermentation

Microbial protein (SCP)

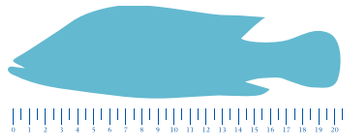
Figure 1:
Soluble substrates have been converted to microbial biomass with protein content more than or equal to 50% containing all essential amino acids for fish feed. This was tested successfully in aquaculture trials using sea bass fingerlings.

Essential amino acids needed for fish cultivation were produced in SCP grown from FWW



Aquaculture trial

- ✓ Dried microorganisms
- ✓ Fish feed additives for trials
- ✓ All essential amino acids



Future directions

Once bench-scale stages have been optimised, an integrated technology pipeline will be created to validate the advanced resource recycling strategy in a controlled laboratory environment. The 100-litre reactor system will be developed as a collaboration between the Nanyang Environment and Water Research Institute, NTU (physicochemical separation technologies), SCELSE (bioengineering systems), and Singapore Institute of Food and Biotechnology Innovation, A*STAR (industrial fermentation systems). This will produce sufficient feed additives for aquaculture trials, while providing the data to assess the sustainability and economic feasibility of the resource recovery pipeline.

In addition to existing partners, local representatives have expressed interest in performing fish trials with the SCP produced in this project in aquaculture testbeds at the St John's Island National Marine Laboratory, Singapore.

Bioresource Technology (2021) 341: 125723.

Routine surveillance of public areas is key to protecting public health from airborne pathgens.
Image credit: Sharon Longford, SCELSE

RESEARCH THEMES

Pathogen surveillance and source tracking

SCELSE is combining multidisciplinary technologies for understanding the transport and transmission of pathogens in waterways, coastal marine environments, and the rapid detection through surveillance of air, surface and sewage.

SURVEILLANCE OF SARS-CoV-2 IN AIR AND ON SURFACES

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Collaborators:
Syed Munir Bin Abdullah Alhamid[2], Jianrong Liu[2], Daniel Daniel[3], Loh Xian Jun[3], Dale Fisher[4], Paul Tambyah[4], David Allen[4], Asim Shabbir[4], Sonja Luz[5], Jessica Lee[5], Ng Lee Ching[6], Judith Wong[6], Ron Tan[6], Joanna Shen[6], Mohammad Nazeem[6], Somasekar Seshagiri[7]

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Introduction

At the commencement of this study in early 2020, the mechanism of transmission of SARS-CoV-2 was believed to occur exclusively via droplets. However, with an increasing number of case studies pointing towards the possibility of infections also spreading via aerosols, a vigorous scientific debate was initiated. To address questions relating to public health and provide guidance for behavioural COVID-19 mitigation measures, such as social distancing, the scientific basis for the detection of aerosols needed to be developed and validated. To address these needs, the SCELSE air microbiome team adapted its bioaerosol analysis protocols and knowhow for the surveillance and analysis of airborne SARS-CoV-2, deploying its fleet of more than 40 air samplers across Singapore.

Main findings

A standard operating procedure (SOP) for air sampling as a diagnostic tool for SARS-CoV-2 surveillance was established involving a novel, robust, highly efficient automated extraction protocol for high-throughput ultra-low biomass RNA extraction from inactivated environmental air and surface swab samples. The extracted RNA underwent SARS-CoV-2 screening by RT-qPCR and metatranscriptomic sequencing. Further, protocols developed for greater resolution than the gold-standard RT-qPCR-based SARS-CoV-2 detection method involved standard PCR, digital droplet PCR (ddPCR) and Illumina MiSeq amplicon sequencing.

Air sampling at a COVID-19 ward in Singapore.

These methods were validated using extensive SARS-CoV-2 environmental surveillance at the Singapore Expo community isolation facility, demonstrating the utility of air surveillance in detecting SARS-CoV-2. Analysis of surface swabs collected in parallel with air samples showed that SARS-CoV-2 was 10 times more likely to be detected in air than on surfaces, suggesting fomites are not as important in SARS-CoV-2 transmission as initially thought. These results were supported by sampling at NUH COVID-19 wards, which produced a higher success rate of environmental SARS-CoV-2 detection (72%) than surface sampling (9.6%), and highlighted high prevalence of the virus in toilet areas of the hospital wards. The results demonstrate that communal screening of air in highly crowded spaces has the potential to provide an early warning of the presence of SARS-CoV-2.

In addition to validating the protocol in COVID-19-positive environments, larger surveillance campaigns were conducted in locations with no confirmed COVID-19 cases but high human occupancy. These included the Singapore Zoo, non-COVID-19 hospital wards (NUH), public places such as hawker centres, wholesale vegetable markets, wet markets and fishery ports, all of which reported zero SARS-CoV-2 detection. Further, SCELSE used surveillance and subsequent results to advise Singapore Zoo operators on post-lockdown reopening protocols, and inform NEA on monitoring sites of food import and distribution.

The air sampling protocol was also utilised in evaluating the efficacy of containment devices designed to protect clinicians by minimising the spread of

Surface swab sampling at a wet market in Singapore.

airborne SARS-CoV-2 in confined hospital spaces (IMRE-A*STAR collaboration), and identifying medical procedures that generate aerosols from patients (e.g., nasal cannula treatment, gastroscopy, endotracheal-intubation) as sources of SARS-CoV-2 exposure.

The SARS-CoV-2 spike (S) protein engages the human angiotensin-converting enzyme 2 (ACE2) receptor to invade host cells, making it highly infectious. A further study found that ACE2 polymorphisms could potentially alter host susceptibility to SARS-CoV-2 by affecting host-virus interactions.

Future directions

The SOPs developed in this study have been used extensively by the Environment Health Institute (EHI) to investigate outbreak sites in Singapore, further validating the reliability and robustness of the protocol. The protocols developed for environmental SARS-CoV-2 surveillance will continue to be refined to further reduce sampling time, simplify sample processing and shorten sample-to-result turnaround times. This further refinement will culminate in a general tool for future 'Disease X' pandemic preparedness.

British Journal of Surgery (2022) 109: 15–20.

Indoor Air (2022) 32: e12930.

Communications Biology (2021) 4: 1–11.

Indoor Air (2021) 31: 1639–1644.

Nature Communications (2020) 11: 2800.

SURVEILLANCE OF SARS-CoV-2 IN WASTEWATER

Janelle R. Thompson[1,2], Stefan Wuertz[1,3], Monamie Haines[4], Eric Alm[5], Karina Gin[6], Germaine Kwok[1], Desmond Chua[1], Jason Ng[1], Dhiraj Nainani[1], Mats Leifels[1], Sera Kim[1], Aisyah Affandi[1], Shobana Gayathri[1], Lee Wei Lin[5], Gu Xiaoqiong[5], Federica Armas[5], Franciscus Chandra[5]

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[4] School of Social Sciences, Nanyang Technological University, Singapore
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Introduction

Wastewater surveillance offers a complementary approach to clinical disease surveillance as it combines population-level health information and provides an unbiased sample of the infected population, including asymptomatic and pre-symptomatic individuals.

Asymptomatic carriers, typical for the Omicron subvariant, can transmit SARS-CoV-2 and carry the virus in faecal matter. These carriers may also test negative in nasopharyngeal samples, and are thus a potential source of local outbreaks. Robust and cost-efficient tools are urgently needed to track the number of infected individuals in a community, without biases introduced by clinical case reporting.

Main findings

Wastewater surveillance as a tool to monitor the spread of COVID-19

Wastewater surveillance (WWS) is a promising tool for indiscriminate monitoring of SARS-CoV-2. To date, WWS has been implemented at more than 3,300 sites in 64 countries worldwide, including Singapore, and developed into a valuable tool for estimating population prevalence and as an early warning for impending outbreaks.

Influent to a wastewater treatment plant (WWTP) reflects a region or community based on the catchment area and the density of the settlement. Hence, an integrated approach to WWS can act as sentinel for the health of the community at large, and a means to identify and manage disease clusters at a neighbourhood or regional scale (Figure 1).

As ongoing research refines the modelling approaches described in Figure 1, actionable insights from viral levels in wastewater can be derived, for implementing health-protective measures at the neighbourhood or regional level. Concurrently, issues of privacy must be considered to ensure appropriate use of wastewater-based surveillance data, which holds the potential to become a new source of 'big data' in our increasingly connected world.

Wastewater surveillance in high-density living environments

Even though theoretically applicable in all (high- and low-income) settings where wastewater is collected or transported to a treatment facility, WWS shows a particular potential for high-density living and situations of prolonged contact among individuals, such as in high-rise residential buildings, university housing facilities and hospitals (Figure 1).

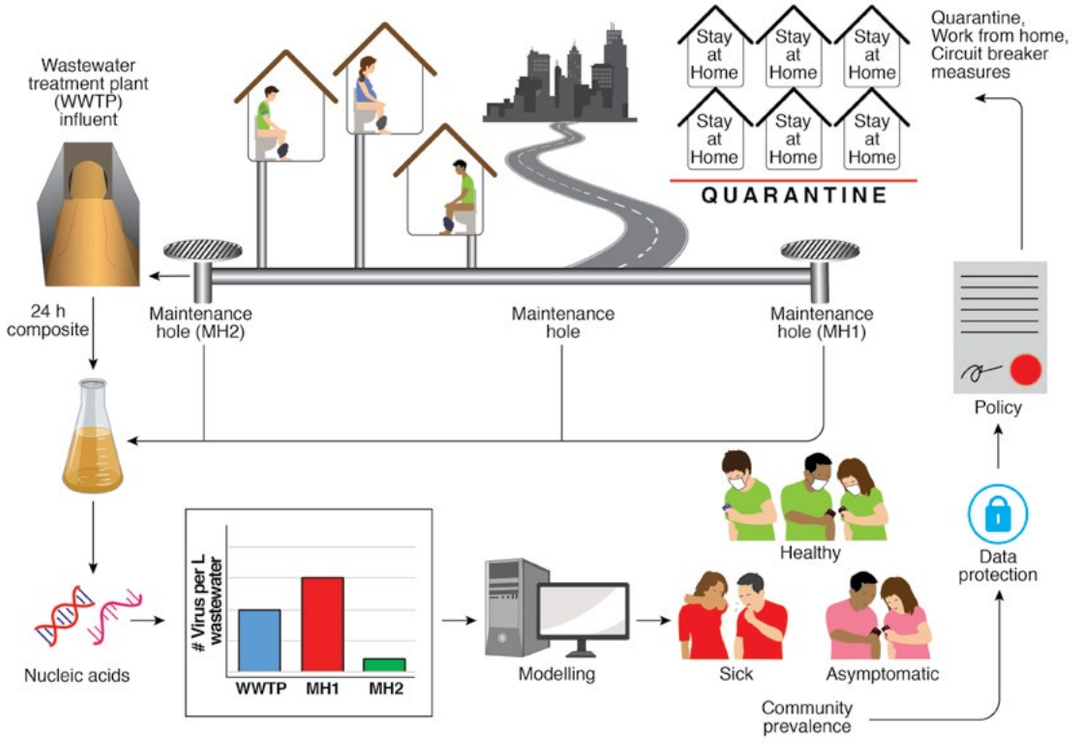

Figure 1:
Wastewater-based surveillance for population-based health management. The SARS-CoV-2 virus enters the sewerage system through faecal shedding. Wastewater sampled at maintenance holes, or the treatment plant, represents an aggregate and average of shed viral titres at the level of the neighbourhood, region or municipality. A composite sample collected over 24 hours captures the daily flux from the population and is purified from a defined volume to recover the RNA associated with wastewater-borne viral particles. The viral genomes are measured in the purified RNA sample using a molecular assay such as quantitative PCR and used to determine the number of viruses in the defined volume of sampled wastewater. Wastewater viral levels are then combined with wastewater flow rates and estimates of excreted viral load per toilet flush to model infection prevalence in the sampled community. Importantly, this estimate of prevalence reflects the excreted viral load from both sick and asymptomatic individuals and complements essential individual testing.

Water Research (2020) 184: 116181-116181.

Environmental Science & Technology Letters (2021) 8: 675-682.

Water Research (2021) 202: 117400.

Science of the Total Environment (2022) 805: 150121.

The risk of transmission in these environments can be reduced due to general (social distancing, face mask, antigen tests) or specific (work from home, remote learning, visitor management rules for healthcare facilities) regulations.

While 'downstream' sampling (i.e., at or near the WWTP) offers the greatest level of population aggregation, it presents several problems, including highly variable sewage travel time, signal degradation and limited spatial resolution. Sampling multiple locations 'upstream', at the neighbourhood level (e.g., at the scale of 2,000–7,000 individuals), alleviates these problems, more accurately tracking signatures of human health and activity, and providing individual or household resolution, while preserving significant anonymity. This spatially resolved approach in sewerage has been deployed for tracking SARS-CoV-2 RNA in Boston and Singapore. In the Boston metropolitan area, SARS-CoV-2 titres in samples collected from maintenance holes correlated to neighbourhood socioeconomic indicators, with less affluent areas showing higher titres, consistent with clinical case surveillance.

WWS in Singapore

SCELSE collaborates with the Singapore National Environment Agency to utilise this integrated approach, combining analysis of wastewater from sewerage maintenance holes with clinical surveillance. Monitoring and management of COVID-19 transmission is undertaken in residential buildings and among migrant workers living in dormitories, which represented the largest infection clusters in the nation at the time.

Once viral SARS-CoV-2 RNA signals were detected in wastewater at a particular dormitory site, clinical testing for COVID-19 was carried out for the corresponding community and if needed, action taken to mitigate further transmission. Conversely, for areas with no detected COVID-19 cases, non-detection of SARS-CoV-2 RNA in the wastewater provided added assurance, given the potential for COVID-19 to be asymptomatic.

'Upstream' surveillance for SARS-CoV-2 can facilitate more granular detection of the virus in catchments with lower overall COVID-19 disease burden, to provide an early warning and guide decision-makers to advance or scale back behaviour-based mitigation efforts, while minimising disruption or risk to the community at large.

Based on the outcomes of this study, a WWS-based monitoring campaign, consisting of 46 sampling sites obtaining 24-hour composite samples each, has been operational downstream of the student housing at NTU since early 2021. With the help of a network of automated sampling units and a dedicated team of researchers in SCELSE, NTU was able to return to in-person learning and onsite gatherings for student activities.

Future directions

Wastewater surveillance for infectious diseases at and upstream from the treatment plant should be a top priority of public health at the local, national and international level, with the aim of acquiring actionable community-level information needed to navigate pandemics like COVID-19.

FATE AND TRANSPORT OF FISH PATHOGENS

Elton Lim Wenxiong[1], Stan Chan Siew Herng[1], Lucía Chávez Díaz[1,2], Maria Yung Pui Yi[1,3], Stefan Wuertz[1,4]

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Figure 1:
Flume mesocosms with water quality sensors. Six 2 m-long rectangular flumes were seeded with sediments from areas not impacted by aquaculture; subsequent biofilms were developed over three weeks before adding the three target organisms.

Introduction

As global demand for seafood is increasing, aquatic food production has transitioned from being primarily based on wild capture of fishes to aquaculture. Yet the environmental factors contributing to disease outbreaks and reservoirs of pathogenic bacterial species in the natural environment are poorly understood. This project addresses the fate and transport of one of the most important fish pathogens in Singapore, *Tenacibaculum maritimum*, at the sediment-water interface in coastal waters.

Main findings

Coastal sediment samples from aquaculture and control sites were collected in the Johor Strait, and analysed using 16S rRNA gene metabarcoding. This established a baseline for sediment bacterial communities and presence of pathogen-like sequences in the Johor Strait that are impacted by aquaculture practices. Putative pathogen-associated sequences of *Mycobacterium* were detected in all samples, including non-aquaculture sites, suggesting a potential threat of the chronic and lethal fish disease, mycobacteriosis. Sequences associated with the genus *Tenacibaculum* were also found at one site.

To study the fate of *T. maritimum*, a laboratory-grown type strain was released in a controlled water-sediment experiment (Figure 1), along with enterococci (a microbial indicator of marine water quality) and bacteriophage P22 (a surrogate for bacterial fish pathogens).

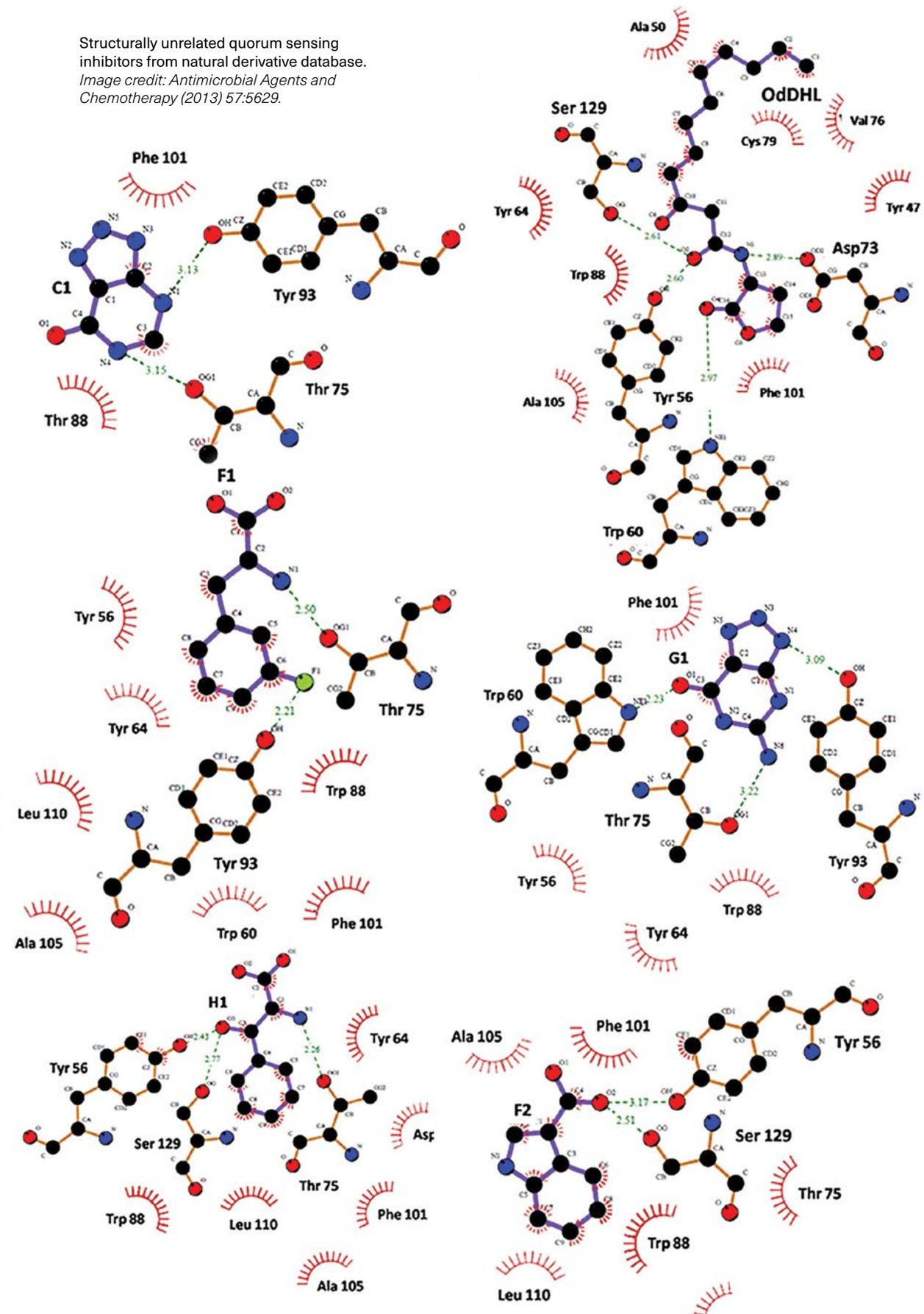
A molecular assay detected viable *T. maritimum*. The decay rates of target organisms in stationary microcosms were compared with those of flow-through mesocosm experiments to determine whether decay rate constants obtained from incubation of stationary flasks without fluid exchange adequately reflect conditions in the natural environment (as approximated in the flumes). This is pertinent as flow-through conditions are almost never considered in decay studies of chemical and biological targets.

Overall, particulate matter facilitates microbial survival and persistence in sediments and seawater in both stationary microcosms and continuous-flow flumes. Sediments should be considered a sink and source of pathogens in the marine environment due to the higher persistence rate of all target organisms. Finally, *T. maritimum* can survive long periods in water and sediments, and this finding suggests a possible threat to fishes and human health.

Future directions

Due to the relevance of aquaculture for sustainable food production and the potential of *T. maritinum* to cause large scale outbreaks that can easily spread between facilities in a high-density farming environment, future studies should investigate means by which *T. maritimum* disease outbreaks can be mitigated.

Structurally unrelated quorum sensing inhibitors from natural derivative database.
Image credit: Antimicrobial Agents and Chemotherapy (2013) 57:5629.



RESEARCH THEMES

Microbial detection and control

Tomorrow's antimicrobials and antibiotics must be effective against biofilm microbes, which are manyfold more resistant than planktonic cells. SCELSE's biofilm detection and control programme identifies and develops compounds that target and interfere with key stages in biofilm development and maintenance. Biofilm-specific assays and a range of novel molecular-based systems are used to report on biofilm-unique targets and traits in populations and communities. The research involves computational and synthetic chemistry, transcriptomics and other specific and efficacy measures, as well as *in vivo* animal models.

NITRIC OXIDE AND ITS ROLE IN BIOFILM CONTROL

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Introduction

Low concentrations of nitric oxide (NO) mediate biofilm dispersal in various single and mixed species biofilms. In *P. aeruginosa*, NO-mediated dispersal is associated with a reduction in intracellular cyclic-di-GMP (c-di-GMP) levels. However, the mechanisms through which NO is sensed, how the signal is transmitted and the effects of NO on downstream processes and responses remain elusive. The project hence explores these questions using a combination of transcriptomics, generation of relevant mutants and structural biology. Further, new classes of NO-releasing compounds and NO-delivery designs and dosing strategies were investigated for their potential and feasibility for use in biofilm control in both industrial and clinical settings.

Main findings

The molecular NO pathway

Intracellular c-di-GMP levels are regulated by the activity of diguanylate cyclases, which catalyse the production of c-di-GMP, and phosphodiesterase (PDEs), which catalyse the hydrolysis of c-di-GMP. As dispersal events are associated with a reduction in c-di-GMP levels, mutants of known PDEs of *P. aeruginosa* thought to be involved in NO sensing were evaluated, although none of the mutants were affected in the NO dispersal response. One of the PDEs evaluated, RbdA, was additionally purified and crystalised for structural elucidation. RbdA was involved in NO-mediated dispersal in these studies but is also involved in general biofilm dispersal.

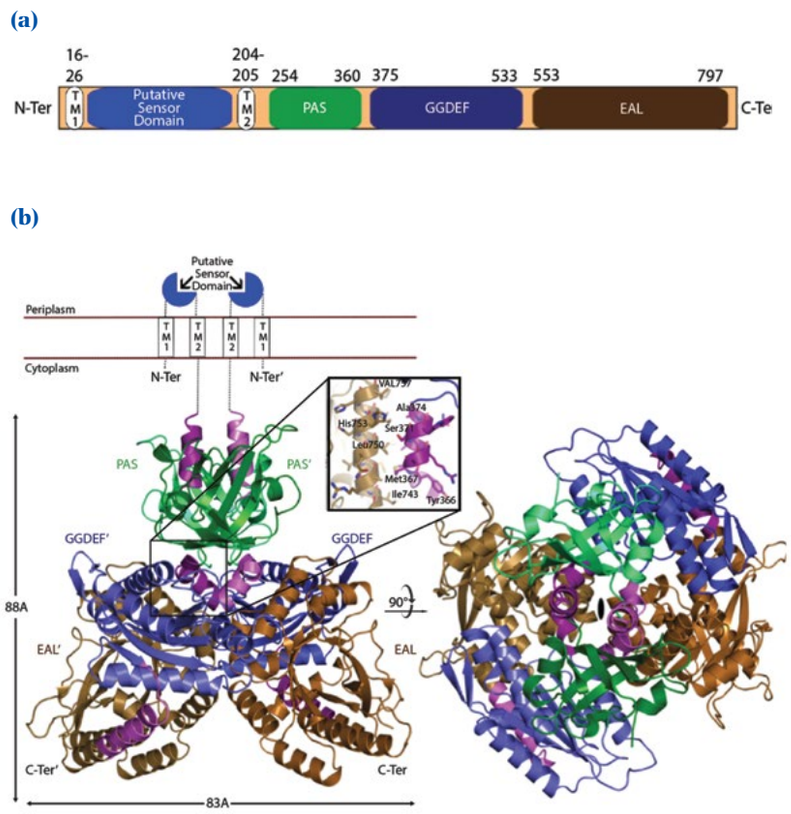

Figure 1:
Structure of the RbdA protein. (a) Domain organisation of RbdA with boundaries given above the amino-acid sequence. Amino-acid catalytic motifs are indicated. (b) The cRbdA dimer from two perpendicular views, with each domain coloured and labelled. The PAS domain is coloured green, GGDEF in cyan, EAL brown, and connecting segments crucial for the protein dynamics (S- and H-helix) in magenta and labelled. The position of the single dyad that runs through the crystallographic dimer is indicated. The region 1-232 (not included in the cRbdA construct) leading to the periplasmic membrane is represented schematically. The inset shows a magnified view of the interactions between helix S and the dimerisation helix α6-E from the EAL' domain.

Structural information derived from the study revealed evolutionary-conserved sites that play important roles in regulating the activity of RbdA and possibly related proteins and could pave the way to find molecular inhibitors or promoters of biofilm dispersal.

The biofilm never completely disperses with the use of NO, and repeated doses do not improve dispersal efficacy. Therefore, to better understand the role of NO in dispersal responses and resistance to NO treatment, transcriptomic profiles of untreated, planktonic and biofilm *P. aeruginosa* cells were compared to those of NO-induced dispersed bacteria and cells remaining within biofilm structures after treatment with the NO donor, spermine NONOate (SP-NO). Transcriptomic analysis revealed that (1) a flavohemoglobin protein, Fhp, was highly induced in NO-exposed cells; (2) the expression levels of most iron acquisition–related genes and in NO-treated biofilms and dispersed cells were lower than in untreated cells, and (3) several non-coding, small RNAs were strongly altered in expression in dispersed versus non-dispersed cells. These observations promoted further studies.

The Fhp protein is thought to bind to and scavenge NO, and an *fhp* mutant showed increased dispersal at lower NO concentrations than the wild-type (WT).

In addition, while WT *P. aeruginosa* strains displayed resistance to further NO treatment following pre-treatment with low concentrations of SP-NO due to *fhp* upregulation, mutants of both *fhp* and *fhpR*, that encode for a NO-responsive regulator of *fhp*, were defective in this response. The addition of imidazole, which can inhibit the NO scavenging activity of Fhp, also attenuated resistance to NO following NO pre-treatment and sensitised the biofilm to NO treatment.

Iron and NO were found to have interactive effects on the regulation of *P. aeruginosa* biofilm development, with supplementation of high levels of iron counteracting NO-mediated dispersal by promoting rapid attachment of planktonic cells. This effect was not due to scavenging of NO by free iron. Instead, iron induces the production of the exopolysaccharide, Psl. It was additionally found that most Psl remained on the substratum after treatment with NO, suggesting that NO-mediated dispersal involved changes in the interaction between Psl and *P. aeruginosa* cells. As such, the data suggest that iron and NO regulate biofilm development via Psl-mediated attachment through different pathways.

NO donors and delivery strategies for biofilm control

The ability of NO to induce dispersal in a wide range of single and mixed species biofilms makes it attractive as a strategy for biofilm control. To this end, (1) a class of NO donors known as furoxans was studied for its ability to disperse *P. aeruginosa* biofilms; (2) various NO donors from a class of compounds known as NONOates were investigated for efficacy in dispersing the biofilm of a complex community from a fouled industrial reverse osmosis (RO) membrane; and (3) cephalosporin-NONOate hybrid NO donors for targeted delivery of NO to bacterial infection sites were developed.

Furoxans with a fast rate of NO release were effective in dispersing *P. aeruginosa* biofilms, although similar dispersal effects were observed using higher concentrations of slow NO-releasing furoxans. However, some furoxans also displayed NO-independent effects on *P. aeruginosa* growth and biofilm formation, promoting *P. aeruginosa* growth in iron-limited minimal medium, leading to a faster rate of biofilm formation and glucose utilisation, and ultimately resulting in early dispersal of biofilm cells through carbon starvation. Additionally, one of the furoxans evaluated repressed pyoverdine production by more than 50-fold, although the exact mechanism through which this was mediated is unknown.

For the second case, membrane biofouling is a major challenge in membrane-based water treatment technologies. In this study, 500 µM of the NO-donor DETA NONOate, an NO donor dispersed more than 50% of complex community biofilms in a continuous flow system. Once-daily treatment with DEA NONOate in a laboratory-scale RO system reduced biofouling and delayed transmembrane pressure increases during constant-flux filtration, NO selection bias of NO was evident on preferentially dispersing certain community, suggesting that NO treatment may be a viable manner to control biofouling.

Lastly, cephalosporin-linked NO donor prodrugs (C3Ds) were developed to improve the specificity of NO release from NONOates, and for co-delivery of antimicrobials and NO for synergistic effects. NO is only released from the NONOate at or near the site of bacterial infection upon cleavage of the β -lactam ring of the associated cephalosporin

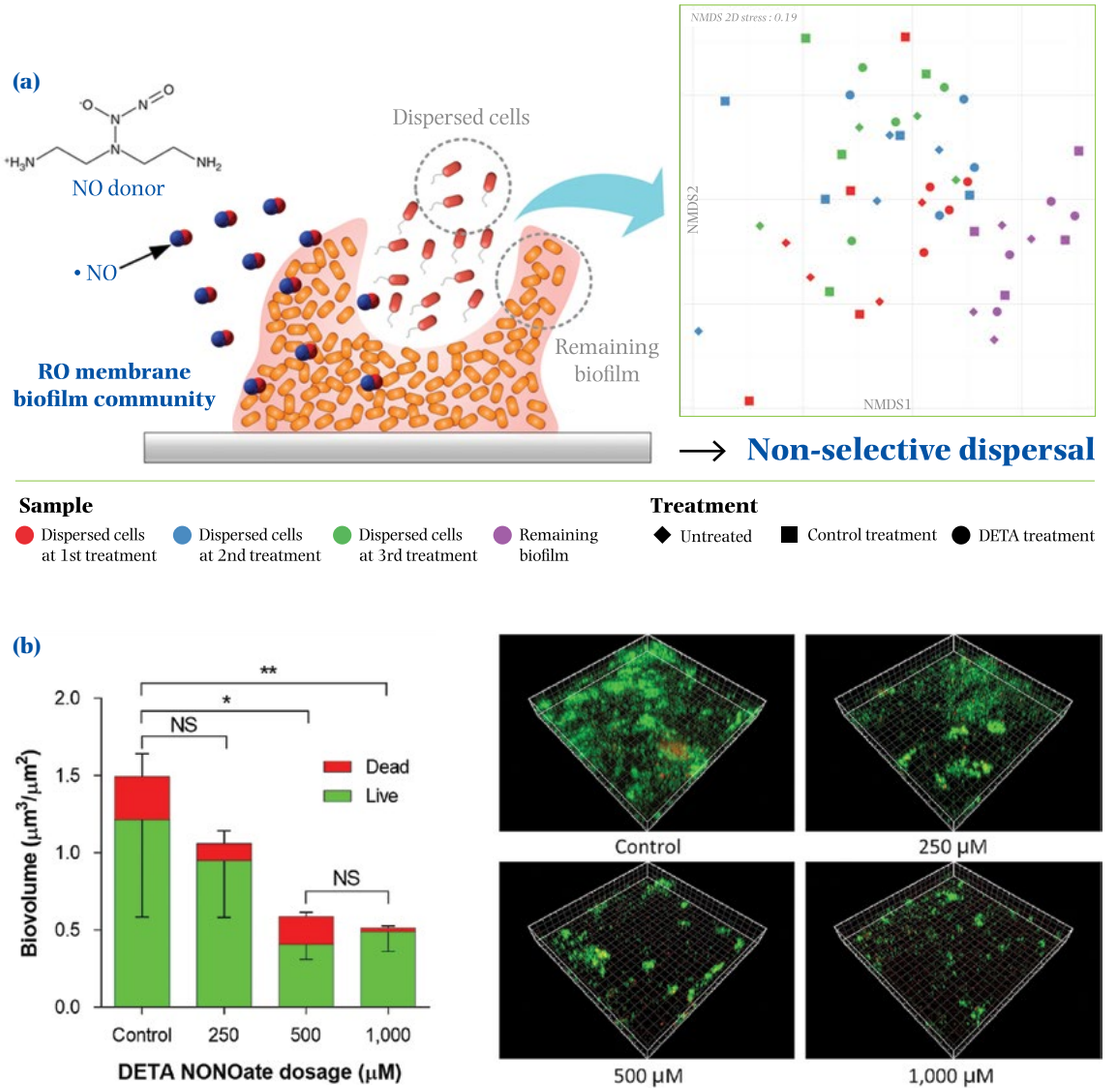

Figure 2:
(a) Schematic treatment of RO membrane community with DETA NONOate. Community analysis was carried out and a NMDS ordination plot based on the Bray-Curtis community dissimilarity of the communities was generated. Bacterial communities from remaining or non-dispersed biofilms differed significantly from the dispersed cells (PERMANOVA multivariate test). However, DETA NONOate treatment had no impact on the overall composition of the bacterial community in either dispersed cells or the remaining biofilms, which indicated there were no significant differences between treatment types when considering overall sample types or each different sample type. (b) The biovolume of live and dead cells left on the glass surface of the flow channel after treatment and associated CLSM images. One-way ANOVA and Tukey's post-tests were performed, and significance is represented as: NS (not significant) padj > 0.05, * padj < 0.05 and ** padj < 0.01).

by bacterial produced β-lactamases, transpeptidases or penicillin binding proteins (PBPs). First generation C3Ds dispersed *P. aeruginosa* biofilms but had no antimicrobial effects. Later generations of C3Ds have both direct antibacterial activity and NO-releasing capabilities. These hybrid C3Ds had higher antimicrobial potency than the parental cephalosporin and had higher PBP reactivity for broad-spectrum antibacterial activity. They were subsequently shown to have activity against ceftazidime-resistant *P. aeruginosa* biofilms and had efficacy in a murine *P. aeruginosa* respiratory infection model.

Nitric oxide as a novel adjunctive therapy for cystic fibrosis patients

Despite aggressive antibiotic therapy, bronchopulmonary colonisation by *P. aeruginosa* causes persistent morbidity and mortality in cystic fibrosis (CF) patients. Chronic *P. aeruginosa* infection in the CF lung is associated with structured, antibiotic-tolerant bacterial biofilms. A proof-of-concept double-blind clinical trial established the effects of non-bactericidal, low-dose NO as a novel adjunctive therapy for *P. aeruginosa* biofilm infection in CF in an *ex vivo*. Sub-micromolar NO concentrations alone caused disruption of biofilms within *ex vivo* CF sputum and a statistically significant decrease in *ex vivo* biofilm tolerance to tobramycin and tobramycin combined with ceftazidime. In the 12-patient randomised clinical trial, 10 ppm NO inhalation caused significant reduction in *P. aeruginosa* biofilm aggregates compared with placebo across seven days of treatment. The results suggest a benefit of using low-dose NO as adjunctive therapy to enhance the efficacy of antibiotics used to treat acute *P. aeruginosa* exacerbations in CF patients. Strategies to induce the disruption of biofilms have the potential to overcome biofilm-associated antibiotic tolerance in CF and other biofilm-related diseases.

Future directions

Using a combination of methods, much understanding of NO-mediated dispersal has been generated. A large amount of data from transcriptomics studies remain to be explored, specifically, the role of sRNA, which plays an important part in gene expression regulation. NO and new NO-delivery methods have been demonstrated as viable strategies for biofilm control and are open to continued studies of existing and new NO drugs.

Antimicrobial Agents and Chemotherapy (2018) 62: e01832-17.

Applied and Environmental Microbiology (2019) 85: e02175-18.

Journal of Membrane Science (2018) 550: 313-321.

Journal of Bacteriology (2018) 200: e00515-17.

ACS Chemical Biology (2017) 12: 2097–2106.

ACS Infectious Diseases (2020) 6: 1460–1479.

Molecules (2022) 27: 674.

FLUORESCENT PROBES FOR BIOFILM DIAGNOSIS

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Introduction

Biofilm cells secrete large amounts of extracellular polymeric substances (EPS) including exopolysaccharides, DNA and amyloids, which are important for surface attachment, initiation of biofilm formation as well as biofilm maturation. In addition, the various EPS components increase biofilm resistance to host defences and antimicrobial agents by serving as a hydrated barrier between cells and their external environment. The function of the EPS includes adhesion, aggregation of microbial cells, cohesion of biofilm, retention of water, sorption of organic and inorganic material, enzymatic activity, nutrient source, exchange of genetic information and export of cell components. The EPS is chemically complex, varying with respect to bacterial species/strains and culture conditions. Discovery of fluorescent probes with specificity for EPS components is a potential strategy to diagnose biofilm-associated infections.

Main findings

SCELSE, together with the Costerton Biofilm Center (CBC), Denmark, hosts a unique chemical library that provides scaffolds turning fluorescent upon binding to macromolecules present in the biofilm mode. Several molecules that target biofilm-unique and strain-specific macromolecular structures have been demonstrated to selectively identify bacteria in the biofilm mode, in particular, by binding to amyloids, exopolysaccharides and eDNA.

As a proof of concept, one molecule (an amyloid targeting probe CDy11) was applied as a functional diagnostic tool in corneal infections. The technology of the invention is readily applicable in clinical settings. Using a simple procedure, the presence of a biofilm infection is indicated by red fluorescence, enabling the physician to make a decision on the required antibiotic intervention strategy to efficiently eradicate the infection.

Bioimaging probes CDy14 and CDr15 for targeting Psl exopolysaccharide and extracellular DNA in biofilms were also developed. Similar to CDy11, CDy14 and CDr15 are feasible as diagnostic tools in the corneal infection model. However, with internal biofilm infections, for example in bones and joints, such simple methods will not suffice. To overcome this challenge, the molecular scaffold contains fluoride atoms that can be substituted with the F18 isotope to lay the foundation for developing the first PET-CT scan-based biofilm diagnostics. In collaboration with the University of Saskatchewan, SCELSE-CBC are developing F18 labelled amyloid targeting probe (the CDy11 compound).

To date, various laboratory-based biofilm detection methods have been established. Among them, optical imaging for effectively monitoring biofilm functions shows great potential and has been widely utilised in biomedical applications. The SCELSE-CBC team has designed unique ß-lactamase-sensitive reporter molecule (ERM-1) that can selectively localise drug resistant pathogens in biofilms. As proof of concept, a typical tetraphenylethylene (TPE) moiety was used as the target fluorophore, which was covalently linked to the cephalosporin structure. Unlike the most commonly used fluorophores, these TPE-based dye molecules exhibit strong emissions in the aggregated state and have thus been extensively applied for biosensing and imaging in living systems. More importantly, aggregated TPE products overcome the problems most existing probes have with random diffusion, and can thus serve as robust fluorogenic probes to real-time biofilm imaging of bacterial pathogens.

Future directions

Antimicrobial peptides (AMPs) are an essential component of the innate immune system to defend against invading pathogens. AMPs are amphipathic molecules that can directly interact with bacterial cell wall components such as lipopolysaccharide and compromise the cell wall integrity. In addition to directly targeting microbial cells, host-derived AMPs modulate the innate immune response and boost the host's capacity for bacterial clearance. One AMP was identified that can interact specifically with the Psl exopolysaccharide of *Pseudomonas aeruginosa*, and thus be used as a diagnostic tool for *P. aeruginosa* biofilms. Molecular dynamics simulation analysis showed how this peptide interacts with Psl. Hence, engineering biofilm EPS-targeting AMPs might provide novel strategies for biofilm detection and targeted treatment of specific pathogens, methods that would limit unwanted, antimicrobial effects on commensal bacteria.

Journal of the American Chemical Society (2016), 138: 402–407.

DECIPHERING QUORUM-SENSING INHIBITORS TO DEVELOP ANTIMICROBIAL AGENTS

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[3] Costerton Biofilm Center, University of Copenhagen, Denmark

Introduction

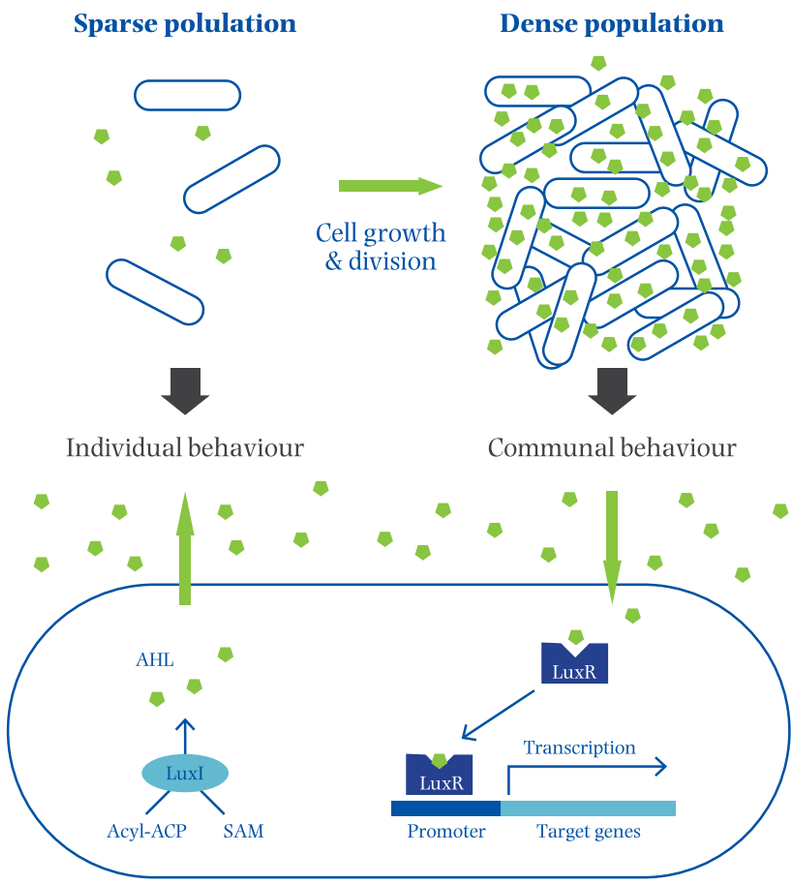
As biofilms hinder antibiotic effectiveness and facilitate the evolution of resistant phenotypes, the discovery and development of anti-biofilm compounds will have a profound impact on human medicine. This project aims to elucidate the intercellular and intracellular signalling mechanisms employed by pathogens to establish the biofilm mode of growth and provide proof of concept for applying signalling-interfering chemical biology approaches to combat biofilm infections.

Main findings

Quorum sensing (QS) is a widely distributed intercellular signalling mechanism where microorganisms regulate gene expression in response to small diffusible signalling molecules. This research established that quorum sensing–regulated activities play a significant role to initiate the formation of drug-tolerant subpopulations in *Pseudomonas aeruginosa* biofilms, highlighting that multicellular behaviour contributes to the development of antibiotic tolerance. Various strategies to discover novel QS inhibitors were thus employed. Through a structure-based virtual screening approach, five natural compounds were found to block QS and its regulated virulence factors for *P. aeruginosa*. In addition, the GacS/GacA two-component system activates expression of two ncRNAs denoted RsmY and RsmZ, which are required to sequester a QS translational repressor, RsmA; and high concentrations of RsmY and RsmZ are required to bind RsmA and abolish its function. The first natural active compound (iberin) that inhibits the expression of these two ncRNAs was identified and proven to significantly repress *P. aeruginosa* QS, biofilm formation and virulence production. A set of disulfide bond-containing ajoene analogues that inhibit the expression of these two ncRNAs at sub-micromolar concentrations were also discovered. These compounds are effective in treating infection caused by *P. aeruginosa* in mouse models.

Cyclic di-GMP (c-di-GMP) is a secondary messenger that mediates signal transduction in many Gram-negative species of bacteria, and is demonstrated

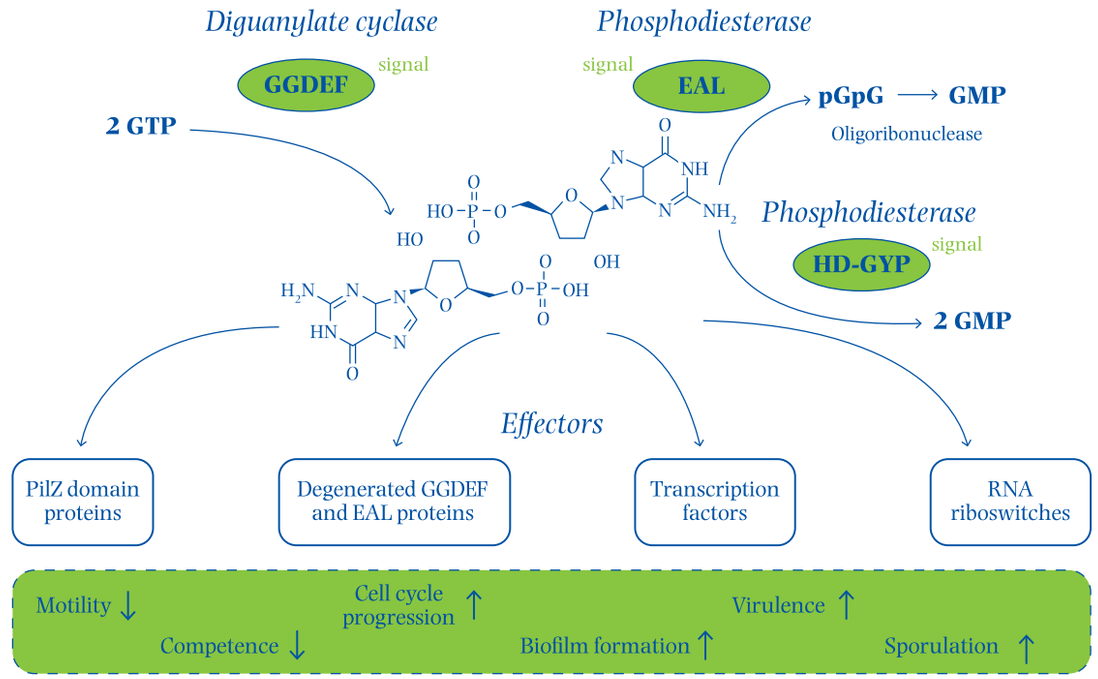
to play an essential role in determining the lifestyles of a wide range of bacteria. When the intracellular c-di-GMP content is high, bacterial cells tend to down-regulate motility and increase synthesis of extracellular polymeric substance and thus facilitate biofilm formation. In contrast, lowering the intracellular c-di-GMP content will enhance bacterial motility and cause biofilm dispersal. The intracellular c-di-GMP content is determined by a number of proteins equipped with diguanylate cyclase (DGC) activity. Such proteins can catalyse the synthesis of c-di-GMP, whereas proteins equipped with phosphodiesterase (PDE) activities degrade c-di-GMP. Intracellular c-di-GMP content was found to play a role in regulation of antimicrobial peptide resistance in *P. aeruginosa*. The induction of biofilm dispersal by lowering intracellular c-di-GMP content was demonstrated as a potential therapeutic strategy to restore antimicrobial efficacy. *In vitro* and *in vivo* protocols detail the generation and characterisation of dispersed cells from



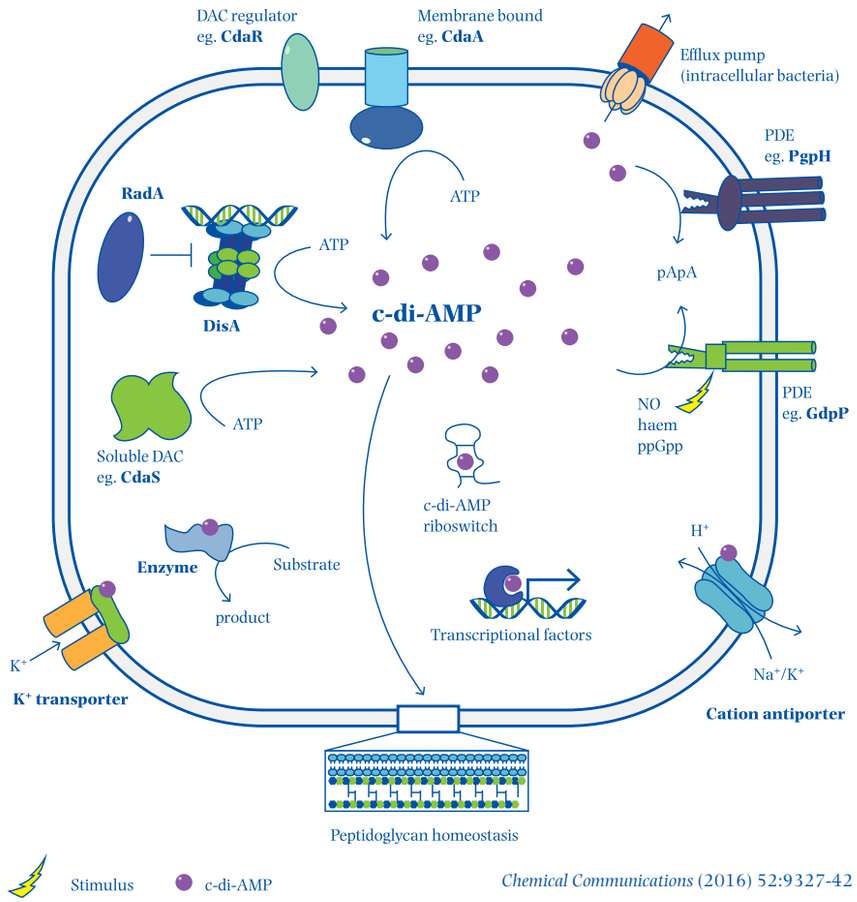
Molecular Biology (Third Edition), 2019

P. aeruginosa biofilms by reducing the intracellular c-di-GMP content through modulation of PDE activity via chemical molecules such as nitric oxide donor sodium nitroprusside. Unlike conventional protocols that demonstrate biofilm dispersal by biomass quantification, these protocols enable physiological characterisation of the dispersed cells. Using single-nucleotide resolution transcriptomic analysis, the physiology of dispersed cells from *P. aeruginosa* biofilms was found to be highly different from that of planktonic and biofilm cells. Biomarkers of dispersed cells have been identified and quantified, serving as potential targets for treating the dispersed cells.

Cyclic di-AMP (c-di-AMP) is a secondary messenger in Gram-positive bacterium and belonging to a subset of Archea. C-di-AMP's pleiotropic role as both an intracellular and extracellular molecule modulates many biological processes, including virulence, cell wall homoeostasis, biofilm formation and host immune responses. C-di-AMP is synthesised by DisA_N or DAC domain-containing diadenylate cyclases (DACs) and hydrolysed by DHH/DHHA1 or HD-domain-containing phosphodiesterases (PDEs). Increased intracellular c-di-AMP levels were shown to sensitise *Streptococcus gallolyticus* subsp. *gallolyticus* (an emerging pathogen involved in rectal colon cancer and infective endocarditis in the elderly) to osmotic stress and reduce biofilm formation and adherence on intestinal cells. C-di-AMP may constitute a key regulatory molecule for S. *gallolyticus* host colonisation and pathogenesis.



Biological Chemistry (2020) 401:1323-34



Chemical Communications (2016) 52:9327-42

Future directions

Future research projects will investigate how microbial interspecies interactions determine biofilm physiology, which further affects pathogenesis during chronic biofilm-associated infections and the functions of the host-associated microbiota. Different bacterial species can interact with each other via direct contact or diffusible signalling molecules, which can shape microbial community composition, function and host immunity. Advanced imaging tools will be combined with systems biology approaches to elucidate the molecular and biochemical basis of mixed-species biofilm infections as well as the host immune response during the infection course. An ongoing study is examining the cross-kingdom talks between pathogens and host cells. Furthermore, the combination treatment of c-di-GMP modulating compounds with conventional antibiotics on different *in vivo* biofilm infection models is being tested. Further, chronic adaptation of pathogens in the hospital environment were found to select for better biofilm forming strains. Thus, future work will develop genetic tools for monitoring the spread of major pathogenic clones/lineages and elucidate their biofilm formation mechanisms.

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Antimicrobial Agents and Chemotherapy (2013) 57:2066-75.

Antimicrobial Agents and Chemotherapy (2013) 57:5629-41.

ANTIMICROBIALS FROM MICROALGAE FOR BIOFILM INFECTION CONTROL

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Introduction

Bioactive natural products have long been identified through bioprospecting marine organisms, uncovering novel drugs for use in medical and antimicrobial applications. Pathogens such as *Pseudomonas aeruginosa* that are involved in chronic bacterial infections predominantly live as biofilms. Bacterial quorum sensing is among the key factors that induce bacterial biofilm formation. The use of small organic molecules that interfere with bacterial quorum sensing represents an alternative approach in treating pathogenic infections.

Many marine organisms successfully defend themselves against microbial colonisation and microfouling by the production of secondary metabolites. The surfaces of most marine benthic macroorganisms and microbial prokaryotes, such as sponges and cyanobacteria, are devoid of fouling, indicating that these organisms might produce compounds to prevent or inhibit microfouling. The primary objective of this research was to screen marine organisms, such as microalgae, bacteria and sponges, for potential anti-infective compounds that exhibit completely new modes of action to disrupt bacterial biofilm formation via inhibition of quorum sensing systems in *P. aeruginosa*.

Main findings

More than 80 extracts/chemical fractions derived from various filamentous marine cyanobacterial strains and sponge species were screened for quorum sensing inhibitory (QSI) activity. Of these, at least 40 fractions/extracts exhibited QSI activity, with numerous compounds subsequently purified and their structures determined. For instance, a series of novel cyclic peptides, known as the trikoramides, possessed significant QSI activity. Trikoramide D (Figure 1), in particular, showed a dose-dependent response in the QSI assay based on *P. aeruginosa lasB-gfp* and *rhlA-gfp* biosensor strains.

In addition, sponge-derived molecules, such as psammaplin A and bisaprasin (Figure 1), revealed exceptional QSI activity. These compounds inhibited the production of the enzyme elastase, in a phenotypic elastase assay when tested at 50 µM. Moreover, from mass spectrometric-based molecular networking analysis, structurally novel compounds were detected from marine-derived fractions with significant QSI activity. This is illustrated by five QSI active fractions derived from the cyanobacterium *Symploca* sp., where 899 ions were detected in the molecular networking clusters.

Future directions

This project resulted in the discovery of new structural classes of compounds having quorum sensing inhibitory activities. Future directions include: (1) Further biological evaluation of QSI active compounds in preventing biofilm formation in *P. aeruginosa* as well as their regulation of gene expression based on transcriptomic studies. (2) The mode of action and structure-activity relationship of QSI active compounds will be further investigated. (3) An *in vivo* assay of bioactive compounds will be conducted to assess their ability to prevent bacterial infection, also in conjuction with known antibiotics as a combination therapy strategy for combating bacterial infections.

Journal of Natural Products (2019) 82:3482–3488.

Molecules (2022) 27:1721.

Frontiers in Microbiology (2021) 12:10.3389/fmicb.2021.631445.

CHEMICAL BIOLOGY APPROACH FOR MICROBIAL CONTROL TECHNOLOGIES

July Fong[1,2], Li Chen[1,2], Yuan Mingjun[1,2], Kim T. Mortensen[1,2], Chua Song Lin[2], Joey Yam Kuok Hoong[2], Scott Rice[2], Tan Choon Hong[1], Yang Liang[2,3], Michael Givskov[2,4], Thomas E. Nielsen[2,4]

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[3] Southern University of Sciences and Technology, China
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Introduction

Since the inauguration in 2011, the chemical biology programme at SCELSE has focused on identifying small molecules as modulators and probes to control and study bacterial biofilms. Such compounds may serve as leads for the development of antimicrobial probes and drugs with entirely new modes of action. Based on pioneering insights and understanding of biofilm biology, SCELSE researchers have developed screening assays to gauge the anti-biofilm activities of compounds available from both commercial sources and in-house medicinal chemistry programmes.

Screening projects based on assay technologies and exchange of chemical resources between the Costerton Biofilm Center (CBC), University of Copenhagen, SCELSE, and the Division of Chemistry and Biological Chemistry at NTU have been particularly successful in this context, featuring the construction of an in-house compound library with thousands of unique compounds, which were plated and stored in a format suitable for drug discovery. Three of many highlights of the fruitful collaboration of SCELSE, CBC, and the Division of Chemistry and Biological Chemistry at NTU are presented below.

Main findings

Disulfide bond-containing ajoene analogues as novel quorum sensing inhibitors of *P. aeruginosa*

The bacterial cell-to-cell communication system, also termed quorum sensing (QS), holds potential as an antipathogenic target. Previous studies at CBC demonstrated (in a series of previous papers) that ajoene from garlic inhibited QS in the opportunistic human pathogen *Pseudomonas aeruginosa*. Screening the in-house compound library revealed sulfur-containing QS inhibitory compounds with structural resemblance to the natural product ajoene. Following structure activity relationship (SAR) studies, a range of analogues were synthesised and tested for QS inhibition activities, leading to a benzothiazole derivative with a submicromolar IC_{50} value. The compounds reduce QS-regulated virulence factors (elastase, rhamnolipid and pyocyanin) and successfully inhibited *P. aeruginosa* infection in murine model of implant-associated infection.

Itaconimides as novel quorum sensing inhibitors of *P. aeruginosa*

A collection of compounds containing the itaconimide scaffold was identified to exhibit novel antivirulence activity against *P. aeruginosa*. The compounds suppressed the *las*, *rhl* and *pqs* QS systems of *P. aeruginosa*, and effectively abolished virulence expression activities. Two lead compounds (**12a:** R=4-Br-C_6H_4, and **18a:** R=$C_{14}H_{29}$; Figure 2) showed low micromolar IC_{50} values against all three QS reporter strains with only minimal toxicity against macrophages at the administrated concentration. Moreover, a synergistic effect with tobramycin was observed for the killing of *P. aeruginosa* biofilms, including the otherwise tolerant and hard to target subpopulation cells. Overall, these findings pointed to a new class of hit compounds of relevance to the development of new drugs against the superbug *P. aeruginosa*.

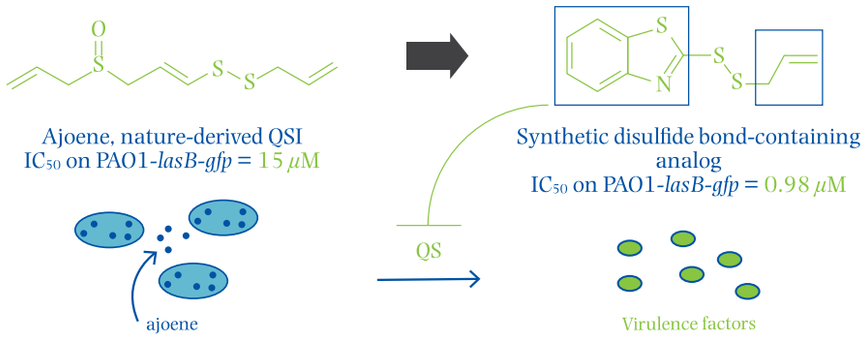
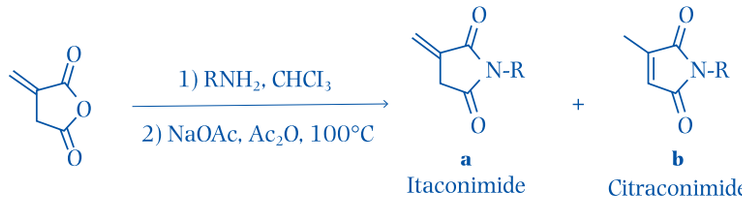


Figure 1:
Development of novel quorum sensing inhibitors (right) resembling the natural product ajoene (left) through design, synthesis and screening.



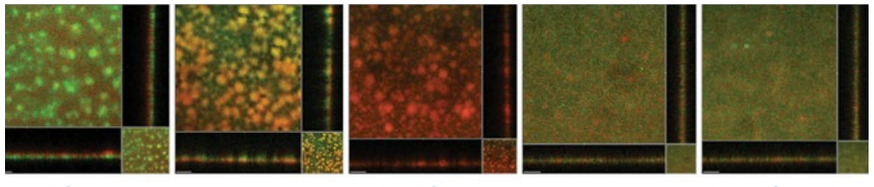


Figure 2:
Synthesis of itaconimides; and representative images of *P. aeruginosa* biofilms treated with antibiotic (tobramycin) and itaconimide compounds. Live cells *P. aeruginosa* (green); dead cells (red).

Repurposing the anti-cancer drug cisplatin with the aim of developing novel *P. aeruginosa* infection control agents

The repurposing of the widely used antitumor drug cisplatin was demonstrated as a potentially effective antimicrobial, effectively killing strains of *P. aeruginosa*. In such experiments, transcriptomic profiling showed upregulation of the *recA* gene, which is important for DNA repair, indicating that cisplatin could interfere with DNA replication in *P. aeruginosa*. Cisplatin treatment significantly repressed the type III secretion system (T3SS), which is important for the secretion of exotoxins. Furthermore, cisplatin also eradicated biofilms *in vitro* and *in vivo* in a mouse keratitis model. Hence, cisplatin could be effectively used to eradicate biofilm infections that were otherwise difficult to treat by conventional antibiotics. Although cisplatin is highly toxic for humans upon systemic exposure, a low toxicity was demonstrated with topical treatment. This indicated that higher-than-minimal inhibitory concentration (MIC) doses of cisplatin could be topically applied to treat persistent and recalcitrant *P. aeruginosa* infections.

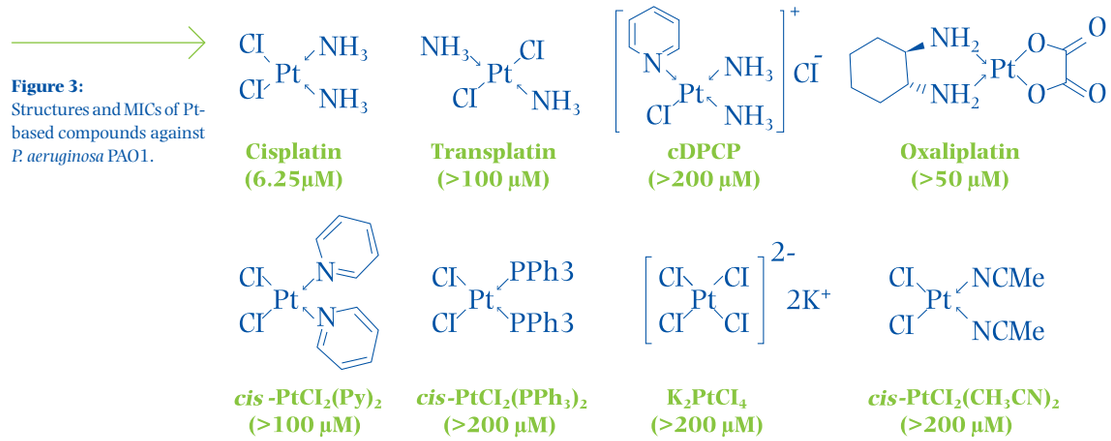


Figure 3:
Structures and MICs of Pt-based compounds against *P. aeruginosa* PAO1.

Future directions

Ongoing research efforts in the chemical biology programme focus on metallodrugs, where many novel analogues have been successfully synthesised and characterised. The compounds generally inhibit the *las*, *rhl* and *pqs* encoded QS systems in dose-dependent manners and inhibit the production of important QS-virulence factors such as elastase, rhamnolipid and pyocyanin. By interfering with QS, some compounds further repress the development of antibiotic-resistant subpopulations. Through thorough molecular optimisation, less toxic compounds have now been developed to exhibit strong anti-biofilm efficacies using the mouse implant model *in vivo*. Ongoing research will illuminate the synergistic combination with approved antibiotics against *P. aeruginosa* biofilms using the glass bead assay developed at SCELSE. For applications in cosmetics and household healthcare products, SCELSE is already leading the dialogue with internationally prominent companies in the field, but there may also be potential for pharmaceutical applications.

In the future, the chemical biology programme will continue to explore biofilm-perturbing tool compounds to study pathogenic bacteria, anti-biofilm additives to health care products and hit-to-lead compounds for antimicrobial drug development. With an infrastructure at SCELSE adequately positioned to support important aspects of early drug discovery, early activities will be translated into medicinally relevant research through testing of the newly discovered compounds in SCELSE's unique biofilm animal models, and valuable IPR of novel compounds that are safe and efficacious *in vivo* will lay the foundation for new antimicrobial medicines.

Journal of Medicinal Chemistry (2017) 60: 215–227.

Frontiers in Cellular and Infection Microbiology (2018) 8: 443.

Journal of Organic Chemistry (2018) 14: 3059–3069.

NOVEL MEMBRANE-TARGETING ANTIMICROBIAL AGENTS

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Introduction

The emergence of multidrug resistant pathogens has posed a threat to the sustainable use of antibiotics to treat serious infections. This is confounded by the lean pipeline of molecules that successfully reach the last stage of clinical trials during the discovery and development of novel therapeutics. Conjugated oligoelectrolytes (COE) are a class of compounds, developed in the last decade, that have potential for use as antimicrobial agents. COEs comprise a hydrophobic conjugated backbone and terminal polar pendant groups (Figure 1). They inhibit microbial growth, by inserting into and disrupting microbial membranes. The length of the conjugated component in hydrophobic core modulates antimicrobial activity, with shorter COEs typically more inhibitory. The minimum inhibitory concentration (MIC) for *Escherichia coli* K12 decreases from 512 $\mu g\,mL^{-1}$ to 64 $\mu g\,mL^{-1}$ as the phenylene units decrease from 5 to 3. The initial studies correlating the COE structures with antimicrobial effects lay the foundation for the rational design of the pre-existing library to generate more COEs that encompass structural variations that impact the balance of solubility in aqueous media, cell membrane intercalation and antimicrobial activity. This project investigated the interplay between these physicochemical and functional endpoints with new COEs that only comprise two phenylene units.

Main findings

Three COEs (D4, D6 and D8) belonging to a previously unreported series were designed and synthesised (Figure 1). The MIC against *E. coli* K12 are 128, 16 and 4 $\mu g\,mL^{-1}$ for D4, D6 and D8, respectively. Antimicrobial activity significantly increased with increasing alkyl chain length. Interestingly, D8 is equally effective against both Gram-negative *E. coli* and the Gram-positive organism *Enterococcus faecalis* (MIC = 4 $\mu g\,mL^{-1}$). Scanning electron microscopy (SEM) of D8-treated *E. coli* K12 revealed significant changes in morphology that were characterised by pitting and membrane rupture, suggesting that COE insertion disrupts membrane integrity. Liposomes comprising bacterial phospholipids that mimic the Gram-negative bacterial membrane were titrated with D8 and measured for the changes in membrane thermodynamics using differential scanning calorimetry. The main gel-to-liquid-crystal transition peaks broadened with increasing D8, indicating strong perturbation to the cooperative transition of the lamellar phase, which is due to the increased partitioning of D8 into hydrophobic domains of the lipid bilayer. D8 achieved the highest uptake in *E. coli* K12 (72%), in contrast to only 16% and 3% of D6 and D4, respectively. Epifluorescence microscopy also revealed that D8-treated cells demonstrated the highest fluorescence intensities, followed by D6 then D4, which corroborated well with the per cent uptake. These data provide strong evidence to show that differential membrane accumulation influences the ability of the COE to disrupt the membrane, and whereby the COE with the best antimicrobial activity (lowest MIC) has the greatest degree of association.

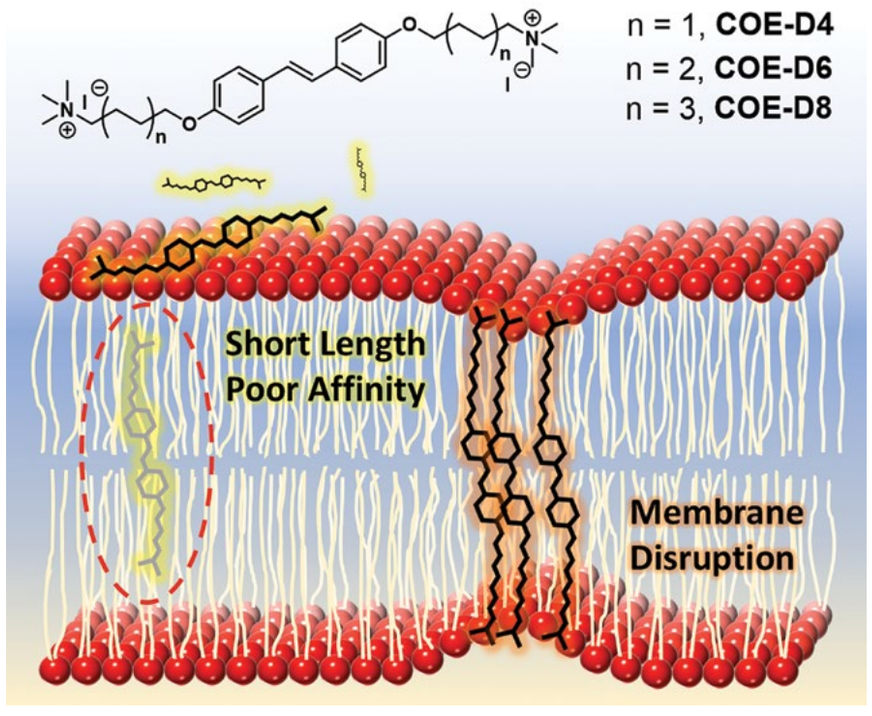

Figure 1: COEs that are shorter than the dimensions of the lipid bilayer disrupt the membrane due to the hydrophobic mismatch when they insert themselves between the phospholipids. The membrane affinity of COEs is also dependent on their aqueous solubility, which can be modulated by the balance between the hydrophobic chain lengths (n = 1 - 3) and the number of charged groups at the end of the molecule.

Angewandte Chemie International Edition English (2018) 57:8069.

Future directions

While strong antimicrobial activity has been demonstrated for COEs, refinement of molecular designs will lead to even more efficacious antimicrobial COEs. Novel COEs can be constructed with a different conjugated core (e.g., azobenzene with photo-switchable properties). The antimicrobial activity of COEs can also be extended to the biofilm context, which is often responsible for difficult-to-treat infections. Selective microbial inhibition with low mammalian cytotoxicity should also be considered a research priority.

BIO-INSPIRED COATINGS TO PREVENT MARINE BIOFOULING IN TROPICS

Snehasish Basu[1], Bui My Hanh[1], J.Q. Isaiah Chua[1], Dan Daniel[2], Muhammad Hafiz Ismail[3], Manon Marchioro[4], Shahrouz Amini[5], Scott Rice[3,6], Ali Miserez[1]

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[5] Max Planck Institut of Colloids and Interfaces, Germany
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Introduction

The firm adhesion of marine organisms on sea-immersed structures such as shipping vessels, port and aquaculture infrastructures, or oceanographic sensors is known as marine biofouling, and has serious economic and environmental consequences. Worldwide, it is estimated that the economic cost of biofouling on shipping alone is US$ 30 billion per annum. Further negative outcomes of additional fuel consumption due to marine biofouling are increasing greenhouse gas emissions that contribute to climate change. In addition, hard foulers such as barnacles and mussels can severely damage pipelines in coastal power plants, reduce heat exchange efficiency, or even clog water distribution pipelines. Singapore is particularly prone to biofouling, and thus, ideally suited to conduct biofouling research due to: (1) the warm tropical water conditions allowing year-round testing; (2) the presence of a wide range of fouling organisms with a high spatial density; and (3) it is a major shipping centre that further aggravates fouling stresses, such as the translocation of invasive species.

Slippery Liquid-Infused Porous Surfaces (SLIPS) have emerged as a very promising class of repellent coatings with excellent anti-marine biofouling characteristics. In this technology, a porous substrate (for example, a polymeric gel) is infused with a low-surface-energy fluid usually made of a silicon oil, which remains entrapped within the porous substrate owing to the strong chemical affinity between substrate and lubricant.

This project had two main directions. First, commercially available synthetic oils/lubricants for SLIPS are usually fluorinated and their slow leakage into the environment over time is a matter of concern. Thus, fully eco-friendly versions of SLIPS surfaces were developed by replacing the silicon-based lubricant with bio-based lubricants, demonstrating that these coatings can deter biofouling and are safe for the marine environment. Second, the resistance against marine biofouling of a range of biocide-free SLIPS coatings recently developed by our commercial partner on this project – Adaptive Surface Technologies (AST) in Cambridge, Massachusetts, in the United States – were investigated, notably in the prolific biofouling environment of Singapore.

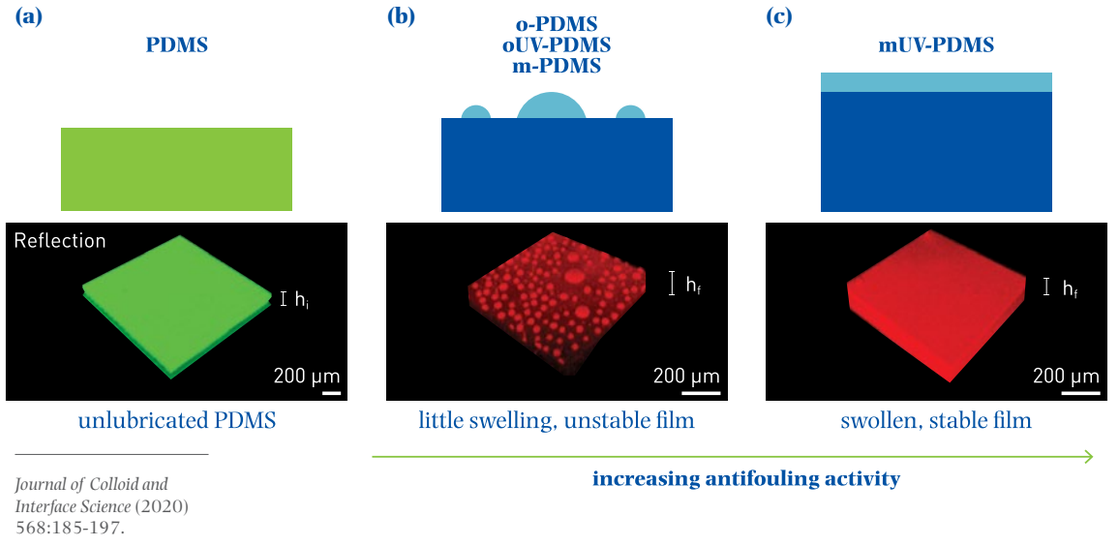
Main findings

SLIPS coatings using bio-based lubricants as infusing phases, namely oleic acid (OA) and methyl oleate (MO), were developed. The infusion efficiency was verified with confocal microscopy, surface spectroscopy, wetting efficiency and nanocontact mechanics. Using green mussels as a model organism, UV-treated polydimethylsiloxane (PDMS) infused with MO was found to provide the most uniform infused film (Figure 1), in agreement with the lowest interfacial energy among all surface/biolubricants produced. These surfaces exhibited excellent antifouling properties in the laboratory, as defined by the lowest number of mussel adhesive threads attached to the surface as well as by the smallest surface/thread adhesion strength. A direct correlation existed between antifouling performance and the substrate/biolubricant interfacial energy.

The fouling resistance performance of AST's coatings was evaluated both in the laboratory and in the field, by conducting multi-month immersion tests in high-fouling pressure environments. In the laboratory, the coatings were predominantly able to deter the settlement of marine mussels, one of the most invasive marine biofouling organisms, and to weaken their adhesion strength. The key design parameter of slippery coatings to minimise fouling is the thickness of the entrapped lubricant overlayer, which was assessed through depth-sensing nano-indentation measurements.

Conversely, the surface energy (i.e., hydrophobic versus hydrophilic) did not significantly affect the antifouling performance of these coatings. After immersion in the field in stagnant waters, all coatings exhibited efficient foul-release capacity against macrofoulers, whereas under stronger hydrodynamic flow conditions, only weakly attached biofilms were detected with a bacterial community composition that was independent of the surface energy.

Figure 1:
Schematic and confocal microscopy images of SLIPS surfaces infused with various biolubricants. (a) Unlubricated PDMS gel. (b) PDMS gel infused with oleic acid (o-PDMS) or methyl oleate (m-PDMS), or UV-treated PDMS infused with oleic acid (oUV-PDMS). (c) PDMS gel infused with methyl oleate (mUV-PDMS). (Reproduced from *Journal of Colloid and Interface Science* (2020) 568:185-197)



Journal of Colloid and Interface Science (2020) 568:185-197.

Sampling the Singaporean native hawksbill turtle for insights into the genetic diversity of local populations, to inform conservation efforts.
Image credit: Regine Tiong, SCELSE

RESEARCH THEMES

Higher organism, climate and conservation genomics

SCELSE's expertise in genomics technologies extends to investigating higher organisms across a broad scope of research domains, from threatened species conservation to personalised medicine.

CLIMATE AND CONSERVATION GENOMICS

Stephan C. Schuster[1,2], Daniela I. Drautz-Moses[1], Kim Hie Lim[1,3], Regine Tiong[1], Charlotte Lindqvist[4], Webb Miller[5], Aakrosh Ratan[1,6]

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Overview

Global losses in biodiversity are increasing at an unprecedented rate. While some causes are still unknown, others can be attributed to changes in habitat due to climate change, overexploitation of the natural environment, destruction of habitats and pollution. Genomic data can provide an opportunity to understand the causes as well as predict potential vulnerable populations. As such, genomic methods can be used to validate species identity, estimate effective population size, and understand genetic diversity of a population. Genomic data also provide a glimpse into historical speciation and hybridisation events and changes in population dynamics coinciding with key climate and environmental changes in a species' history. Conservation genomics paired with the recovery and sequencing of ancient DNA can offer insights into the evolution and adaptation of species such as the polar bear and woolly mammoth populations to their present-day counterparts. The SCELSE has aided in conservation efforts by extending its unique expertise in processing and sequencing to both contemporary mammalian and ancient DNA samples.

Genome sequencing aids in conservation efforts for the local hawksbill turtle population

Kim Hie Lim[1,3], Regine Tiong[1]

Introduction

The Singaporean native hawksbill turtle is a globally critically endangered species that makes its nests in the sandy beach areas around East Coast Park and Sentosa. Between 2017 and 2021 there have been as few as 210 sightings reported by National Parks Board (NParks). Conservation initiatives will aid in the understanding of how best to protect the hawksbill turtle by studying its habitat and its susceptibility to environmental risks such as disease.

Newly hatched hawksbill turtles provide an opportunity to assess the genetic diversity of local populations to aid conservation efforts. *Image credit: Regine Tiong, SCELSE*

Main findings

Since the beginning of 2019, the SCELSE team has worked closely with NParks Singapore to aid in the conservation efforts of the critically endangered hawksbill turtle. Genetic material was collected from egg shells, liver of stillborn hatchlings, and yolk samples from unhatched eggs, and assembled into the first genome sequence of this species. The result was a genome assembly of approximately two billion base pairs, substantially larger than the human genome of 3.2 billion base pairs. The

sequenced genetic material provides insights into the genetic diversity of local populations and aids in the investigation of factors related to possible disease susceptibility.

Research on the hawksbill turtle is shedding light on the species' genetic diversity to better direct conservation efforts for the nesting population in Singapore. Specifically, the sequencing of the Singapore turtles will help understand global diversity to help with larger conservation efforts.

Polar bear genomics and conservation

Collaborator: Charlotte Lindqvist[4]

Introduction

Because of an altered Arctic environment, which has experienced the strongest effects of global climate change in recent decades, the polar bear (*Ursus maritimus*), with its uncertain long-term status, has become a symbol of the threat to biodiversity from climate change. Understanding polar bear evolutionary history may therefore provide insights into apex carnivore responses and prospects during periods of extreme environmental perturbations. In recent years, genomic studies have examined bear speciation and population history, including evidence for ancient admixture between polar bears and brown bears (*Ursus arctos*). While interspecific hybridisation is a widespread phenomenon, measuring its extent, directionality and adaptive importance remains challenging. Ancient genomes, however, can help illuminate the history of modern organisms.

Ancient polar bear genomes provide insights into the history modern organisms.

Main findings

The genome of a ~120,000-year-old polar bear was sequenced to 10X coverage, and genome analyses were performed for the modern polar bear and its close relative the brown bear, throughout their geographic ranges. The study revealed a dramatic decline in effective population size for the ancient polar bear's lineage, followed by a modest increase just before its demise. A slightly higher genetic diversity in the ancient polar bear suggests a severe genetic erosion over a prolonged bottleneck exists in modern polar bears. Further, at least one ancient introgression event from brown bears into the polar bear ancestor was identified, possibly dating back more than 150,000 years. This suggests that gene flow was likely bidirectional, but allelic transfer from brown into polar bear is the strongest detected signal. These findings contrast with other published works, and have implications for understanding climate change impacts, such that polar bears, a specialist Arctic lineage, may not only have undergone severe genetic bottlenecks, but may also have been the recipient of generalist, boreal genetic variants from the brown bear during critical phases of Northern Hemisphere glacial oscillations.

Woolly mammoth genomics

Collaborator: Webb Miller[5]

Introduction

Genetic changes that contributed to woolly mammoth adaptations to extreme cold were identified in a study characterising the major phenotype difference of woolly mammoths and living elephants, allowing them to inhabit very different environments. However, identifying such genetic changes that underlie morphological evolution is challenging, particularly in non-model and extinct organisms.

Main findings

The nuclear genomes of two woolly mammoths that died ~20,000 and ~60,000 years ago and three Asian elephants were sequenced, thereby expanding coverage from previously analysed samples. Amino acid changes unique to woolly mammoths were identified, some of which likely contributed to woolly mammoth–specific traits and adaptations to extreme cold such as small ears, thick fur and altered temperature sensation. Genes with mammoth-specific amino acid changes were enriched in functions that allowed woolly mammoths to adapt to life in the high Arctic. The mammoth and ancestral elephant TRPV3 gene encoding for thermal sensation and hair growth was resurrected and functionally tested, with an amino acid substitution that may have contributed to cold tolerance. The genomic data generated through this study will provide a useful resource for future studies to explore the genetic changes that underlie woolly mammoth morphology, physiology and demography.

HUMAN GENOMICS

The Khoisan people

Stephan C. Schuster[1,2], Daniela I. Drautz-Moses[1], Kim Hie Lim[1,3]

Introduction

Another example of the influence of climate on populations involves the history of human populations in Africa. The Khoisan people from southern Africa, also known as Bushmen, have maintained ancient lifestyles as hunter-gatherers or pastoralists up to modern times, though little else is known about their early history. Their population has long been in decline owing to land loss and the effects of European colonisation. Yet, for tens of thousands of years, the ancestors of the modern Khoisan people were members of the largest population on the planet and have maintained the greatest genetic diversity known among human populations, despite the current Khoisan speakers population numbering just 100,000.

Through a collaborative effort with Pennsylvania State University, SCELSE researchers sequenced the complete genomes of five Khoisan hunter-gatherers from Namibia and one Bantu speaker (of southern Africa), and compared their DNA with that of 1,462 genomes of people from around the world. The study revealed that two of the Khoisan genomes inherited their DNA only from Khoisan ancestors in the northern Kalahari region, with no genetic mixing with non-Khoisan speakers, thereby preserving the ancient diversity inherited entirely from their direct ancestors.

Using several different methods of analysis, the study found that the Khoisan and their ancestors have been the largest populations since their split with the non-Khoisan population ~100–150 kyr ago. In contrast, the ancestors of the non-Khoisan groups, including Bantu-speakers and non-Africans, experienced population declines after the split and lost more than half their genetic diversity.

Future directions

In collaborative efforts with local groups in Singapore and continued partnership with conservation biologists around the world, we hope to continue to contribute to conservation efforts of critically endangered species. In addition, further research into the population structure of the Ju/'hoansi and related Khoisan groups with larger sample sizes will be essential for a more comprehensive understanding of the deep divergence and population history of modern humans.

Cell Reports (2015) 12: 2, 217–228.

GenomeAsia100K

GenomeAsia100K Consortium

Introduction

Asians – despite being less than 40% of the world's population – are significantly underrepresented in current genomic studies and reference genome databases. The unique genetic diversity prevalent in South and East Asia provides a valuable source of clinical insights into rare and inherited diseases across global populations, as well as complex diseases such as cancer, diabetes and neurological disorders. GenomeAsia100K (GA100K) is a consortium founded by SCELSE-NTU in collaboration with MedGenome and Emerge Ventures to sequence and analyse 100,000 Asian genomes to help accelerate Asian-population-specific medical advances and precision medicine.

Main findings

The pilot phase of the GenomeAsia100K project catalogued genetic variation, population structure, disease associations and founder effects from 219 population groups and 64 countries across Asia. The analysis addressed a wide range of questions regarding the history of specific Asian population groups and informed on strategies for additional sequencing efforts.

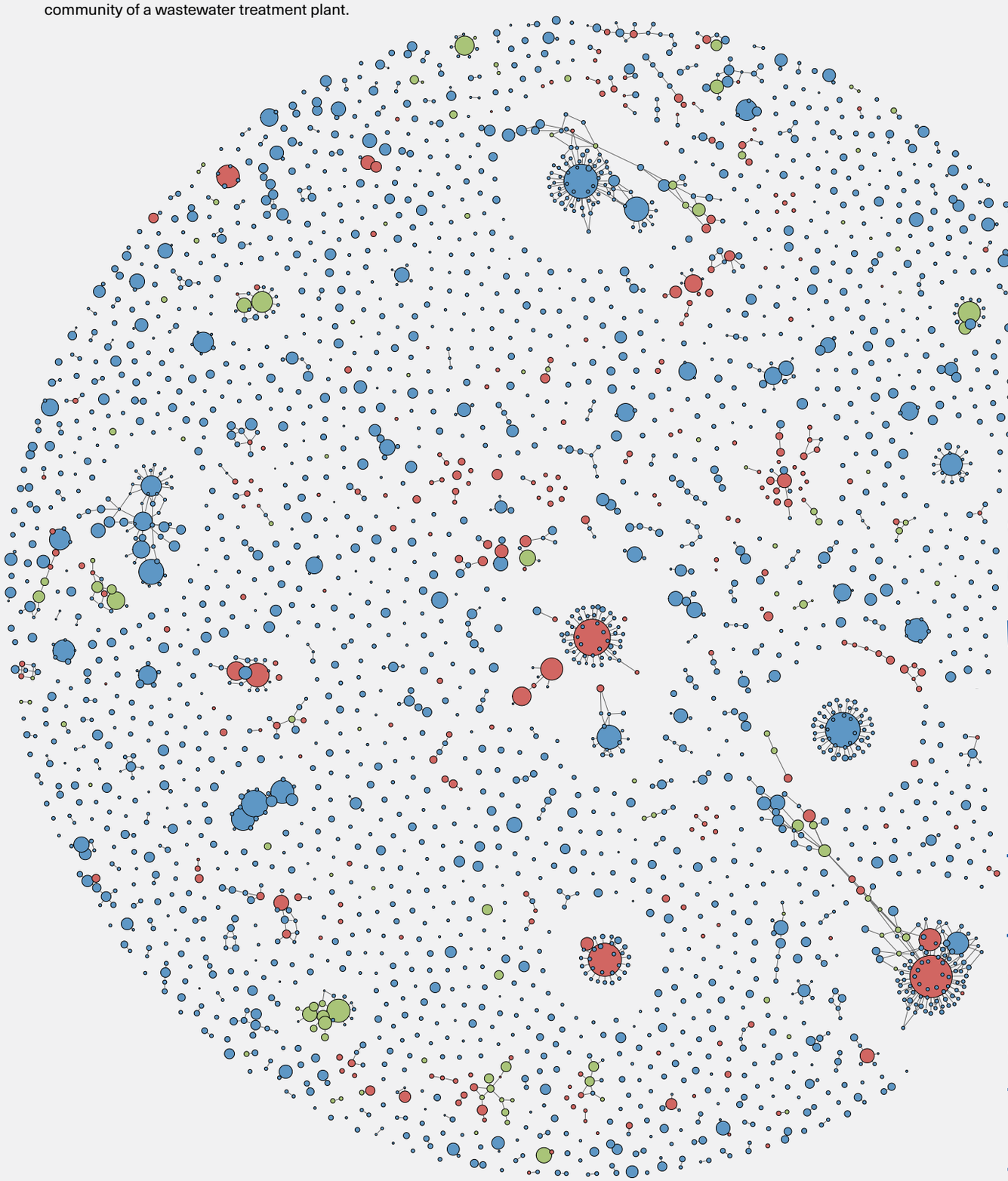
The ability to define gene function in humans through the study of the phenotypic effects of loss-of-function mutations is becoming an increasingly valuable approach, and the study of additional variants and populations in which homozygosity occurs at high rates will add to the global resources for human genetic and phenotypic studies.

Future directions

Future GA100K efforts involve a staged and coordinated approach, to include the generation of genomic population-specific reference data sets and imputation panels for disease-association studies.

Nature (2019) 576: 106–111.

A network model for the complex microbial community of a wastewater treatment plant.



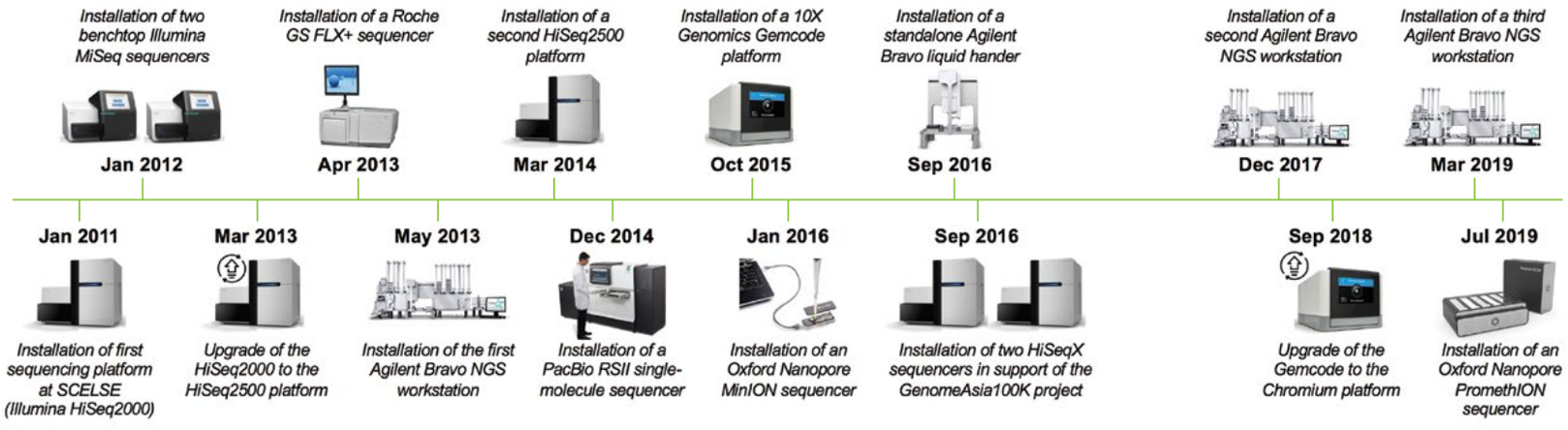
STATE-OF-THE-ART CAPACITY FOR MULTI-DISCIPLINARY RESEARCH

High-throughput Sequencing Facility

Complex microbial communities in both natural and engineered systems harbour a wealth of genomics and transcriptomics knowledge for biofilm and microbiome research, while also presenting key challenges in sample extraction and the sheer volume of data to acquire and process. SCELSE's High-throughput Sequencing Facility is designed to provide researchers and collaborators access to cutting-edge, next-generation sequencing technologies.

The facility was established in 2011, starting with a single next-generation sequencing instrument, an Illumina HiSeq2000, and two technical staff. Over the years, the sequencing team has expanded to up to 10 highly skilled technical staff, and additional sequencing platforms were added to SCELSE's sequencing portfolio. These include an Illumina HiSeq2500 instrument, two Illumina MiSeq benchtop sequencers, a Roche GS FLX+ pyro sequencer as well as single-molecule sequencing platforms from Pacific Biosciences (RSII) and Oxford Nanopore Technologies (MinION and PromethION). The facility's instrumenttion can generate long-read lengths of 15,000 base pairs and more, with sequencing capabilities to support *de novo* sequencing of novel genomes without the need of reference sequences for alignment. In addition, in 2015, SCELSE was the first research centre in Asia to receive the 10X Genomics GemCode instrument, which allows for artificial long reads to be generated by barcoding DNA molecules for standard short-read sequencing. These can then be assembled *in silico* into long reads.

SCELSE staff member Yap Zhei Hwee operating one of the centre's Agilent Bravo next-generation sequencing liquid handlers.



Expansion of SCELSE's sequencing facility since 2011.

Between 2013 and 2019, the facility implemented a fleet of liquid handlers, consisting of three Agilent Bravo NGS workstations and one standalone Bravo unit, allowing library preparation processes as well as library QC to be fully automated, thereby eliminating human error while maintaining the highest sequencing quality. Another milestone was reached in 2016 when two Illumina HiSeqX high-throughput sequencers were installed in SCELSE's sequencing facility in support of the GenomeAsia 100K human genome sequencing initiative, which is led by a consortium of industry partners and academics, including NTU (see *Higher organism, conservation and climate genomics* section). To date, SCELSE's sequencing facility has contributed more than 6,000 complete genomes to this project.

SCELSE's sequencing facility has come a long way since its humble beginnings in 2011 when a total of 24 samples were sequenced during the first year. Today, the facility processes 8,000 to 10,000 samples on average per year, and provides sequencing services not only to researchers at SCELSE but also at NTU, in Singapore and beyond. It now offers a variety of sequencing applications, including metagenomic and metatranscriptomic sequencing, whole genome sequencing, *de novo* sequencing, small RNA sequencing, amplicon sequencing, RNA-seq, ChiP-seq, ultralow biomass sequencing, and bisulfide sequencing.

SCELSE High Performance Computing (HPC) Facility

SCELSE's HPC infrastructure was first developed in 2011 to provide computational capacity to handle the data stream from the genomics laboratory and for genomics and bioinformatics analyses. Over the past 10 years, the high-throughput DNA sequencing machines have produced many terabytes of sequencing data. The resulting data sets need to be stored, processed and analysed to provide biological insights and confirm or refute experimental hypothesis. Some analysis techniques are computationally expensive and require hundreds of CPU-cores for timely processing, while others required hundreds of gigabytes of memory to accommodate the essential data in the server. Throughout its lifetime, the various aspects of the HPC have been managed in-house, encompassing all facets of hardware management, software development and optimisation, application troubleshooting, and new technology exploration.

Computer servers

The requirements for computing resources have grown in tandem with SCELSE's sequencing needs. In 2019, SCELSE purchased and implemented an HPE Cray Hybrid supercomputer, upgrading from its exiting CPU-cores to more than 13,000 CPU-cores. The high performance computing cluster (HPCC), named HADLEY, is a physical network comprising both AMD and Intel CPU, featuring 2 x 1.5 TB of high-memory nodes, 1 x 3 TB of high-memory node to serve memory intensive research such as genome assembly, and 2 x NVIDIA GP-GPU nodes to serve AI/ML needs. These nodes are linked to a high-throughput network with a bandwidth of 100 GB/s. The distribution of the workload is managed by Altair's PBS pro scheduler with a customised policy for each category node available in the HPC (Figure 1).

From its inauguration in 2011 to 2018, the computer cluster has provided more than 12 million CPU-hours of computational time annually for SCELSE's researchers and operated with an annual average of 95.6% uptime. After the implementation of the HPCC HADLEY in 2019, the SCELSE HPC provides four times the computational capacity, while the average annual uptime is increased to 98.5%.

The increase in computational power combined with continual optimisation of analysis pipelines has reduced computing time and aided SCELSE in its commitment towards sustainability by reducing the amount of energy necessary for analysis. To illustrate this, the implementation of the HADLEY HPCC paired with pipeline optimisation has reduced the time needed for SCELSE's air microbiome research team to process a set of metagenomic samples from approximately 5.4 months to 16 hours, while reducing the total estimated energy usage by 15-fold. This translates to energy savings equivalent to about eight years of a single household energy usage in Singapore.

Data storage

Another essential component of an HPC facility is the data storage system, primarily used for DNA sequencing data and subsequent downstream analysis. The genomics laboratory currently houses several sequencing instruments with various data throughput rates of up to hundreds of gigabytes of data per day. Consequently, a high-performance data storage system is able to handle multiple instruments simultaneously streaming data, and the analytics workload submitted by researchers. In addition, the system is easily expandable to accommodate evolving sequencing technology and increasing data throughput. SCELSE HPC facility houses high-performance data storage for primary data and high-throughput analytics. Currently SCELSE HPC facility houses two types of high-performance data storage: (1) primary storage – used as landing storage that is linked directly to the DNA sequencing instruments. (2) High-throughput analytics data storage capable of handling high-throughput analytics jobs and accessed by hundreds of servers simultaneously without any performance penalty. The latter is using IBM's general parallel file system (GPFS) and is connected to all computer nodes in the cluster. This high level of performance is crucial as every analytics job running in the computer node will be reading and writing its data to the data storage.

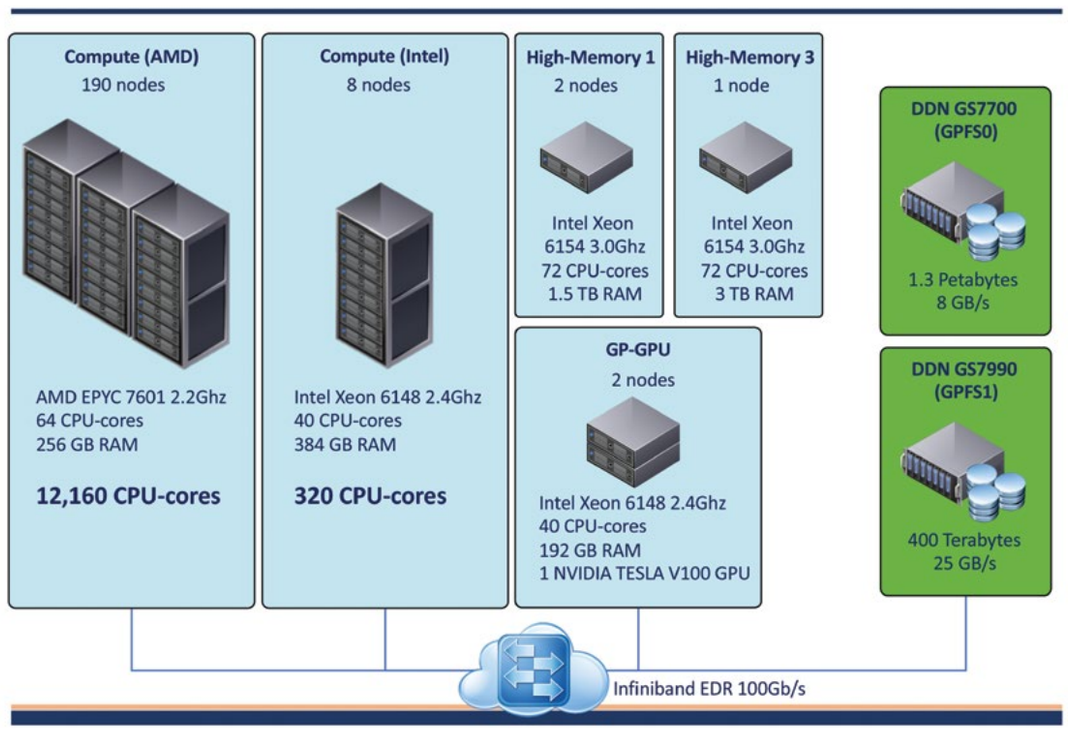
Network interconnect system

The network system is the link between the computer nodes, data storage and other supporting infrastructure. The network could easily become a bottleneck in any HPC system if it cannot deliver the data from the data storage to the computer node fast enough. To avert potential data loss through bottleneck complications, a 100 GB/s backbone network system was implemented to connect to all the HPC hardware components and have a high-speed fibre connection to NTU's Experimental Medicine Building and HPC facility, and the National Supercomputing Centre of Singapore (NSCC Singapore), for high-speed data transfer.

Collaboration

While the HPC infrastructure has served SCELSE well in terms of processing capacity, the exponential rise of data throughput and the number of projects SCELSE runs, necessitates a secondary site for processing during peak workloads. The centre's collaboration with the National Supercomputing Centre Singapore provides access to the ASPIRE-1 computing system, which consists of 30,000+ CPU-cores, sufficient to accommodate overflow from SCELSE. A 40 GB/s fibre link between SCELSE's and NSCC's remote login node enables data transfer. SCELSE also has future plans for collaboration with NSCC to share more computing resources and storage space from ASPIRE-2 once it begins operation later this 2022.

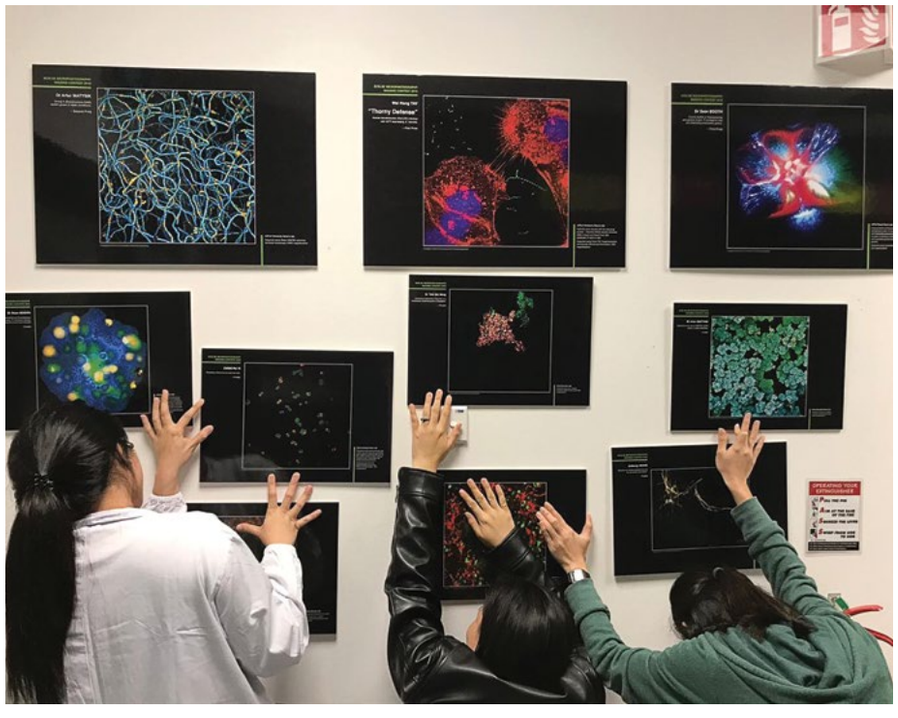
Hadley Cluster Configuration



Advanced Biofilm Imaging Facility

SCELSE's Advanced Biofilm Imaging Facility (ABIF) provides access to state-of-the-art microscopes, image processing and analysis software. Importantly, this is supported by the in-house comprehensive microscopy expertise of ABIF staff, who train and instruct users in microscope operation, and advise and assist researchers with sample preparation, advanced imaging techniques, processing and analysis of microscopy images.

ABIF started in 2012 as a partnership with leading microscope manufacturer Carl Zeiss and has since remained at the forefront of imaging technologies to address the diverse needs of SCELSE researchers. Currently, the facility houses more than 10 microscopes, including basic light and epi-fluorescence microscopes, laser scanning and spinning disc confocal microscopes, a microscope for super-resolution imaging techniques, structured illumination microscopy (SIM) and localisation microscopy (e.g., PALM, STORM), a scanning electron microscope and a unique custom-designed system for confocal Raman and Brillouin micro-spectroscopy. Microscopes can be fitted with incubation chambers, temperature control units and flow cell systems simulating both natural and engineered habitats for biofilms and microbial communities, to observe live events unfolding over extended periods of time at high spatial and temporal resolution.

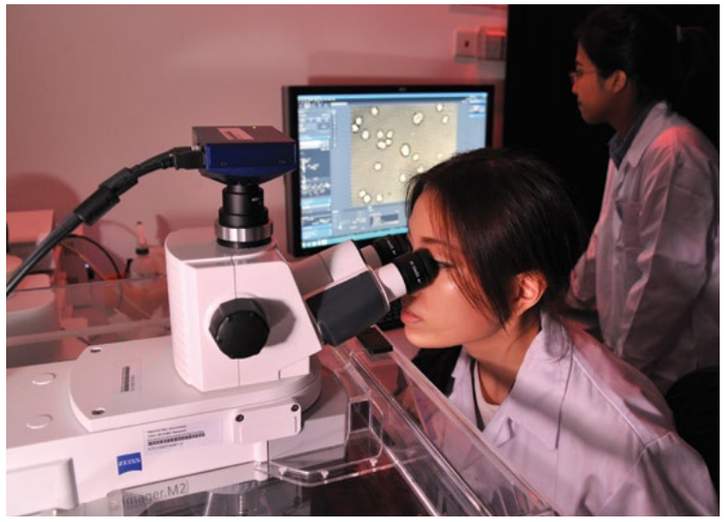


Showcasing the winners of the SCELSE Microphotography Imaging Contest 2018.

ABIF – a member of SingaScope

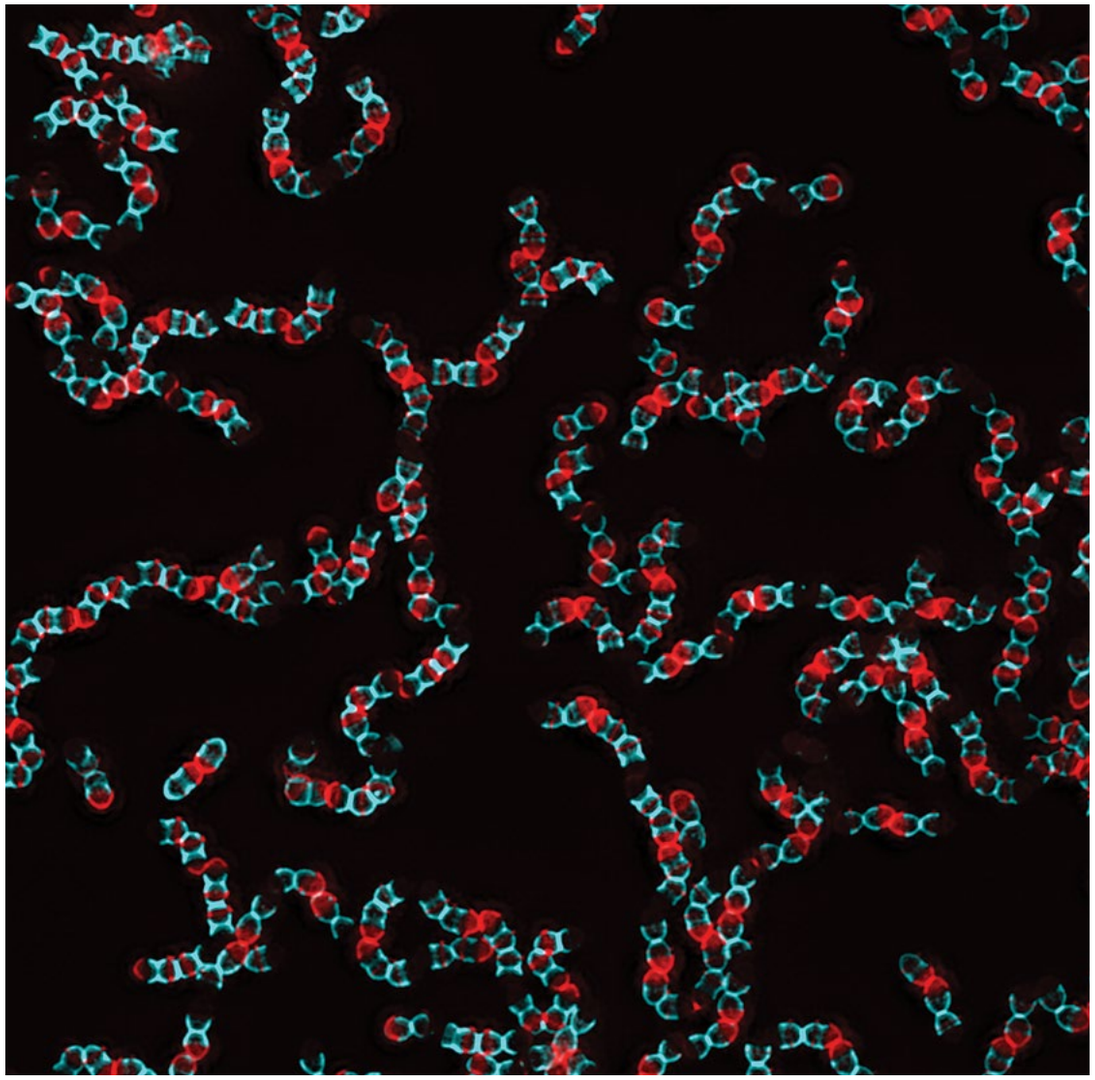
While ABIF caters primarily to research in microbial cells, populations and communities conducted at SCELSE, the advanced microscopes available at the facility can address a broad range of research questions in life and material sciences, attracting collaborators from outside SCELSE. The accessibility of ABIF's microscopes to external users is a key part of the facility's participation in SingaScope, a Singapore-wide microscopy facilities network funded through a National Research Foundation–shared infrastructure grant, and which SCELSE has been a founding partner. SingaScope's mission is to provide researchers in Singapore access to cutting-edge microscopy equipment and relevant expert knowledge, through the partnership of Singapore's leading microscopy platforms. This goal is achieved not only by opening the partner facilities to external users, but also by organising courses and workshops, promoting the best practices in partner facilities and funding unique equipment to extend the capabilities within the network. ABIF's Raman and Brillouin microscope is an example of SingaScope-funded equipment. In 2019, SingaScope joined Global BioImaging, a worldwide network of similar open-access initiatives, placing ABIF on the global map of microscopy facilities.

To further strengthen their microscopy capabilities and expertise, SCELSE and Lee Kong Chian School of Medicine (LKCMedicine) at NTU joined forces in establishing NTU Optical Bio-Imaging Centre (NOBIC) in 2020. As a part of NOBIC, ABIF can leverage on the joint expertise of NOBIC's team of scientists and engineers, who can design, develop and build bespoke microscopy equipment, as demonstrated by ABIF's unique Raman and Brillouin microscope, tailored to meet the specific needs of NOBIC users. A two-photon microscope, designed and built by NOBIC, is expected to be installed in 2022, with more unique custom-built setups expected in the future.



Microscopy at SCELSE's ABIF: Dr Radek Machán (top); Dr Chew Su Chuen (bottom foreground) and A/Prof. Yang Li Yang.

Promoting microscopy and disseminating microscopy knowledge have always formed an integral part of ABIF's mission. In 2017, SCELSE held its inaugural Microscopy Seminar with course instructors from academia and industry. Later courses were organised under the NOBIC banner, starting with the virtual course Introduction to FIJI in September 2021 and followed by the hybrid physical/virtual NOBIC Microscopy Course, in 2022. A monthly series of short microscopy-related talks, called NOBIC µ-Talks, encourages even closer interactions between the facility and the researcher community. All these events were promoted by SingaScope to address a broader audience.



3rd place winner of the NOBIC Image Contest 2021. Image was taken by Choo Pei Yi (SCELSE). The image shows strings of *Enterococcus faecalis* cells, taken on the Carl Zeiss ELYRA PS.1 and processed via structured illumination microscopy (SIM) at 100x magnification. Scale bar: 2 µm.

Showcasing the aesthetic beauty of microscopy images of microbial life is another part of ABIF's outreach activities. The tradition was started by the SCELSE Microphotography Imaging Contest in 2018 and continued with the NOBIC Image Contest 2021. In the latter event, images of microbes submitted by SCELSE researchers scored well against images of apparently more photogenic samples, such as zebrafish embryos, contributed by contestants from LKCMedicine. A SCELSE image achieved third place in this competition, and three out of ten finalist images were from SCELSE research, a testimony to the skill of the centre's researchers as well as to the capabilities of ABIF's equipment.

SCELSE Bioreactor Facility

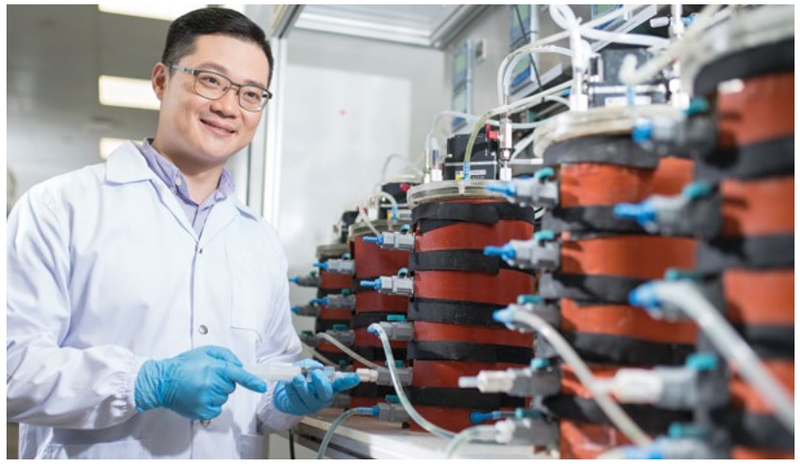
SCELSE conducts research on microbial communities and their roles in treating wastewater (sewage, food production streams) and providing ecosystem functions in natural systems. Many relevant questions can only be answered by considering different scales. Consequently, SCELSE's environmental bioengineering and bioreactor laboratories cater for a range of experimental scales, and an extensive range of laboratory-scale reactors is available, including off-site mesoscale flumes (formerly at the Van Kleef Aquatic Science Centre, and presently at the St John's Island National Marine Laboratory).

SCELSE has a wide range of bioreactors in-house for various research needs in its state-of-the-art facilities. The bioreactor setup offers online monitoring and precise control of operational parameters (i.e., temperature, aeration, pH, agitation and dissolved oxygen, gas flow measurements), providing ample resources for researchers to investigate the temporal variation of microbial community dynamics, structure, and functions as well as the means required to develop predictive models in a controlled environment.

A range of customisable laboratory-scale bioreactors with fully automated and semi-automated monitoring system can be employed for batch, fed batch or continuous systems. SCELSE currently hosts more than 30 laboratory-scale bioreactors associated with a variety of wider research projects ranging from single-cell protein generation to question-relating wastewater treatment: 10 aerobic 6 L sequencing batch reactors (SBR); 18 anaerobic 6 L SBRs; six fully autoclavable 4 L borosilicate glass bioreactors; five aerobic 5 L SBRs; and four aerobic 2 L SBRs.

Apart from this bioreactor setup, SCELSE's laboratories are also fully equipped with various equipment (liquid handler, furnace, gas chromatography, ion chromatography and a broad range of molecular, (real time or digital droplet) polymerase chain reaction–based devices to quantify microorganisms) for the subsequent physicochemical and analytical works.

6 L aerobic replicated SBRs.



5 L anaerobic replicated SBRs.

Integrative Analysis Unit

Krithika Arumugam[1], Irina Bessarab[1], Mindia Haryono[1], Ezequiel Marzinelli[1,2], Nay Min Min Thaw Saw[1], Rohan Williams[1]

[1] SCELSE
[2] School of Life and Environmental Sciences, University of Sydney, Australia

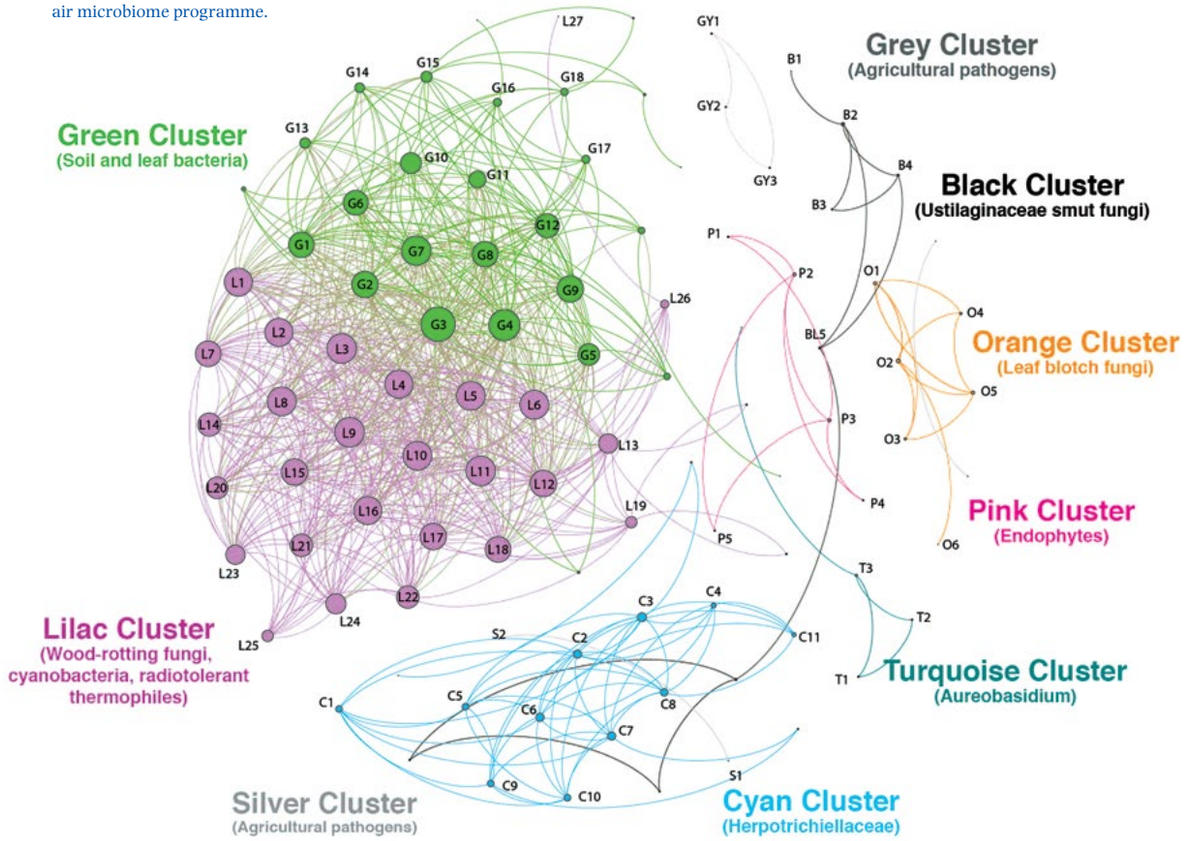
The analysis of biological data generated by modern sequencing and mass spectrometry systems is now routinely required in many research programmes in the life sciences but remains highly challenging. Such analysis is complex and time consuming, and requires a mix of deep biological or bioengineering insight, skills in scientific computing and a knowledge of data science (data analysis and statistics). As research questions often generate problems that require non-standard solutions, standalone bioinformatics software can easily become limiting, and a deeper, more rigorous exploration of the data is needed.

SCELSE has an Integrative Analysis Unit (IAU) to address these challenges, providing a hub for the analysis and interpretation of complex 'omics data sets generated within the centre. The philosophy underpinning the setup and operation of the IAU involved innovating at the boundary of research and service provision, in a manner attuned to the complexity and breath of SCELSE's overall mission.

It is well understood that bioinformatics service units are always challenging to operate and maintain, in part due to the open-ended nature of research activity that does not fit well with the notion of distinct, defined units of work. A deeper underlying problem is the often-blurred boundaries between what can be considered research and what can be routine, service activity, often exacerbated when disciplines interact.

Accordingly, the service component of the IAU focuses on providing bioinformatics supported in the areas of short read mapping, complex community analysis, 16S amplicon sequencing analysis and untargeted metabolomics data, with the focused objective of permitting research teams in the centre to move rapidly beyond dealing with large sets of raw FASTQ or mzXML files and start thinking about downstream analysis and interpretation.

Beyond this explicitly service-oriented activity, the first innovation was to implement a 'hub and spokes' model, whereby staff from other units within the SCELSE can be seconded to work within the IAU, with a view to gaining skills and deeper knowledge of bioinformatics. The second was the development of a series of training workshops to disseminate technical knowledge of bioinformatics methods, procedures and processes with the overall objective of broad 'up-skilling'. The workshops run for a duration of two to three days approximately twice yearly, with a mix of lectures and data-driven practical sessions using the R Statistical Computing Environment. Each workshop typically accommodates 25 to 35 participants, from within the SCELSE and partner institutions, including government agencies. Instructors are IAU staff including SCELSE visiting academic Dr Ezequiel Marzinelli (University of Sydney), a quantitative microbial ecologist; and visiting guest instructors, such as Dr Leo Lahti (University of Turku), a well-known expert in microbiome data science. An ongoing theme of the workshops was on the deep interconnections between experimental design, data analysis and data interpretation, thus providing a generic foundation for the conduct of well-designed, statistically rigorous laboratory and field studies.



Co-occurance network clusters of airborne microorganisms sampled as part of SCELSE's air microbiome programme.

The research activities of the IAU are focused on addressing some fundamental problems in complex microbial community analysis, using a mix of experimental and computational approaches, with a commitment to collaborative and translational research, including national and international collaborations and engagements with industrial partners. The IAU has pursued a core research programme based on the development and application of genome-resolved metagenomics and multi-omics, with the latter incorporating co-sampled genome-resolved metagenomics, metatranscriptomics and metabolome data. In recent years, the IAU has pioneered the use of long read metagenomics, and was the first to show that complete, closed genomes could be assembled from long read metagenome data, thus overcoming a long-standing bottleneck in genome-resolved analysis of complex microbial communities.

Singapore Phenome Centre

The Singapore Phenome Centre (SPC) supports research in the clinical, biological and environmental sciences. The research projects involve the study of phenotypes (the traits that are displayed) by profiling critical biomolecules such as metabolites and lipids. The tools available for such investigations include the state-of-the-art ultra performance liquid chromatography-mass spectrometry (UPLC-MS) and nuclear magnetic resonance (NMR) spectroscopy technologies.

SPC is a unit of the NTU Integrated Medical, Biological, and Environmental Life Sciences (NIMBELS) cluster. It is co-funded by Nanyang Technological University's Lee Kong Chian School of Medicine and the School of Biological Sciences (SBS), and SCELSE, in association with the National Phenome Centre at Imperial College London and Waters Corporation. SPC is an interdisciplinary research platform, focusing on the areas of microbiome health, nutritional metabolism and health, and aiming to enhance analytical capabilities to support research efforts and to deliver world-class metabolic phenotyping services to the NTU's scientific community, Singapore's researchers, and industries in Singapore and beyond.

SPC (www.ntu.edu.sg/spc) houses a suite of instrumentation to meet the varied needs of its interdisciplinary researchers, including:

Liquid chromatography-mass spectrometry (LC-MS)

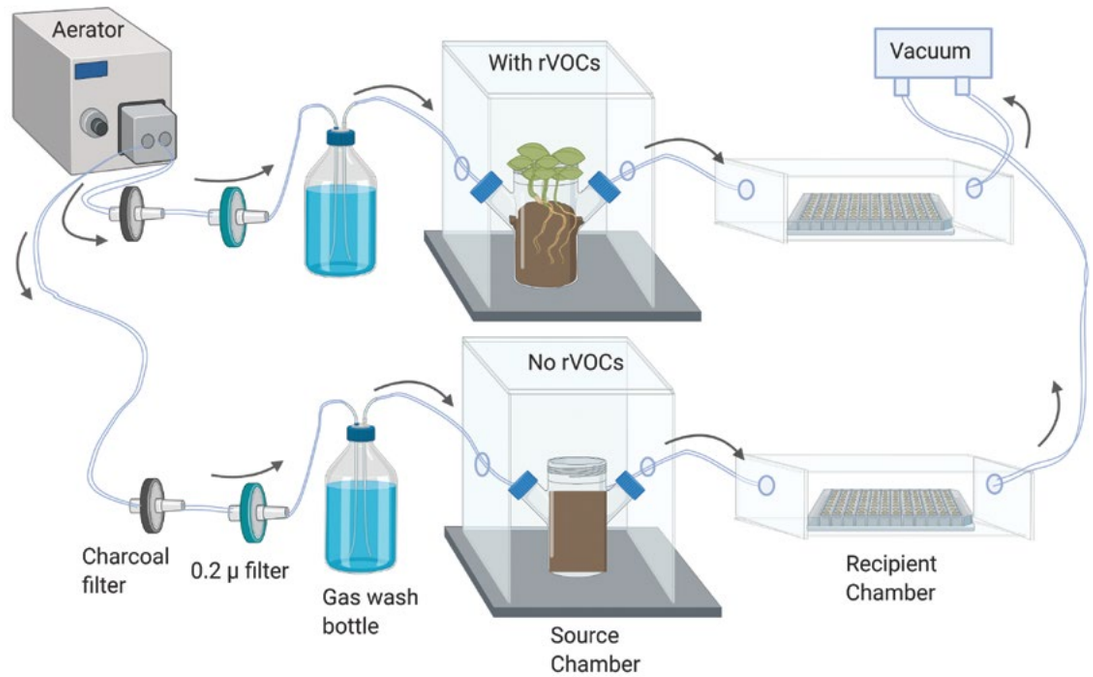
The LC-MS facilities at SPC include four high-resolution quadrupole/time-of-flight (QToF) mass spectrometers for untargeted profiling and biomarker discovery and two tandem quadrupoles (TQ) for quantitative targeted analysis of metabolites. SPC also has two SYNAPT high-definition mass spectrometry instruments with desorption electrospray ioniation (DESI) and matrix-assisted laser desorption ionisation (MALDI) mass spectrometry imagining capability.

Nuclear magnetic resonance (NMR) facility in SPC

SPC is the first centre in Singapore and Southeast Asia to be equipped with the *In Vitro* Diagnostics Research (IVDr) system. This 600 MHz NMR IVDr system can screen complex mixtures by 1H NMR for rapid identification and quantification of metabolites. The system also quantifies 112 lipoproteins, including major components of triglycerides, cholesterol, HDL-cholesterols, LDL-cholesterol, Apo-A1, Apo-A2 and Apo-B100.

Metabolomics, Experimental and Analytical Facilities

SCELSE collaborates with the NUS Environmental Research Institute (NERI), establishing high-throughput metabolomics capabilities to detect, analyse and identify thousands of small molecules and ascribe them to chemical classes and families. Highly complex chemical and metabolite mixtures in liquid or vapour phase can be deconvoluted and resolved to 10s of 1000s of individual chemical moieties. The analytical facilities are further supported by our data handling platforms utilising (1) automated workflows, (2) proprietary and in-house pipelines, and (3) metabolite repositories/database. Integrated workflows have been developed to detect volatiles and other relevant chemical classes from plant and environmental samples. A database has been created with information on more than 30,000 compounds, categorised into several chemical classes of biological relevance. A targeted metabolite panel for investigating plant-microbiome associations has been developed, along with a number of methods to analyse metabolites directly from samples.



Bespoke modular airflow system developed to study volatile-mediated interactions between plant roots and associated soil microbial communities.

Plant microbiome capabilities

A framework for rapid analysis of microbial products has been developed from microbes in diverse urbanised or natural terrestrial habitats. The framework includes various computational pipelines such as genomes from metagenomes assembly, hybrid genome assembly and microbial interaction prediction by genome scale metabolic modelling. An in-house repository houses about 150 bacterial isolates, out of which 50 have their whole genomes assembled. A catalogue of approximately 400 genes has been prepared for identifying plant growth-promoting bacteria and approaches to connect microbial and chemical ecology using biosynthetic gene clusters in phylogenomes. A database of about 7,000 plant-associated bacterial genomes has been created from public databases. Together, these databases and pipelines are currently providing insights about phytobiomes and how to reap benefits in diverse applications in fields, farms and urban greening.

Field-based capabilities – waterways

A wide array of field-based capabilities have been built, ranging from park soil, waterway sediments, greenhouse gas emissions, water and rhizosphere under the study of Plant-Air-Water-Soil-Sediment (PAWS2) axis of urban waterways ecosystems. These capabilities include bespoke workflows and SOPs for on-site field measurements, field sampling, sample storage and transportation. They are further supported with the use of field equipment such as flow meters, water quality probes, soil moisture meter, carbon dioxide analyser and field sensors to acquire real-time data. Custom-developed reactors allow the team to translate field observations into mesocosm experiments to test the findings.

Plant-biofilm relationships

A novel modular airflow system was developed to study volatile-mediated interactions between biological systems, for example, the effect of plant root volatiles (rVOCs) on a soil microbial community. In this system, sterile air is forced through the root-soil system using an aerator and drawn into another chamber using a vacuum. A variety of plant species can be tested in the source chamber that can be scaled for plant size. This setup also allows vapour-absorbing cartridges to be attached, thus providing VOC mixtures to be analysed using mass spectrometry.

Plant-microbiome relationships

A custom-made mesocosm system, named Live-Exudation Assisted Phytobiome Cultromics System (LEAP-CS), is driven by ecological principles governing the interactions within the complex phytobiomes. The system is designed to (1) simulate native associations between the plant hosts and its associated microbiomes; (2) maximise metabolic exchange and chemical dialogue to enhance processes underlying plant-microbe interactions; and (3) enable concurrent data collection of plant phenotypic/molecular traits, microbiome composition and interacting metabolites. It is a modular system that supports (1) easy manipulation of the microbiome communities; (2) chemical complementation assays; (3) different biological and analytical platforms for phenotyping; and (4) non-invasive *in situ* growth profiling.

St John's Island National Marine Laboratory

St John's Island National Marine Laboratory (SJINML) (sjinml.nus.edu.sg) was established in 2016 as a National Research Infrastructure (NRI) to support strategic marine science research in Singapore. Funded by the National Research Foundation, SJINML supports multidisciplinary and multi-institutional marine science research.

Pertinent to SCELSE's research, the marine station includes a state-of-the-art climate-controlled aquaria biosafety-level-2 facilities to support upcoming disease- and pathogen-focused research and a 10 m seawater current flume to aid studies in marine hydrodynamics. The station is also equipped with other indoor and outdoor aquaria with fibreglass tanks of various sizes, and laboratories that enable researchers to conduct analytical chemistry, biomolecular work or take high-resolution bio-imagery.

SCELSE is an active stakeholder of the marine station, in collaboration with other local marine science experts, for example, to study the coral reef microbiome's response to environmental changes, microbial community compositions and function on ecologically engineered seawalls, and to study microbe-sediment interactions in marine flume facilities.

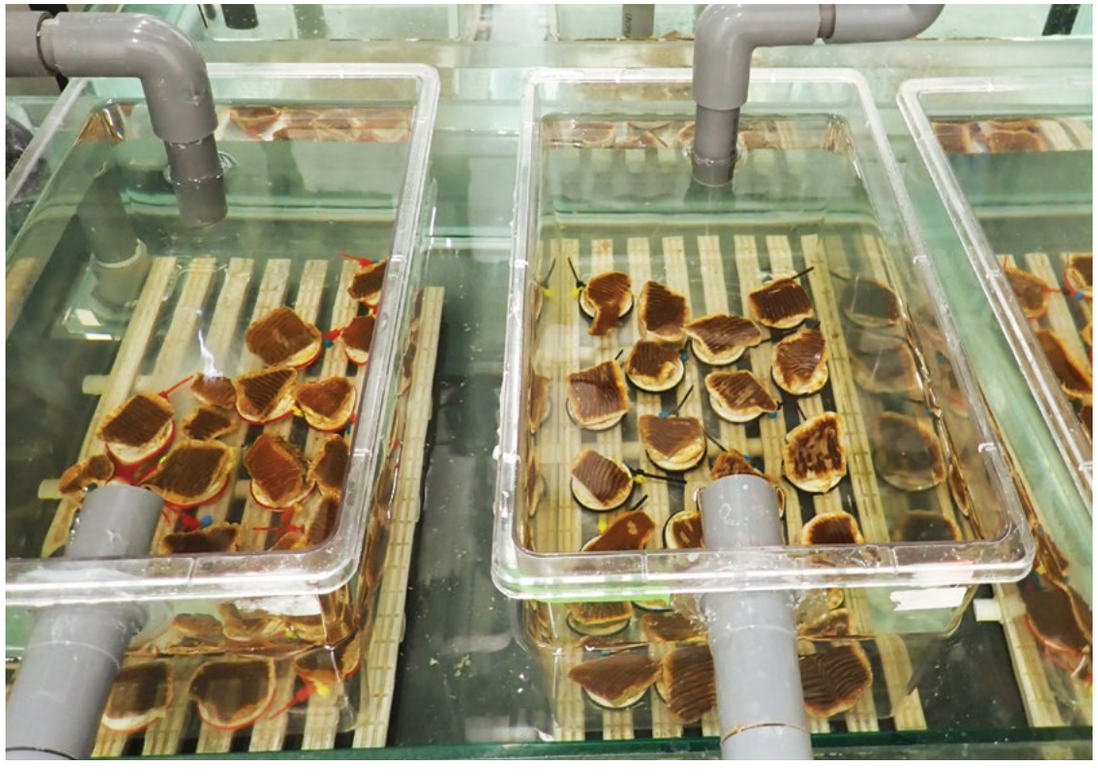


Figure 1: Investigating the coral microbiome response to environmental stress at SJINML. *Image credit: Lindsey Deignan, SCELSE*

SCELSE international Summer Course students.

EDUCATION, TRAINING AND OUTREACH

Mentorship and training

SCELSE as a training ground for research and professional careers

Over the past decade, 50 PhD candidates have graduated and more than 130 post-doctoral researchers have been trained at SCELSE, with about 80 having completed their appointments. More than half of SCELSE's former post-doctoral researchers have taken up positions at universities or research institutes around the world and in Singapore, with the remainder entering private industry as research scientists. SCELSE PhD alumni predominantly take up research positions in universities or research institutes, also in different locations around the world.

The course is open on a competitive basis for graduate students or graduates in engineering/sciences including life sciences, chemical or physical sciences. Placement in the course for local graduate students (in NTU and NUS) and external applicants are competitive, with restricted number of places. The class size is approximately 30 to 35 students.

Undergraduate education and training

SCELSE has broad-ranging education and mentorship programmes designed to equip the next generation of biofilm and microbiome researchers. The centre engages in both classroom and practical/laboratory teaching across the scope of its multidisciplinary programme, providing students with the guidance and training to tackle future complex research questions.

SCELSE scientists contribute actively and extensively in both research and teaching at the undergraduate level in the schools and departments of NTU and NUS. Significantly, SCELSE members have expanded the scope of undergraduate courses available at both universities, to accommodate the broad and multidisciplinary nature of comprehensive microbiology.

These courses draw on the knowledge and experience of multiple expert researchers in their various fields from within and outside SCELSE, and serve to build a strong foundation of upcoming biofilm and microbiome scientists and engineers.

Final year project

A strong culture exists in SCELSE to support undergraduate researchers by hosting Final Year Projects (FYP) from NTU and NUS. Since 2011, more than 180 students from multiple schools and departments have undertaken their FYP projects in SCELSE. This experience has been rewarding for SCELSE investigators and research fellows. These students not only gain exposure to active research and work with top scientists, but they also become well-positioned to make informed choices about developing a career in the domain of environmental life sciences engineering (ELSE) by pursuing a PhD. While SCELSE supervisors gain insights into the suitability and motivation of potential students for future studies, undergraduate researchers are directly involved in publications, which testifies to their level of work and achievement. Additional undergraduate experience and training provided by SCELSE include research attachments under several campus, polytechnic and internship programmes.

Postgraduate education and training

SCELSE's postgraduate student training covers the full scope of ELSE at the PhD level. To enable this, a postgraduate programme was established when SCELSE began, to function across both NTU and NUS, and select high-calibre candidates to be supervised by senior SCELSE personnel, with regular monitoring of their progress.

The postgraduate programme combines a strong foundation in core disciplines with experiential learning in multidisciplinary research. Training in core disciplines is deeply anchored in and integrated with courses from NTU and NUS. This tight integration with multiple academic units is evidenced in the range of the courses adopted by SCELSE and the host departments and schools of graduate research supervisory team members.

International summer course

The annual international SCELSE Summer Course (Fundamentals of Environmental Life Sciences Engineering) has brought together world-renowned scientists and engineers, including SCELSE scientific leaders, and guest experts since the centre began in 2011, acquainting participants with the key principles underlying environmental life sciences and engineering.

Convocation 2015: SCELSE PhD graduate Dr Martin Tay (second left) celebrating with Final Year Project students. SCELSE researchers (left to right): Shifana Raja Abdeen (SCELSE FYP), Dr Martin Tay (SCELSE PhD), Ru Ying Lai (SCELSE FYP), Thow Xin Qiang (SCELSE FYP) and Muhammad Hafiz Bin Ismail (then SCELSE PhD student).

When SCELSE was established, there was a need to promote an understanding of comprehensive microbiology in light of two broad emerging trends. (1) The field was shifting in research focus away from population-based studies in acknowledgement that microbes in nature do not exist as single cell, free living planktonic organisms. Instead, microorganisms predominantly assemble as dynamically structured communities of multiple species at interfaces; they are anchored by extracellular polymeric substances and collectively known as biofilms. (2) Classical microbiology training is based on knowledge of microbes that can be cultured, although it is now widely accepted that many remain uncultured. Hence, studying multicellular biofilms needs new research, teaching and learning approaches. The SCELSE Summer Course was introduced in response to these shifts in focus and continues to provide up-to-date training for the next generation of environmental life sciences and engineering experts.

The course provides a comprehensive overview of basic biofilm concepts and application of biofilm technologies. Participants interact with experienced mentors in their interdisciplinary approach to understand and harness the biology, physics and chemistry of biofilms in environmental, public health and engineered settings.

The course is open on a competitive basis for graduate students or graduates in engineering/sciences including life sciences, chemical or physical sciences. Placements in the course for local graduate students (in NTU and NUS) and external applicants are competitive, with a class size of 30 to 35 students.

SCELSE in local and international translational landscapes

Singapore National Biofilm Consortium (SNBC)

Biofilms are an important part of the global challenges faced by society today, with economic and societal impact, from food and water safety to antimicrobial resistance. The estimated economic impact of biofilms across industry sectors is US$4 trillion to US$5 trillion (NBIC 2022). In healthcare, the direct impact of biofilms is close to US$400 billion, which represents about 5% of global health expenditures (NBIC 2022). Understanding biofilms requires a multidisciplinary approach with researchers from divergent fields.

Novel microbial biofilm and microbiome technologies harness the potential of microbial biofilms and microbiomes in natural, engineered and public health systems for ensuring clean water, a sustainable environment and disease management. To facilitate the translation of such R&D, the Singapore National Biofilm Consortium (SNBC) was established in 2019 to connect biofilm and microbiome researchers with industry, agency and institutes of higher learning (IHLs) across Singapore.

> "Fostering collaborations between institutional research and industry, and enabling technology translation in biofilms and microbiomes."

The SNBC, an NRF technology consortium administered through NTUitive, NTU's technology transfer office, and hosted at SCELSE, is driven by SCELSE's translational capacity and multidisciplinary research on microbial biofilm biology and ecology and microbiology. It serves as a coordinating platform at the convergence of health, engineering, technology and science, that integrates innovation and business to address emerging challenges across diverse industries throughout Singapore. SNBC fosters collaborative research and translational projects involving academics from different IHLs, local agencies and industry partners.

In addition to accessing research expertise and networks, consortium members can participate in training and workshops, and are eligible for seed funding for technology development and applications to foster collaboration and translational innovation between academia and industry. To anchor Singapore as a significant player in addressing the growing global economic impact of biofilms, SNBC is working closely with SCELSE's international network – National Biofilms Innovation Centre in the UK; Costerton Biofilm Center in Denmark; and the Center for Biofilm Engineering, in the US, and their industry partners.

To foster academic-industry R&D, SNBC has granted seed funding for industry-academic research collaborations in domains encompassing water and aqua-technologies, surface technologies, nutrition and bioremediation.

To date, the SNBC has attracted 25 industry members and more than 10 institutional and academic members, which have access to SNBC seed funding and biofilm analysis facilities, participation in conferences, seminars, courses and workshops, student industry attachment programmes, advice and consultancy on technical queries, and network connections to a global network of leading biofilm research centres and industry collaborators.

SNBC partnering with NBIC

SNBC and NBIC signed an MOU in 2019 to foster interactions between the two organisations. NBIC has an extensive network of academic and industry partners across the UK, focusing on surface and materials design, characterisation, imaging, nanofabrication and modelling, and R&D translation into industry and healthcare settings, complementing and supporting the capacities available in Singapore.

SCELSE IN PICTURES

2011

From left: Prof. Yehuda Cohen (SCELSE deputy centre director), Prof. Alexander Zehnder (chair, SCELSE SAB); Prof. Staffan Kjelleberg (SCELSE centre director); Mr Peter Ho (chair, SCELSE GB); Ms Grace Fu (GOH; senior minister of state MND, MOE); Mrs Tan Ching Yee (permanent secretary, MOE); Dr Francis Yeoh (CEO, NRF); Prof. Freddy Boey (provost, NTU); Prof. Barry Halliwell, (deputy president, NUS). NB: the position titles are as per September 2011.

2012

Singapore President, Dr Tony Tan (centre) visited SCELSE in 2012 for an update on the newly opened centre's progress, including the sequencing facilities, guided by Prof. Stephan Schuster (Director, Meta'omics and Microbiomes cluster, SCELSE).

2013

SCELSE centre director, Prof. Staffan Kjelleberg, promoting environmental research for NTU.

2014

Raising awareness of the newly formed *npj Biofilms and Microbiomes* journal, a partnership between Nature Partner Journals (NPJ), Nanyang Technological University, Singapore and SCELSE. From left: Ms Poojah Aggarwal (NPJ), Prof. Yehuda Cohen (SCELSE deputy director), and Martin Delahunty (NPJ).

2015

The NTU Integrated Medical, Biological and Environmental Life Sciences (NIMBELS) cluster is launched to promote life sciences partnerships across NTU. From left: Prof. Staffan Kjelleberg (director, SCELSE), Prof. Bertil Andersson (President, NTU), Prof. James Best (Dean, LKCMedicine), and Prof. Peter Preiser (School of Biological Sciences Chair).

SCELSE Scientific Advisory Board and Research Directorate members during the 2015 SAB meeting.

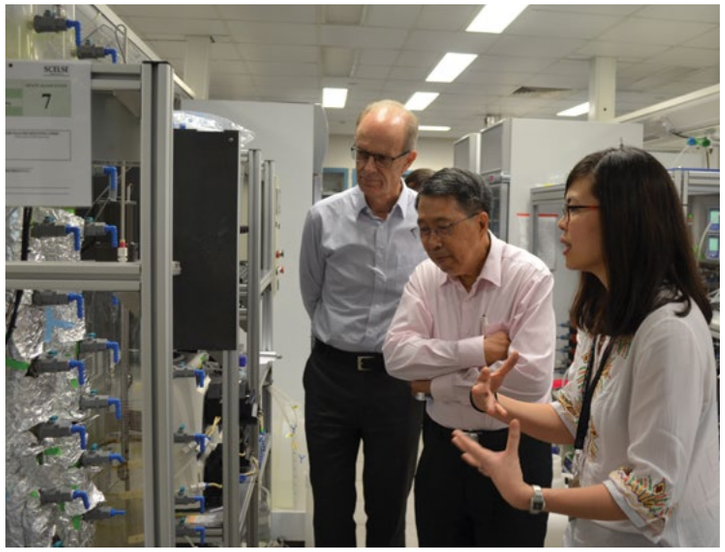
2016

SCELSE's football team made it to the semifinals in the NTU Staff Games 7-a-side football tournament.

SCELSE research fellow Dr Law Yingyu explaining the centre's bioreactor facilities to students from Nanyang Girls High, Singapore. SCELSE regularly hosts outreach education events.

SCELSE industry outreach and networking reception, held at the Life Sciences Institute, National University of Singapore.

2017



NTU President Emeritus Prof. Su Guaning was a key driving force behind the formation of SCELSE during the Research Centre of Excellence grant application process. He is pictured (centre) during a tour of the centre's bioreactor facility, with centre director Prof. Staffan Kjelleberg (left) and research fellow Dr Law Yingyu (right).

SCELSE co-hosted the Nature Conference "Environmental and Human Microbiomes: Drivers of Future Sustainability", at NTU Singapore, February 2017. Prof. Alexander Zehnder (then Chair of NTU's Sustainable Earth Office) speaking at the opening session.

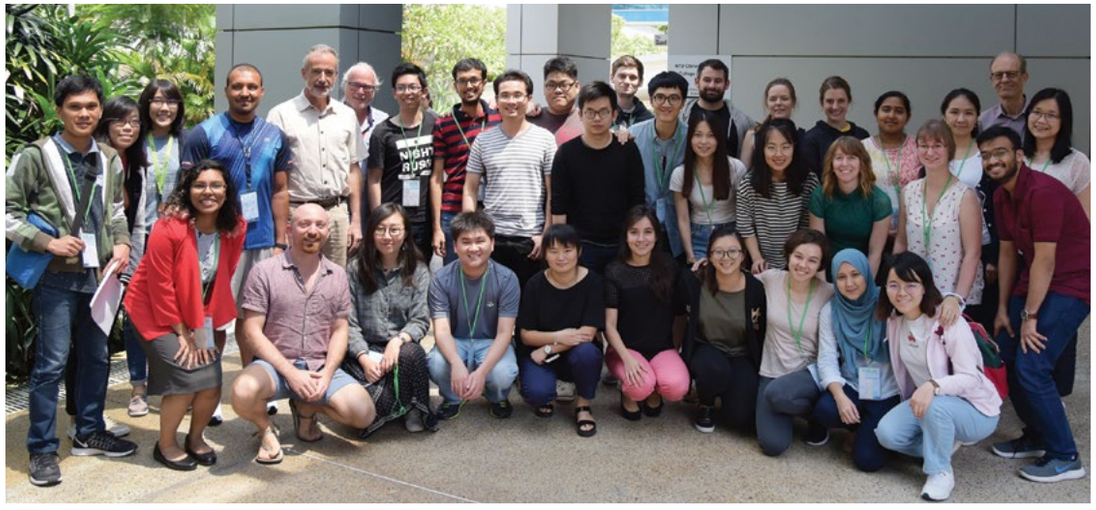
Prof. Staffan Kjelleberg presents outgoing NTU President Prof. Bertil Andersson with a farewell gift in appreciation for his support of SCELSE from the centre's earliest conception.

Singapore Deputy Prime Minister and Chairman of the National Research Foundation Singapore Mr Teo Chee Hean (centre) discussing SCELSE's sequencing capacity with High-throughput Sequencing Facility manager Dr Daniela Drautz-Moses.

2018

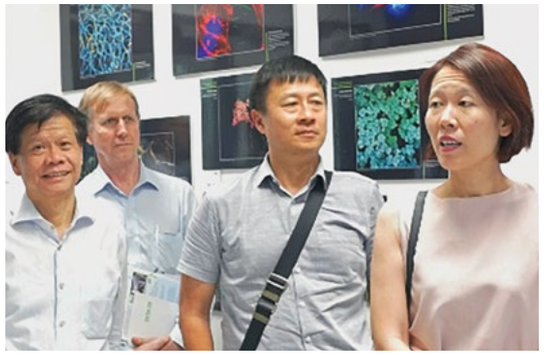
SCELSE and the National Biofilms Innovation Centre (NBIC), UK signed a memorandum of understanding in 2018, for research, academic and education collaborations between the two centres. Signing the MOU (from left): Dr Mark Richardson, CEO, NBIC, Prof. Lam Khin Yong (Senior Vice President (Research), NTU), and Prof. Staffan Kjelleberg (centre director, SCELSE).

NTU President Prof. Subra Suresh (right) in SCELSE's Bioreactor Facility during a familiarisation tour of the centre, pictured with (from left) Dr Samarpita Roy (SCELSE) and Dr Ezequiel Santillan (SCELSE).



Since 2011, SCELSE has welcomed postgraduate students and instructors from around the globe as part of its annual Summer Course, creating an international network of young environmental life scientists and engineers. The 2018 Summer Course participants are pictured here.

2019



Singapore's Second Permanent Secretary, Ms Lai Wei Lin, visited SCELSE, May 2019. From left, touring the Advanced Biofilm Imaging Facility with Prof. Lam Khin Yong (NTU), Prof. Timothy White (NTU), and Mr Ong Tze-Ch'in (MOE).

SCELSE's annual Open House showcases the centre's research and facilities to university and general public audiences. PhD student Zeus Nair (right) explaining medical biofilm research to prospective students.

Singapore National Biofilm Consortium's (SNBC) inaugural seed fund information session, attended by the consortium's industry and academic members.

2021

The 2021 launch of the L'Oréal-SCELSE joint laboratory, the culmination of a long-standing skin microbiome research collaboration. From left: Dr Viduthalai Rasheedkhan Regina (SCELSE), Prof. Staffan Kjelleberg (SCELSE), A/Prof. Scott Rice (SCELSE), Dr Mark Phong (L'Oréal Asia), Dr Tarun Chopra (L'Oréal Singapore).

2022

SCELSE and SNBC co-hosted a Procter & Gamble (P&G) delegation in 2022 to discuss research collaborations. Standing: Prof. Stephan Schuster (SCELSE); P&G representatives seated front row from right: Yuko Nakamura (Vice President, R&D, Singapore Innovation Centre, P&G); Andrew Weatherston (Senior Vice President, R&D, P&G Hair Care & Asian Innovation); and Victor Aguilar (Chief R&D and Innovation Officer, P&G).