SCIENTIFIC PROGRAM

Asia-Pacific Biofilms 2022

October 18-23, 2022 | Guangzhou, China

On behalf of the Organizing Committee, you are cordially invited to attend the virtual conference of the 4th International Conference on Biofilms (Asia-Pacific Biofilms 2022), held on October 18-23 of 2022, in Guangzhou, China.

This conference aims to bring together leading academic scientists, engineers, and clinicians globally, primarily from the Asia-Pacific area including China and Singapore from Asia, Australia and New Zealand from Oceania, United States and Canada from America, and many other countries/regions to share new knowledge and research progresses in microbial biofilms. Scope of APB 2022 includes molecular biology of biofilms, quorum sensing, industrially and clinically relevant biofilms and emerging technologies for biofilm mitigation. This conference will serve as a major platform that create collaborative opportunities for biofilm researchers in the Asia-Pacific area, and to facilitate our interactions with colleagues from Europe (Euro Biofilms) and the United States (ASM Biofilms). For the first time, APB will be organizing a signature program for the conference. The signature program for APB 2022 is Biofilms in Australia, from Biomaterials-Microorganism Interface to Recalcitrant Infections, co-organized by the Department of Infectious Diseases, the Alfred Hospital and Monash University.

Highlighted topics include:

1. **Bioinformatics analysis in biofilms**
2. **Biofilms development and control**
3. **Biofilms antimicrobial resistance**
4. **Communication and signaling factors in biofilms**
5. **Rapid detection and application to biofilms and microorganisms**
6. **Virulence and toxins on clinical biofilms**
7. **Evolution and stress tolerance in Biofilms**
8. **Industrial and applied biofilms research**

The Organizing Committee are making every effort to make this a memorable and valuable biofilm conference.

Sincerely yours,

Birthe Kjellerup
Liang Yang
Yue Qu
Zhenbo Xu
The Organizing Committee

Organization

Organizers

South China University of Technology

Southern University of Science and Technology

The Singapore Centre for Environmental Life Sciences Engineering (SCELSE)

Department of Infectious Diseases (DID), the Alfred Hospital and Monash University

Co-Organizers

Academic Exchange Information Centre (AEIC)

Supporting parties

ESCMID Study Group for Biofilms

China Society for Microbiology (CSM)

Overseas Chinese Society for Microbiology (SinoMicro)

ELSEVIER

Microbiology Australia (The official journal of Australian Society for Microbiology)

Monash-WMU Alliance

Organizing Committee

Founder and Honorable President

Mark Shirtliff

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Founder and Honorable President

Mark Shirtliff

(1969-2018)

Dr. Mark Shirtliff was a professor at University of Maryland-Baltimore, where he held a primary appointment in the Department of Microbial Pathogenesis in the University of Maryland School of Dentistry and a secondary appointment in the Department of Microbiology and Immunology in the University of Maryland School of Medicine. Mark was also the lead inventor and co-founder of the vaccine company Serenta Biotechnology, LLC that was established in 2017. The license is based on a multivalent vaccine strategy against infections caused by *Staphylococcus aureus*. Further development of the vaccine is continued by Dr. Jan Harro in the Shirtliff-Harro Lab at UMSOD and by Birthe Kjellerup-Shirtliff as Chief Scientific Officer in Serenta LLC.

Mark Shirtliff was a leading expert in the field of biofilm in the US and internationally. His childhood in the foothills of the Canadian Rocky Mountains lead him to University of Alberta, where he graduated with a bachelor in Geo-microbiology. After this, he moved to Texas, US to continue his education. Originally Mark wanted to pursue medical school to become a medical doctor, but he quickly learned that he over time would be able to help more people by performing research thus his goal became to develop diagnostics and vaccines to prevent biofilm infections. Mark graduated with his Ph.D. in 2001 from University of Texas Medical Branch, Galvaston TX in the Department of Microbiology and Immunology. His thesis was titled "*Staphylococcus aureus*: Roles in Osteomyelitis."

During graduate school Mark was introduced to a fellow Canadian biofilm researcher Dr. Bill Costerton, who at that time was the Director of Center for Biofilm Engineering (CBE) at Montana State University in Bozeman, Montana. This was the beginning of an inspirational work relationship and friendship between Mark and Bill and a very prolific career in *Staphylococcus aureus* biofilm

research for Mark-but it was way too short!

Bill convinced Mark that she should move to Bozeman, Montana and the CBE in 2001 to continue working on biofilms and was initially funded to work on drinking water biofilms in Dr. Anne Camper's lab. He quickly obtained his own funding and returned to S. aureus research that was so important for him. The years at the CBE were instrumental in developing molecular tools, having important biofilm centered discussions and to get out in the wilderness to get great ideas. In 2003, Mark moved to Maryland and entered a tenure track position at UMB-Baltimore.

Mark was actively involved in leading the biofilm field forward. His engaging and very energetic way of behaving made him easy to talk to both about biofilms, science and completely other topics. He was very interested in listening to the junior scientists and to connect with the next generation in science. Therefore, mentoring and training of the next generation of biofilm scientists was a mission that he took seriously. Over the years, Mark trained many scientists in his lab-both graduate students, post docs and visiting scientists from around the world. He also initiated many collaborations globally and many of these excellent scientists are present at ChinaBiofilms 2019. He also organized numerous biofilm workshops at international conferences such as ASM Biofilms (American Society of Microbiology), ECCMID (European Congress of Clinical Microbiology & Infectious Diseases), EuroBiofilms and of course ChinaBiofilms 2017. Over the years, Mark was the author of more than 120 peer-reviewed scientific papers and book chapters on pathogenic microorganisms. He explored the biofilm mode of growth and the chronic diseases they cause.

Mark never forgot his Canadian roots and was a proud and energetic hockey (i.e. ice hockey) fan, who would travel far distances to cheer on his favorite team Edmonton Oilers. He also loved to take his family back to the Canadian Rockies to climb on glaciers and to tell great stories from his childhood and about interesting tree-ring counting studies and field trips as a geology major in college. He also kept in touch with colleagues at the CBE, which allowed him (and his family) to spend time in Montana during the summers. This would recharge his batteries with lots of fly fishing, floats on majestic rivers and good times with friends and family.

In Mark's honor, we have started the "Mark Shirtliff Memorial Biofilm Foundation" (https://markshirtliffbiofilmfoundation.org/). Donations can be made via the website. The goal of the foundation is to support and encourage junior biofilm researchers to travel and initiate collaborations with other biofilm groups on a global scale.

The Board of Trustees include several members, who are present at ChinaBiofilms 2019: Birthe V. Kjellerup, Chair (Mark's wife), Garth D. Ehrlich, Secretary and Treasurer, Kendra Rumbaugh, Vice President, James Kaper, Zhenbo Xu and Annette Moter. Please feel free to contact any of us if you have any questions or would like to share a good story or memory about Mark.

We hope that you will participate in making Asia-Pacific Biofilms 2022 a successful follow-up to the China Biofilms series conferences that Mark was an important founder for.

South China University of Technology

South China University of Technology (SCUT) is a leading educational institution in China, a public research-intensive university directly governed by the Chinese Ministry of Education. Located in Guangzhou, the center of southern China, it covers a total area of 405 hectares, consisting of three campuses: Wushan Campus, University Town Campus, and Guangzhou International Campus.

SCUT was first founded in 1952 by merging the engineering schools and departments of a number of major universities and polytechnic universities from five provinces in central and southern China. In 2016, SCUT was ranked the world's top 300 universities by the Academic Ranking of World Universities, with its Engineering at 22nd. According to Thomson Reuters' Essential Science Indicators, SCUT has chemistry, materials science, engineering, agricultural science, physics, biology and biochemistry, computer science, and environment and ecology ranked in the global top 1%.

School of Food Science and Engineering (SFSE) was established newly in November 2015 through the reorganization of the School of Light Industry and Food Science, however, its history can be dated back to 1952, the beginning of the University. A national evaluation of key discipline carried out in 2012 showed that the Food Science and Engineering in the School were ranking No.3 among Chinese universities.

Southern University of Science and Technology

Southern University of Science and Technology (SUSTech) is a research-oriented public university founded in Shenzhen, China's innovation center. From their inception, SUSTech has

attached enormous importance to attracting high-quality talents to its faculty. Through continuing efforts to introduce world-class educators, SUSTech is proud to have over 800 faculty members on staff, and they continue to expand its faculty with amazing talent from all over the world. Many of their faculty have been honored with selection into national or regional talent programs, and SUSTech is keen to nurture and cultivate its talented staff.

The Singapore Centre for Environmental Life Sciences Engineering (SCELSE)

SCELSE

Singapore Centre for Environmental Life Sciences Engineering

The Singapore Centre for Environmental Life Sciences Engineering (SCELSE) is a unique interdisciplinary Research Centre of Excellence (RCE), funded by National Research Foundation, Singapore Ministry of Education, Nanyang Technological University (NTU) and National University of Singapore (NUS). Hosted by NTU in partnership with NUS, SCELSE is linking new insights from the life sciences with expertise from the emerging technologies in engineering and natural sciences to understand, harness and control microbial biofilm communities and microbiomes. The union of these fields has established a new discipline of environmental life sciences engineering (ELSE). SCELSE mission is “To discover, control and direct the behavior of microbial biofilm communities and microbiomes for sustainable environmental, engineering, public health and medical applications.”

SCELSE research focuses on the universality of microbial biofilm communities. Unravelling microbial biodiversity and function in complex microbial communities enables SCELSE researchers to identify key mechanisms involved in biofilm biology. The exploratory power available to SCELSE researchers, from laboratory-scale to full-scale environmental, medical and engineered systems, combined with an unrivalled level of interdisciplinary expertise places SCELSE in a unique position, to deliver a comprehensive understanding of all aspects of a microbial system.

SCELSE is deciphering the biology of microbial biofilm communities and microbiomes in environmental and engineered systems. Importantly, the use of new molecular tools (genomics, proteomics, and metabolomics) for prospecting biofilms will demonstrate communal metabolic capacity and diversity, far surpassing the combined activities of individual member species. Moreover, obtaining high-resolution information from huge multi-layered databases, now possible through significant advances in analytical, bio-informatics, and bio-computational tools, will facilitate our understanding of community behavior in complex natural and engineered habitats.

Information gained on basic mechanisms of microbial community signaling interactions at micro-scales will be evaluated, integrated and quantified in large-scale experiments. Since microbial interactions with the environment are governed by surface chemistry, SCELSE's approach also accommodates the merging of nano-technological tools. Ecological theories that link natural processes at these different scales predict biofilm community behavior in the face of environmental stresses.

Department of Infectious Diseases (DID), the Alfred Hospital and Monash University

MONASH University

The Department of Infectious Diseases (DID)

Monash is a modern, global, research-intensive university, delivering education and research excellence in Australia and across the Indo-Pacific. We're making a positive impact on today's global challenges – whether that's by mitigating climate change, easing geopolitical insecurity or fostering healthy communities.

The Department of Infectious Diseases (DID) integrates clinical services with biomedical research and teaching. With researchers in Alfred Health's Infectious Diseases Unit, our expertise spans general infectious diseases to HIV/AIDS and tuberculosis. The Department of Infectious Diseases is a premier centre for clinical and biomedical research and education, offering undergraduate and postgraduate study programs. We integrate clinical services with clinical and basic science research. The clinical services work closely with research staff and laboratories are based within the Burnet Institute building, with a presence within the Central Clinical School.

AEIC Academic Exchange Information Center

AEIC Academic Exchange Information Center, also known as AEIC, is a well-developed international exchange platform co-founded by colleges, scientific research institutions and enterprises. We concentrate on global professional academic forces and devotes to the academic exchange activities such as scientific and technological information dissemination, scholars scientific research exchanges and social hotspots analysis. Now we have received big support from many colleges and research institutes. Adhering to the spirit of professional, focus and concentrate, we provide an international professional exchange platform for scientific and technological academic communication to realize the transformation of academic achievements.

AEIC cooperates with many international presses including Springer, Elsevier, IEEE, Taylor & Francis Group, IOP, EDP, ASME, SPIE, Academic Press, American Scientific Publishing, DEStech Publications, TTP and Atlantis Press. AEIC calls for papers from academic conferences and publishs papers for EI or CPCI index. Outstanding papers will be recommended for publication in well-known international journals such as the ones indexed by SCI, EI, etc.

ESCMID Study Group for Biofilms (ESGB)

The objective of ESGB is to increase knowledge on various aspects of microbial biofilms with as ultimate goals improved diagnostic tools for biofilm infections, and better approaches to prevent and treat such infections. In order to obtain these goals, a multidisciplinary approach is necessary and one of the objectives of the ESGB to facilitate cooperation between scientist working on biofilms in different disciplines.

Chinese Society for Microbiology (CSM)

Chinese Society for Microbiology (CSM) is a national, academic and public welfare legal person social organization voluntarily formed by National Microbiology scientific and technological workers and units and registered by the Ministry of civil affairs of the people's Republic of China according to law. It is a non-profit social organization and a social force for the development of Microbiology in China. The Chinese Society for microbiology was established on December 18th, 1952. As early as 1928, initiated by Wu Liande, Xie Heping and Lin Zongyang, pioneers of modern medicine in China, the Chinese society of microbiology was established in Beijing. In 1937, it was renamed the Chinese society of pathology and microbiology, and moved to Shanghai. It has more than 50 members and held academic seminars. In 1945, the conference was held in Guangzhou, attended by more than 100 people. After the founding of new China in 1949, the Chinese society of microbiology was established at the capital assembly of the Chinese Medical Association in 1950. This is the gestation stage before the official establishment of the Chinese society of Microbiology in 1952.

Overseas Chinese Society for Microbiology

海外华人微生物学会

Overseas Chinese Society for Microbiology

Overseas Chinese Society for Microbiology (Sino-Micro) is a registered non-for-profit organization formed by overseas Chinese researchers who study microbiology. Our goal is to establish a social network that will facilitate the advancement of our research programs and the development of our careers. In addition, we wish to work as a group to create a platform for enhancing scientific interactions with our colleagues in China. Current Sino-Micro members are primarily principal investigators in the USA. However, our organization is open to all overseas Chinese microbiologists.

Microbiology Australia (The official journal of Australian Society for Microbiology)

Microbiology Australia, the journal of the Australian Society for Microbiology, is produced online and in print four times a year. The journal contains scientific papers, technical notes, book reviews, conference information, data on new products and services in microbiology, and material for tertiary students, in addition to providing detail on ASM activities. Microbiology Australia comprises mostly thematic issues focused on the areas of greatest importance to microbiology. Themes are determined by the Editorial Board and Guest Editor(s), with the exception of a biennial issue that features Breaking Research of ASM's Early Career Researchers.

The Australian Society for Microbiology (ASM) is a not-for-profit organisation, formed in 1959 as a learned society devoted to furthering the science of microbiology. In 1976, the ASM became an incorporated professional society, and has a membership approaching 2000. The society functions in "bringing microbiologists together" with the objective of advancing the science of microbiology in Australia.

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Elsevier as a global leader in information and analytics, Elsevier helps researchers and healthcare professionals advance science and improve health outcomes for the benefit of society. We do this by facilitating insights and critical decision-making for customers across the global research and health ecosystems. In everything we publish, we uphold the highest standards of quality and integrity at scale to ensure value to our customers.

Antibiotics

antibiotics
an Open Access Journal by MDPI

Antibiotics is an international, peer-reviewed, open access journal published online by MDPI, Basel, Switzerland. The scope of Antibiotics includes but is not limited to pharmacodynamics, uses of antibiotics, antimicrobial stewardship, antibiotic resistance, and novel antimicrobial agents. The journal's Impact Factor is 5.222 (2021), ranking Q1 in 'Pharmacology & Pharmacy" in JCR.

Membranes

membranes
an Open Access Journal by MDPI

Membranes (ISSN 2077-0375) is an open access journal with its latest impact factor of 4.562, ranking 21/88 (Q1) in Polymer Science and Q2 in Chemical Engineering (miscellaneous). It provides an interdisciplinary forum for publishing papers which advance the fields of: Membrane Processing and Engineering, Membrane Applications, Biological Membrane Functions, Biological Membrane Dynamics and Computation, Biological Membrane Composition and Structures, Biofilms.

Agenda

Time and date shows here refers to China Standard Time (GMT+8).
Asia: GMT+9 for JST, GMT+7 for WIT, GMT+5:30 for IST
Oceania: GMT+10 for AEST, GMT+12 for NEST
U.S. and Canada: GMT-4 for EDT, GMT-5 for CDT, GMT-6 for MDT, GMT-7 for PDT
Europe and U.K.: GMT+2 for CEST, GMT+1 for BST

Oct 18th Registration	
16:00-18:00	**Registration and Meeting platform test**
Oct 19th Workshop	
8:00-9:30	**Animal Models in Biofilm Research** **Modeling biofilm-associated wound infections** **Kendra Rumbaugh, Texas Tech University, Lubbock** **Animal models of orthopedic infection** **Janette Harro, University of Maryland, Baltimore**
9:30-10:00	**Meet the speakers / Coffee break**
10:00-11:30	**Control strategies for bacterial biofilms – the need for standard methods?** **Why the need for standard methods?** **Paul Stoodley, The Ohio State University, Columbus** **Standardized Biofilm Methods** **Kelli Buckingham-Meyer, Montana State University, Bozeman** **Statistical considerations in image analysis** **Albert Parker, Montana State University, Bozeman**
11:30-15:00	**Meet the speakers 11:30-11:45 / Lunch 11:45-14:00**

15:00-16:00	**Construction of microbial biofilms and detection- How to do it in a correct way?** Yulong Tan, Qingdao Agricultural University, Qingdao Zhenbo Xu, South China University of Technology, Guangzhou Renyou Gan, Singapore Institute of Food and Biotechnology Innovation, Singapore Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou Xuejie Li, South China University of Technology, Guangzhou
16:00-16:30	Meet the speakers / Coffee break
16:30-17:30	**Biofilms mediated infection- Difference between *in vitro* and *in vivo*** Yulong Tan, Qingdao Agricultural University, Qingdao Ke Wang, First Affiliated Hospital of Guangxi Medical University, Nanning Renyou Gan, Singapore Institute of Food and Biotechnology Innovation, Singapore Yao Sun, First Affiliated Hospital of Wenzhou Medical University, Wenzhou Yu Li, Qiqihar Medical University, Qiqihar
17:30-19:00	Meet the speakers 17:30-17:45 / Dinner & Networking 17:45-19:00
19:00-20:30	**Getting your article published in Biofilm** Tom Coenye, Ghent University, Ghent Birthe Kjellerup, University of Maryland, College Park
20:30-20:45	Meet the speakers

Oct 20th Medical Microbiology

Session 1	
Chair	**Chuanwu Xi, University of Michigan, Ann Arbor** **Zhenbo Xu, South China University of Technology, Guangzhou**
9:00-9:10	**Opening ceremony** **Birthe Kjellerup, University of Maryland, College Park**
9:10-9:40	**Free-floating biofilm-like aggregates: expanding the biofilm conceptual developmental model** **Paul Stoodley, The Ohio State University, Columbus**
9:40-10:10	**Understanding biofilms in wounds** **Kendra Rumbaugh, Texas Tech University, Lubbock**
10:10-10:25	**Modeling polymicrobial infection in the CF-like airway of *Scnn1* transgenic mice** **Janette Harro, University of Maryland, Baltimore**
10:25-10:40	**Immunity to *S. aureus* skin infections** **Nathan Archer, Johns Hopkins University, Baltimore**
10:40-10:55	**Meet the speakers / Coffee break**
Session 2	
Chair	**Liang Yang, Southern University of Science and Technology, Shenzhen** **Guanglei Qiu, South China University of Technology, Guangzhou**
10:55-11:25	**Interbacterial interactions that enhance resistance to host defense** **Daniel Wozniak, The Ohio State University, Columbus**
11:25-11:55	**New insight into the biofilm matrix of *P. aeruginosa*** **Matthew Parsek, University of Washington, Seattle**
11:55-12:10	**Candida biofilms: importance, regulation, and evolution** **Clarissa Nobile, University of California, Merced**
12:10-12:25	**Lysocin E - a novel antibiotic potentiated in the host** **Hiroshi Hamamoto, The University of Tokyo, Bunkyoku**
12:25-14:00	**Meet the speakers 12:25-12:40 / Lunch 12:40-14:00**

Session 3	
Chair	**Wei Hu, Shandong University, Jinan** **Yulong Tan, Qingdao Agricultural University, Qingdao**
14:00-14:30	**Reduced antimicrobial susceptibility in microbial biofilms: where are we and where should we be going?** **Tom Coenye, Ghent University, Ghent**
14:30-15:00	**The clinical importance of interkingdom biofilms in the oral cavity and beyond** **Gordon Ramage, University of Glasgow, Glasgow**
15:00-15:30	**Bacterial second messenger cyclic-di-GMP and its regulation and inhibition in *Pseudomonas aeruginosa*** **Luyan Ma, Institute of Microbiology of the Chinese Academy of Sciences, Beijing**
15:30-15:45	**Quorum sensing as a target for controlling biofilm formation in *Acinetobacter baumannii*** **Celia Mayer, University of Santiago de Compostela, Galicia**
15:45-16:00	**Meet the speakers / Coffee break**
Session 4	
Chair	**Yue Qu, Monash University, Melbourne** **Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou**
16:00-16:30	**Regulation of biofilm formation by cyclic di-GMP signaling** **Ute Römling, Karolinska Institute, Stockholm**
16:30-17:00	**Pathogenesis of polymicrobial biofilm-associated infections** **Kimberly Kline, Nanyang Technological University, Singapore**
17:00-17:30	**To be determined** **Po-Ren Hsueh, National Taiwan University Hospital, Taipei**
17:30-17:45	**Spatial transcriptome uncovers rich coordination of metabolism in bacterial community** **Jintao Liu, Tsinghua University, Beijing**
17:45-18:00	**Engineered polyurea (PURE) dendrimers are a potential alternative to conventional antibiotics** **Sandra Pinto, University of Lisbon, Lisbon**
18:00-18:15	**Evolution of biofilm cells in response to antibiotics showcases the role of biofilms as diversity incubators for the microbial world** **Anahit Penesyan, Macquarie University, Sydney**
18:15-20:00	**Meet the speakers 18:15-18:30 / Dinner & Networking 18:30-20:00**

Oct 21st Biofilms in Australia

Session 1	**Biomaterial and microorganism interface**
Chair	**Helmut Thissen, CSIRO, Canberra**
7:55-8:00	**Opening: Welcome to the Australian Biofilms meeting** **Yue Qu, Monash University, Melbourne**
8:00-8:40	**Colloidal crystal based micro- and nanostructured surfaces to control bacterial colonization** **Peter Kingshott, Swinburne University of Technology, Melbourne**
8:40-9:10	**Osteoblasts response on two-tiered bactericidal architecture fabricated on titanium surfaces** **Elena Ivanova, Royal Melbourne Institute of Technology, Melbourne**
9:10-9:40	**Biointerfaces – opportunities for the effective control of medical device related infections** **Helmut Thissen, CSIRO, Canberra**
9:40-10:00	**Meet the speakers / Coffee break**
Session 2	**Microbial factors influencing biofilm formation**
Chair	**Yue Qu, Monash University, Melbourne**
10:00-10:30	**The genetic basis of mixed species biofilm development** **Scott Rice, CSIRO, Canberra**
10:30-11:00	**Assessing the role of pharyngeal cell surface glycans in Group A *Streptococcus* biofilm formation** **Heema Vyas, The University of Sydney, Sydney**
11:00-11:30	**Biofilms, *luxS* gene and virulence of the oral bacterium *Campylobacter concisus*** **Taghrid Istivan, Royal Melbourne Institute of Technology, Melbourne**
11:30-11:50	**Meet the speakers / Coffee break**
Session 3	**Biofilms and medical device-related infections**
Chair	**David McGiffin, Monash University, Melbourne**
11:50-12:30	**Development and clinical trials of antimicrobial contact lenses** **Mark Wilcox, The University of New South Wales, Sydney**

12:30-13:00	**Biofilm-related VAD driveline infections and phage therapy** **Anton Peleg, Monash University, Melbourne**
13:00-13:30	**N-acetyl cysteine as a biofilm disruptor and an aid to eradication** **Jim Manos, The University of Sydney, Sydney**
13:30-14:00	**Meet the speakers / Coffee break**
Session 4 **Chair**	**Biofilms and other chronic infections** **Xenia Kostoulias, Monash University, Melbourne**
14:00-14:30	**The dysbiotic polymicrobial biofilm nature of chronic oral disease** **Stuart Dashper, Melbourne University, Melbourne**
14:30-15:00	**Development of stimuli-responsive hydrogel for treatment of mature biofilms in murine wound infection models** **Zlatko Kopecki, University of South Australia, Adelaide**
15:00-15:30	**Do biofilms play a role in the recurrent vulvovaginal candidiasis (RVVC)?** **Yue Qu, Monash University, Melbourne**
15:30-16:00	**Bench to bedside and back again: targeting host-microbial interactions to treat biofilm infections** **Ruth Thornton, The University of Western Australia, Perth**
16:00-16:15	**Closing remarks: What we need from biofilm research: From clinicians' perspective** **David McGiffin & Anton Peleg, Monash University, Melbourne**
Chair	**Oct 21st Early Career Researchers and Students** **Yulong Tan, Qingdao Agricultural University, Qingdao** **Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou**
19:00-19:08	***Lactobacillus Plantarum* inhibits *Candida albicans* filamentation** **Xuejie Li, South China University of Technology, Guangzhou**
19:08-19:16	**Biofilm formation and control of *Bacillus licheniformis* in the dairy industry** **Luyao Fan, Yangzhou University, Yangzhou**
19:16-19:24	**Antibiotics-free nanoparticles eradicate *Helicobacter pylori* biofilms and intracellular bacteria** **Shuqi Zhang, Sun Yat-sen University, Guangzhou**
19:24-19:32	**Persistence of *Listeria monocytogenes* ST5 in Ready-to-Eat Food Processing Environment** **Xin Liu, University of Shanghai for Science and Technology, Shanghai**
19:32-19:40	**The role of Flagella in biofilm formation of emetic *Bacillus cereus*** **Yangfu Li, Jinan University, Guangzhou**
19:40-19:48	**Hypoxia-sensitive antibiotic adjuvant loaded liposomes eradicate *Pseudomonas aeruginosa* biofilms** **Yingying Sun, Sun Yat-sen University, Guangzhou**
19:48-19:56	**Charge switchable nanoparticles anti-biofilm and anti-virulence activities for chronic *Pseudomonas aeruginosa* Lung Infection management** **Pengyu Li, Sun Yat-sen University, Guangzhou**

Oct 22nd Foodborne Microbiology

Venue: Nanyue Hall

Session 1	
Chair	**Zhenbo Xu, South China University of Technology, Guangzhou** **Lei Yuan, Yangzhou University, Yangzhou**
9:00-9:30	**A novel method for controlling *Listeria monocytogenes* on lettuce** **Steve Flint, Massey University, Palmerston North**
9:30-9:45	**To be determined** **Boce Zhang, University of Florida, Gainesville**
9:45-10:00	**Study on the removal of bacterial biofilm by photodynamic sterilization** **Yong Zhao, Shanghai Ocean University, Shanghai**
10:00-10:15	**Mechanism of Acid and Alkali Electrolyzed Water on the Elimination of *Listeria monocytogenes* Biofilm Based on Proteomic Analysis** **Jianxiong Hao, Hebei University of Science and Technology, Shijiazhuang**
10:15-10:30	**The Molecular Mechanism of Inhibition on Staphyloxanthin and Biofilm of *Staphylococcus aureus* by Naftifine Derivative JX08806** **Chunlei Shi, Shanghai Jiaotong University, Shanghai**
10:30-10:45	**Meet the speakers / Coffee break**
Session 2	
Chair	**Xihong Zhao, Wuhan Institute of Technology, Wuhan**
10:45-11:00	**Inhibitory effect of biofilm-degrading enzyme on the biofilm formation and eracidation of *Vibrio parahaemolyticus*** **Zunying Liu, Ocean University of China, Qingdao**
11:00-11:15	**Study on the quorum sensing regulation mechanism of dominant spoilage bacteria in aquatic products processing** **Hongman Hou, Dalian Polytechnic University, Dalian**
11:15-11:30	**Cold atmospheric plasma to remove bacterial biofilms** **Anne Mai-Prochnow, Sydney University, Sydney**
11:30-11:45	**Control of some of foodborne pathogens in planktonic and biofilm Form by electron beam irradiation and natural antibacterial substances** **Xin Wang, Northwest Agriculture and Forestry University, Xianyang**
11:45-12:00	**Progress of *Listeria monocytogenes* biofilm risk** **Qingli Dong, University of Shanghai for Science and Technology, Shanghai**
12:00-12:15	**Effects of freezing stress on *Staphylococcus aureus* biofilm formation and the inhibitory effect of biochanin A** **Na Guo, Jilin University, Changchun**
12:15-12:30	**Multi-omics Reveals the *Bifidobacterium* Biofilm Formation Mechanism and Fermentation Regulation** **Wenwei Lu, Jiangnan University, Wuxi**
12:30-14:00	**Meet the speakers 12:30-12:45 / Lunch 12:45-14:00**

Session 3	
Chair	**Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou**
14:00-14:30	**Biofilm resilience does not rely exclusively on bacterial viability** **Manuel Simões, University of Porto, Porto**
14:30-14:45	**A combined study on the antibiotic resistance and biofilm-forming abilities of *C. jejuni* and *C. coli* isolates from retail raw chicken samples** **Efstathios Giaouris, University of the Aegean, Mytilini**
14:45-15:00	**Regulatory mechanism of quorum sensing system and second messenger on biofilm formation in *Listeria monocytogenes*** **Xiaomei Bie, Nanjing Agricultural University, Nanjing**
15:00-15:15	**The Rcs system in Enterobacteriaceae: envelope stress responses and morphology regulation** **Jingyu Chen, China Agricultural University, Beijing**
15:15-15:30	**Risk identification and biofilm control of *Listeria monocytogenes*** **Moutong Chen, Guangdong Institute of Microbiology, Guangzhou**
15:30-15:45	**Meet the speakers / Coffee break**
Session 4	
Chair	**Yulong Tan, Qingdao Agricultural University, Qingdao**
15:45-16:15	**Spatio-temporal diversification of *Bacillus subtilis* cell types in surface-associated communities** **Romain Briandet, University of Paris-Saclay, Paris**
16:15-16:45	**The training and researching on biofilms in Vietnam: the current status and the need of international collaboration** **Dinh Toi Chu, Vietnam National University, Hanoi**
16:45-17:00	**Battle against viable but nonculturable state in rice and flour products: control and detection** **Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou**
17:00-17:15	**Discovery of antibacterial and anti-biofilm natural products** **Renyou Gan, Singapore Institute of Food and Biotechnology Innovation, Singapore**
17:15-17:30	**Screening of quorum-sensing inhibitors and construction of *luxS* gene knockout vector in *Leuconostoc citreum*** **Rihua Xu, Inner Mongolia University, Hohhot**
17:30-17:45	**How Gram-Negative Bacterial Cell Envelope Respond to Antimicrobial Stress** **Mingming Guo, Zhejiang University, Hangzhou**
17:45-19:00	**Meet the speakers 17:45-18:00 / Dinner & Networking 18:00-19:00**

Oct 22nd Basic Microbiology and Anti-Biofilms (Venue 2)

Venue: Nanhu Hall

Session 1	
Chair	**Yulong Tan, Qingdao Agricultural University, Qingdao**
9:00-9:30	**Targeting *Fusobacterium nucleatum* through Chemical Modifications of Host-Derived Transfer RNA Fragments** **Xuesong He, Dental Medicine of Harvard University, Boston**
9:30-10:00	**Interspecies interactions during bacterial biofilm formation** **Liang Yang, Southern University of Science and Technology, Shenzhen**
10:00-10:30	**Regulation of Pf Phage and Phage Defense in *Pseudomonas aeruginosa* Biofilms** **Xiaoxue Wang, South China Sea Institute of Oceanology, Guangzhou**
10:30-10:45	**The quorum sensing inhibitors from the medicinal and food plants** **Aiqun Jia, Hainan University, Haikou**
10:45-11:00	**Meet the speakers / Coffee break**
Session 2	
Chair	**Liang Yang, Southern University of Science and Technology, Shenzhen**
11:00-11:30	**Self-organized canals enable long range directed material transport in bacterial communities** **Yilin Wu, Chinese University of Hong Kong, Hongkong**
11:30-12:00	**Antibiotic resistance and pathogenicity assessment of various Gardnerella sp. strains in local China** **Lichuan Gu, Shandong University, Jinan**
12:00-12:15	**Novel drug delivery strategies against biofilm infections** **Haiyan Hu, Sun Yat-Sen University, Guangzhou**
12:15-12:30	**Interactions between live and dead bacterial cells** **Xiangjun Gong, South China University of Technology, Guangzhou**
12:30-14:00	**Meet the speakers 12:30-12:45 / Lunch 12:45-14:00**

Session 3 **Chair**	**Zhenbo Xu, South China University of Technology, Guangzhou**
14:00-14:30	**To be determined** **Maëlle Molmeret, University of Toulon, Toulon**
14:30-14:45	**Regulation of the T3SS and quorum sensing systems by a CspA family protein CspC in response to host environment in *Pseudomonas aeruginosa*** **Weihui Wu, Nankai University, Tianjin**
14:45-15:00	**To be determined** **Mariagrazia Di Luca, University of Pisa, Pisa**
15:00-15:15	**Inhibiting effect of pH responsive materials on oral biofilm** **Lei Cheng, Sichuan University, Chengdu**
15:15-15:30	**To be determined** **Sanna Maria Sillankorva, International Iberian Nanotechnology Laboratory, Braga**
15:30-15:45	**A red fluorescent small-molecule for visualization of c-di-GMP tetramer in live bacterial cells and real-time monitoring of biofilm formation on biotic and abiotic surfaces** **Ning Sun, Guangzhou First People's Hospital, Guangzhou**
15:45-16:00	**Meet the speakers / Coffee break**
Session 4 **Chair**	
16:00-16:15	**EPS from Biofilm: Structure and Functional Relationships** **Qingbin Guo, Tianjin University of Science and Technology, Tianjin**
16:15-16:30	**Joint cavity infection and biofilm treatment** **Qingjun Wei, Guangxi Medical University, Nanning**
16:30-16:45	**Self-produced dextranase prevents *Streptococcus mutans* biofilm and dental caries** **Nan Liu, Shandong Provincial Hospital, Jinan**
16:45-17:00	**To be determined** **Jing Lin, Guangzhou University, Guangzhou**
17:00-17:15	***Pseudomonas aeruginosa* Quorum Sensing Systems and Iron Homeostasis as Drug Discovery Targets** **Pinghua Sun, Jinan University, Guangzhou**

17:15-17:30	Nanoparticle-stabilized encapsulation of borneol and citral: Physicochemical characteristics, storage stability, and enhanced antibacterial activities Jianyu Su, South China University of Technology, Guangzhou
17:30-17:45	Highly surface-functionalized antimicrobial peptide formulations and their antibacterial mechanism against local infections Chao Lu, Jinan University, Guangzhou
17:45-18:00	Growth, biofilms and virulence factors of *Pseudomonas aeruginosa* suppressed by the synergistic interaction between bioactive plant extract and antibiotics Iqbal Ahmad, Aligarh Muslim University, Aligarh
18:00-19:00	Meet the speakers 18:00-18:15 / Dinner & Networking 18:00-19:00
Oct 22nd Early Career Researchers and Students	
Chair	Zhenbo Xu, South China University of Technology, Guangzhou Xuejie Li, South China University of Technology, Guangzhou
19:00-19:08	The AhR ligand phthiocol and vitamin K analogs as *Pseudomonas aeruginosa* quorum sensing inhibitors Tianyuan Jia, Southern University of Science and Technology, Shenzhen
19:08-19:16	Acquisition of Daptomycin Resistance by Enterococcus faecium Confers Collateral Sensitivity to Glycopeptides Yao Sun, Affiliated Hospital of Wenzhou Medical University, Wenzhou
19:16-19:24	Effect of sub-MIC of antibiotics on *Staphylococcus aureus* biofilm formation Yaqin Li, South China University of Technology, Guangzhou
19:24-19:32	SPR detection on microbial biofilms: an initial study Haoyue Xue, South China University of Technology, Guangzhou
19:32-19:40	*In vitro* antimicrobial activity and resistance mechanisms of the new generation tetracycline agents, eravacycline, omadacycline, and tigecycline against clinical *Staphylococcus aureus* isolates Weiliang Zeng, Affiliated Hospital of Wenzhou Medical University, Wenzhou
19:40-19:48	Metabolism of Periodontal Pathogens: Their Regulatory Roles in Dysbiotic Subgingival Biofilm Jing Ding, Sun Yat-sen University, Guangzhou
19:48-19:56	Antimicrobial resistance and biofilm formation in *Candida* strains Jiaying Hong, South China University of Technology, Guangzhou
19:56-20:04	Bacterial community in surface water and sediments in highly urbernized subtropical region and their potential roles in nitrogen cycle Sijia Ji, South China University of Technology, Guangzhou
20:04-20:12	Carbon uptake bioenergetics of PAOs and GAOs in full-scale WWTPs Liping Chen, South China University of Technology, Guangzhou
20:12-20:20	Pathogenesis and biofilm formation in clinical *Klebsiella pneumoniae* strains Feifeng Zhong, South China University of Technology, Guangzhou
20:20-20:28	Microbial interaction between *L. plantarum* and *S. cerevisiae*: Transcriptome level mechanism of cell-cell antagonism Nixuan Gu, South China University of Technology, Guangzhou
20:28-20:36	Decorating probiotic based on Manganese Dioxide Nanozymes as Intelligent Cytoprotective Shells Jie-Yan Shi, Shantou University, Shantou
20:36-20:44	Biofilm tolerance, resistance and infections: how to deal with the increasing threat of public health Yang Shanshan, Shenyang Agricultural University, Shenyang
20:44-20:52	The *dexA* Gene Regulates Exopolysaccharides Metabolism in *Streptococcus mutans* Mature Biofilm Xiamengying, West China School & Hospital of Stomatology, Sichuan University, Chengdu
20:52-21:00	Dysregulated glycogen metabolism in *Candida albicans* impacts innate immune signaling via β-(1→3)-glucan unmasking Jian Miao, University of Tennessee Health Science Center, Memphis
21:00-21:08	Detection of biofilm in Hypervirulence *Klebsiella pneumoniae* isolated from hospital Yuzhu Mao, University of Maryland, College Park

Oct 23rd Environmental Microbiology

Venue: Nanyue Hall

Session 1	
Chair	**Guanglei Qiu, South China University of Technology, Guangzhou** **Zhenbo Xu, South China University of Technology, Guangzhou**
9:00-9:30	**To be determined** **Stefan Wuertz, Nanyang Technological University (SCELSE), Singapore**
9:30-10:00	**To be determined** **April Gu, Cornell University, New York**
10:00-10:15	**To be determined** **Diane McDougald, University of Technology Sydney, Sydney**
10:15-10:30	**To be determined** **Enrico Marsili, University of Nottingham, Ningbo**
10:30-10:45	**Meet the speakers / Coffee break**
Session 2	
Chair	**Yichao Wu, Huazhong Agricultural University, Wuhan** **Faqian Sun, South China University of Technology, Guangzhou**
10:45-11:15	**Biofilms & Beer** **Darla Goeres, Montana State University, Bozeman**
11:15-11:45	**Extracellular DNA in Natural and Engineered Environmental Systems** **Bin Cao, Nanyang, Technological University (SCELSE), Singapore**
11:45-12:00	***Staphylococcus aureus* biofilm cell wall phenotypic changes associated with biofilm age and water stress result in increased disinfectant tolerance** **Honghua Hu, Macquarie university, Sydney**
12:00-12:15	**To be determined** **Rongchang Wang, Tongji University, Shanghai**
12:15-12:30	**Interactions between prescription drugs and biofilms in sewer system** **Yuan Ren, South China University of Technology**
12:30-14:00	**Meet the speakers 12:30-12:45 / Lunch 12:45-14:00**

Session 3	
Chair	**Shanquan Wang, Sun Yat-sen University, Guangzhou** **Guanglei Qiu, South China University of Technology, Guangzhou**
14:00-14:30	**Novel insight into the microbiology of flocs and biofilms in global wastewater treatment systems** **Per Halkjær Nielsen, Aalborg University, Aalborg**
14:30-15:00	**Microbial reductive dechlorination of polychlorinated biphenyls in polluted urban rivers** **Shanquan Wang, Sun Yat-sen University, Guangzhou**
15:00-15:15	**Subsurface biofilm community assembly driven by microbial interaction** **Yichao Wu, Huazhong Agricultural University, Wuhan**
15:15-15:30	**Enhanced phenol biodegradation from industrial wastewater by resuscitation promoting factor (Rpf) under stressful conditions** **Faqian Sun, Zhejiang Normal University, Jinhua**
15:30-16:00	**Meet the speakers / Coffee break**
Session 4	**<Environmental Science and Ecotechnology> Special Session**
Chair	**Yu Tao, Harbin Institute of Technology, Shenzhen**
16:00-16:30	**Charging memory effect of microbial communities in wastewater treatment systems** **Aijie Wang, Harbin University of Technology, Shenzhen**
16:30-16:45	**Microbiome Research of Activated Sludge Flocs and Biofilm in Wastewater Treatment Systems** **Feng Ju, Westlake University, Hangzhou**
16:45-17:00	**How emerging contaminants affect the dissemination and evolution of antimicrobial resistance genes?** **Shuhong Gao, Harbin University of Technology, Shenzhen**
17:00-17:15	**Low-carbon resource recovery technology based on extracellular biopolymers derived from granular sludges** **Cuijie Feng, Sun Yat-sen University, Zhuhai**
17:15-17:30	**<Environmental Science and Ecotechnology> Meet the editor** **Yu Tao, Harbin Institute of Technology, Shenzhen**
17:30-17:45	**Closing ceremony**

Oct 23rd Applied Microbiology

Venue: Nanhu Hall

Session 1	
Chair	**Shezmin Ismail, Monash University, Melbourne**
9:00-9:30	**Why do we have to apply engineered biofilms to ecosystems and the environment?** **Gamini Seneviratne, National Institute of Fundamental Studies, Kandy**
9:30-9:45	**Crude oil degrading microbial biofilms: a synthesis** **Madushika Perera, University of Colombo, Colombo**
9:45-10:00	**Soil carbon sequestration in lowland paddy cultivation: a Biofilm biofertilizer approach** **Sidath Ekanayake, National Institute of Fundamental Studies, Kandy**
10:00-10:15	**Biofilmed Azorhozobial biofertilizer to replace 50% urea requirement for rice (*Oryza sativa*)** **Thilini A. Perera, University of Colombo, Colombo**
10:15-10:30	**Synthetic Cyanobacteria / Hetrotroph Communities: Engineering in characteristics from biofilming species towards improved consortia robustness** **Danny Ducat, Michigan State University, East Lansing**
10:30-10:45	**Meet the speakers / Coffee break**
Session 2	
Chair	**Radha Prasanna, ICAR-Indian Agricultural Research Institute, New Delhi**
10:45-11:15	**SPR detection in microbial biofilms** **Chii-Wann Lin, National Taiwan University, Taipei**
11:15-11:30	**Cellulolytic activity of fungal-bacterial biofilm developed from brown rot fungi and soil bacteria** **Amila P. Henagamage, Uva Wellassa University, Badulla**
11:30-11:45	**Microbial biofilms can shape gut microbiota better than diet-based interventions** **Mahesh Premarathna, National Institute of Fundamental Studies, Kandy**
11:45-12:00	**Development of Biofertilizers to Strawberries: a microbial biofilm approach** **Darshani Singhalage, Uva Wellassa University, Badulla**
12:00-14:00	**Meet the speakers 12:00-12:15 / Lunch 12:15-14:00**

Session 3	
Chair	**Gamini Seneviratne, National Institute of Fundamental Studies, Kandy**
14:00-14:30	**Fungal-bacterial biofilms: promises, progress and prospects** **Shezmin Ismail, Monash University, Melbourne**
14:30-14:45	**Prospecting cyanobacterium-based biofilms as climate-smart options under elevated CO_2 environments** **Radha Prasanna, ICAR-Indian Agricultural Research Institute, New Delhi**
14:45-15:00	**Application of Cunninghamella elegans biofilms in drug metabolite production and pollution removal** **Cormac Murphy, University College Dublin, Dublin**
15:00-15:15	**Verification of Fermentation Time of Kombucha 'Tea Fungus'** **Viduranga Waisundara, Australian College of Business & Technology, Kandy**
15:15-15:30	**Meet the speakers / Coffee break**
Session 4	**Biofilms and synthetic biology**
Chair	**Yanrui Ye, South China University of Technology, Guangzhou** **Zhenbo Xu, South China University of Technology, Guangzhou**
15:30-15:45	**Metabolic engineering *Corynebacterium glutamicum* co-culture system to utilize lignocellulose hydrolysate for efficient production of α-carotene** **Cheng Li, Massachusetts Institute of Technology, Cambridge**
15:45-16:00	**Exploiting living materials by engineering Gram-positive pili** **Yuanyuan Huang, Shenzhen Institutes of Advanced Technology, Shenzhen**
16:00-16:15	**Intense pulsed light for inactivation of foodborne gram-positive bacteria in planktonic cultures and bacterial biofilms** **Xuejie Li, South China University of Technology, Guangzhou**
16:15-16:30	**Meet the speakers / Coffee break**

Asia Pacific Biofilm Workshop: Control strategies for bacterial biofilms – the need for standard methods?

Title: Why the need for standard methods?

Presenter: Paul Stoodley

Abstract: Bacterial biofilms are generally associated with many problems affecting human, animal and plant health and industrial processes. They also can be used for beneficial purposes such as nitrogen fixation in agriculture, pollutant bioremediation, fuel cell and biofuel power generation and waster water treatment. Due to the many applications of treatments or manipulations biofilms many researchers and industries are developing technologies to control biofilms. However, due to the huge diversity in different types of biofilms, their growth context, and treatment strategies researchers and companies often develop their own treatment assessment methods, usually tailored to the treatment mode of action. This makes it difficult for regulatory agencies and investors difficult to judge how any of these technologies compare to each other and existing methods for biofilm. In this section of the workshop these issues will be presented and discussed.

Title: Standardized Biofilm Methods.

Presenter: Kelli Buckingham-Meyer

Abstract: The Standardized Biofilm Methods Laboratory at Montana State University developed six ASTM biofilm methods for biofilm growth and/or disinfectant efficacy treatment. An overview of biofilm standard methods components, definitions and a description of each method's growth parameters, advantages and limitations will be provided.

Title: Statistical Analysis and Experimental Designs for Biofilm Methods

Presenter: Al Parker

Abstract: Standard methods are rigorously validated for several characteristics: Relevance, Reasonableness, Resemblance, Repeatability, Responsiveness, Ruggedness, Reproducibility. Results are used for determining experimental designs. A new method for growing Legionella biofilms that is undergoing standardization is used in examples.

Free-floating biofilm-like aggregates: expanding the biofilm conceptual developmental model

Paul Stoodley

Abstract: Lab experiments and real world observations find that bacteria are commonly found in free-floating aggregates held together in an extracellular matrix produced by the bacteria themselves or from molecules in the local environment. These free-floating bacterial aggregates demonstrate many of the attributes associated with bacterial biofilms that form from growth on surfaces, including tolerance to antimicrobial treatments. Free-floating aggregates can form in fluid suspension or be shed from attached biofilms. Similarly they can move from the fluid phase to the surface, initiating attached biofilm formation. In this seminar the mechanism of formation of aggregates in synovial fluid with possible consequences for orthopedic periprosthetic joint infections and the broader implications of how we think about biofilm developmental dynamics will be discussed.

Candida biofilms: importance, regulation, and evolution

Clarissa J. Nobile

Abstract: An infection is often treated as if it is composed of a single microbial species in isolation, yet in reality, infections are immensely complex ecosystems composed of many interacting microbes. Research in the Nobile lab is directed towards understanding the molecular and mechanistic bases of biofilm microbial communities. We are most interested in investigating how transcriptional networks underlie the regulation of gene expression during the development of biofilms. Much of this work is carried out in the *Candida* clade species, consisting of some of the most prevalent fungal pathogens of humans. In this talk, I will discuss some of the major questions my lab is currently pursuing, including: How are *Candida* biofilms regulated? How are they built ? How are their unique and specialized properties maintained? How have they evolved ?

Lysocin E-a novel antibiotic potentiated in the host

Hiroshi Hamamoto (Teikyo University Institute of Medical Mycology)

Abstract: We identified a novel antibiotic named lysocin E. Lysocin E had identified using the silkworm *Staphylococcus aureus* infection model. Lysocin E belongs to cyclic depsipeptide and targets a menaquinone, a bacteria-specific co-factor of the electron transport chain. The mode of action of lysocin E is ultra-rapid bactericidal activity, killing almost all of *S. aureus* within a minute. From this feature, lysocin E may act against biofilm-formed *S. aureus*. In addition, it showed potent therapeutic activity compared with other anti-MRSA drugs. The unique feature of lysocin E is that the antimicrobial activity of lysocin E is enhanced by apolipoprotein A-I, which contributes therapeutic efficacy of lysocin E. In this symposium, I will introduce these unique bioactivities and the underlaid mechanism of lysocin E.

References

1 Geberetsadik, G. *et al.* Lysocin E targeting menaquinone in the membrane of *Mycobacterium tuberculosis* is a promising lead compound for antituberculosis drugs. *Antimicrob. Agents Chemother.*, e0017122, doi:10.1128/aac.00171-22 (2022).

2 Hamamoto, H. *et al.* Serum apolipoprotein A-I potentiates the therapeutic efficacy of lysocin E against *Staphylococcus aureus*. *Nat Commun* 12, 6364, doi:10.1038/s41467-021-26702-0 (2021).

3 Itoh, H. *et al.* Development of a high-throughput strategy for discovery of potent analogues of antibiotic lysocin E. *Nat Commun* 10, 2992, doi:10.1038/s41467-019-10754-4 (2019).

4 Hamamoto, H. *et al.* Lysocin E is a new antibiotic that targets menaquinone in the bacterial membrane. *Nat. Chem. Biol.* 11, 127-133, doi:10.1038/nchembio.1710 (2015).

Bacterial second messenger cyclic-di-GMP and its regulation and inhibition in *Pseudomonas aeruginosa*

Dejian Liu, Yu Zhang, Pramod Bhasme, Anming Xu, Di Wang, Luyan Z. Ma*

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Abstract: *Pseudomonas aeruginosa* is an environmental microorganism. It is also an opportunistic pathogen that is notorious for its ability to form biofilm and its resistance to antibiotics. Cyclic diguanosine monophosphate (c-di-GMP) is a bacterial second messenger that plays critical roles in biofilm formation. *P. aeruginosa* contains 41 genes that encode enzymes to participate in the metabolism of c-di-GMP (biosynthesis or degradation), yet it lacks tools to investigate the systematic expression pattern of those genes. My group constructed a promoter-gfp transcriptional fusion reporters' library that consists of 41 reporter plasmids. Each plasmid contains a promoter of corresponding c-di-GMP metabolism-related (CMR) genes from *P. aeruginosa* PAO1 strain, thus each promoter-Gfp fusion reporter can be used to detect the promotor' activity as well as the transcription of corresponding gene. My presentation will focus on the application of this library to determine the influence of different temperatures, growth media, and sub-inhibitory concentrations of antibiotics on transcriptional profile of the 41 CMR genes in *P. aeruginosa* as well as to define the mechanisms of anti-biofilm compounds. My talk will also include a few mechanisms that we discovered in past few years to regulate the intracellular c-di-GMP in *P. aeruginosa*.

Spatial transcriptome uncovers rich coordination of metabolism in bacterial community

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Center for Infectious Disease Research, School of Medicine, Tsinghua University, Beijing 100084, China

Abstract: Microbial communities often display region-specific properties, which give rise to complex interactions and emergent behaviors that are critical to the homeostasis and stress response of bacterial communities. However, systems-level understanding of these properties still remains elusive. Here, we established RAINBOW-seq and profiled the transcriptome of Escherichia coli biofilm communities with high spatial resolution and high gene coverage. We uncovered three modes of community-level coordination, including cross-regional resource allocation, local recycling, and feedback signaling, which were mediated by strengthened transmembrane transport and spatially specific activation of metabolism. As a consequence of such coordination, nutrient limited region of the community maintained unexpectedly high level of metabolism, enabling it to act as a signaling hub and to activate many latent sociality functions. Our work provides a comprehensive understanding of the metabolic interplay in biofilms and presented a new approach of investigating complex interactions in bacterial communities on the systems level.

Evolution of biofilm cells in response to antibiotics showcases the role of biofilms as diversity incubators for the microbial world.

Anahit Penesyan

Abstract: Considering that majority of microorganisms live as biofilms in their native environment, including in infection settings, understanding the dynamics of biofilms exposed to antibiotics is important for developing infection control strategies. With this in mind, we exposed biofilms of *Acinetobacter baumannii*, a pathogen responsible for up to 20% of infections in Intensive Care Units worldwide, to sub-inhibitory concentrations of two antibiotics: ciprofloxacin and tetracycline. Phenotypic and genomic analyses were undertaken on cells dispersing from biofilms, while the biofilms were investigated using transcriptomics.

We have shown that a three-day exposure of *A. baumannii* biofilms to sub-inhibitory concentrations of antibiotics had a profound effect, increasing biofilm formation and antibiotic resistance in the majority of biofilm dispersal isolates. Genome sequencing identified multiple mutations in these cells. By using network analyses we were able to link mutations to specific phenotypes. Transcriptomics of biofilms confirmed the network analysis results, revealing novel gene functions of relevance to both resistance and biofilm formation.

Rapid evolution of biofilm cells in response to external stressors, such as antibiotics, highlights a new fundamental property of biofilms as incubators of genotypic and phenotypic diversity in the microbial world. I will demonstrate that biofilms are unique life forms with an unparalleled role as both evolutionary hotspots and testing grounds for ongoing mutation and selection. The role of biofilms as diversity incubators is under-appreciated but can be central for microbial evolution and adaptation.

Engineered polyurea (PURE) dendrimers are a potential alternative to conventional antibiotics

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Infectious diseases caused by bacteria are a disturbing societal burden that puts in risk millions of people worldwide. This is a problem of major concern and utmost priority especially due to the emergence of novel resistant pathogens and infectious diseases. In this sense, the development of new antimicrobial agents with novel mechanisms of action is of critical importance. In 2017, the World Health Organization (WHO) made an appeal to the scientific community, stating that R&D strategies should focus on the discovery and development of new antibiotics.

Considering this scenario, we developed engineered polyurea (PURE) dendrimers, densely positive-charged core-shell nanoparticles that mimic antimicrobial peptides, as a novel class of antimicrobial agents. Novel cationic core-shell PURE dendrimers were synthetized following a sustainable protocol. The antimicrobial capacity (MIC and MBC values and colony count kinetic assay) was tested against several multidrug resistant (MDR) bacteria. In addition, we also preformed preliminary assays (e.g. confocal microscopy) to study the effectiveness of PURE dendrimers against *L. monocytogenes* biofilms.

The results obtained point towards a high efficiency of dendrimers in the treatment of MDR infections. We also found that polycationic core-shell dendrimers have an impact on cell density and biofilm adhesion. Using electron microscopy and coarse-grained molecular dynamics simulations it was possible to observe that the dendrimers display a disruptive action at the membrane level.

In a scenario of increasing resistant pathogens and infectious diseases, cationic core-shell PURE dendrimers emerge has a step forward in the development of effective and reliable antibiotics.

Progress on resistance mechanism and control technology of *Listeria monocytogenes* biofilm

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Abstract: *Listeria monocytogenes* is an important foodborne pathogenic bacterium that is widely found in nature and can cause listeriosis in humans. The biofilm is a resilience protection mechanism for microorganisms that protects them from or reduces the negative effects of environmental stresses (e.g. low temperature, low acid, dry conditions, high osmotic action, disinfectants, etc.) and increases their survival rate. *Listeria monocytogenes* can form biofilms on various food contact surfaces, and once formed, they are difficult to remove, thus posing a potential threat to food safety and human health. In this paper, we summarize the common detection methods of biofilm and analyze the resistance mechanism of *Listeria monocytogenes* biofilm; we also summarize the existing prevention and control techniques from physical, chemical and biological aspects, in order to provide a theoretical reference for the effective control of the spread of *Listeria monocytogenes* biofilm.

Keywords: *Listeria monocytogenes*； biofilm； resistance mechanism; detection method; control technology

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The role of Flagella in biofilm formation of emetic *Bacillus cereus*

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Abstract: *Bacillus cereus*, an important foodborne pathogen, poses a risk to food safety and quality. The food poisoning incident caused by *B. cereus* ranks fourth among the food poisoning incidents caused by microorganisms in China, and there are reports of death cases. Robust biofilm formation ability is one of the key properties that is responsible for the food contamination and food poisoning caused by *B. cereus*, especially the emetic strains. To investigate the mechanism of biofilm formation in emetic *B. cereus* strains, we screened for the mutants that fail to form biofilm by using random mutagenesis towards *B. cereus* 892-1, an emetic strain with strong biofilm formation ability. A mutant with a transposon insertion site for the flagellar hook coding gene *flgE* lacks biofilm-forming ability. When knocking out *flgE*, the mutant showed disappearance of flagellar structure and swimming ability. Compared with the flagellar paralytic strains Δ*motA* and Δ*motB*, the inhibition of biofilm formation by Δ*flgE* is not only caused by the inhibition of motility. The deletion of *flgE* not only reduced biofilm formation, but also significantly down-regulated cereulide production. Therefore, we speculated that *flgE* may serve as an important contributor to both biofilm formation and cereulide production, which suggests that the two phenotypes are possibly governed by a common system within the cell. The relationship between biofilm formation and cereulide production in the emetic strain of *B. cereus* is still unclear and the mechanism of *flgE* in these two processes needs to be further investigated in the future.

A novel method for controlling *Listeria monocytogenes* on lettuce

Steve Flint

Massey University, Palmerston North

Abstract: The ability of *L. monocytogenes* to form biofilms is a concern in terms of fresh produce safety. The use of hydroponics (growing plants in nutrient solution without using soil) in fresh produce production is a more controlled environment than soil, and therefore may be easier to prevent pathogen contamination. The aim of this study was to determine whether hydroponic grown lettuce and UV-C stress can prevent or reduce the colonization, growth and biofilm formation of three fresh produce related strains of *L. monocytogenes*: PFR O8A06 (coleslaw isolate), PFR O8A07 and PFR O8A08 (cabbage isolates) on lettuce.

There was no significant difference ($p > 0.05$) in *L. monocytogenes* attachment to both hydroponic and soil grown lettuce leaves under minimal exposure times. Exposure of lettuce to 5 log CFU/ml for just one second resulted in at least 0.77 log CFU/cm^2 attachment. *L. monocytogenes* was able to survive and grow on both lettuce leaf surfaces at 4 and 10°C. Both hydroponic and soil grown lettuce leaf extracts enhanced the survival, growth and biofilm formation of *L. monocytogenes* on stainless steel coupons, representing surfaces in lettuce processing plants. The results of this study demonstrate the ability of *L. monocytogenes* to colonize and form biofilms on lettuce irrespective of the growth system used.

A novel control measure using UV-C (1.3 kJm^{-2}) on lettuce produced a stress response in the plants that reduced *L. monocytogenes* attachment, survival and growth at pre-harvest. Further exploration of this technique may enhance the microbial safety of lettuce.

Progress of *Listeria monocytogenes* biofilms risk

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Abstract: *Listeria monocytogenes* (LM) is a major problem for the food industry. LM biofilms are a persistent source of cross-contamination in housing storage and food processing environments. In order to investigate the prevalence of LM in meat products in China, a meta-analysis method was used to systematically analyze the contamination information. Focusing on the cross-contamination caused by LM biofilm, systematic research has been conducted in food processing and housing storage. Firstly, the biofilm formation of LM in simulated meat processing was explored, and the biofilm formation probability boundary model of LM under simulated meat processing conditions was established. Then beef juice and standard medium were used as cultures to observe formation process and microstructure of LM biofilms. Secondly, the biofilm formation model of *Pseudomonas aeruginosa* and LM in a simulated chicken environment was established, and the formation of LM biofilm and the expression of related genes were studied under nutritional stress conditions. Finally, the transfer and residual of biofilm cells after a single and successive contacts at specific stages was studied. Our findings provide a theoretical reference for the prevention and control of LM biofilms in food processing.

In recent years, much chemical, physical and biotechnologies have been used as practical methods to control LM in food. Probiotics and their derivatives have great potential for the control of LM and provide better options for biological control strategies in food. By imparting "sterilization", "anti-adhesion" or a combination of the two functions on the food contact surface, the formation of biofilm can be reduced by reducing the reversible adhesion of foodborne pathogens in the primary stage, so as to realize the prevention of foodborne pathogens. In-depth research on the mechanism and modeling of biofilm formation and transfer is required. Taking environmental factors into full consideration, and setting standards for cleaning and disinfection are conducive to reducing the potential risk of food borne pathogens transmission

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Effects of freezing stress on *Staphylococcus aureus* biofilm formation and the inhibitory effect of biochanin A

Na Guo

Abstract: *Staphylococcus aureus* (*S. aureus*) is one of the most common foodborne pathogens related to foodborne disease outbreaks via contaminated food. It is worth noting that the frozen and refrigerated food is contaminated by *S. aur*eus sometimes. Freezing doesn't seem to be entirely safe due to the cold tolerance of *S. aureus*. Once the frozen food is thawed or out of cold chain temperature control, some cold tolerance bacteria may grow immediately, if present, which represents a potential hazard of *S. aureus* to consumers. Biofilm-forming *S. aureus* can easily accumulate on various food contact surfaces which induces cross-contamination and are difficult to eliminate. Thus, it is necessary to elucidate the *S. aureus* biofilm formation mechanism in response to freezing stress for better controlling them. Biochanin A, an isoflavonoid, derived from chickpea (Cicer arietinum), peanuts, and other legumes, has been reported to exhibit bioactive potentials including anti-microbial. But, the anti-biofilm activity has not been reported.

This study aimed to explore the *S. aureus* biofilm formation in response to freezing stress and evaluate the activity of the biochanin A against *S. aureus* biofilm. This study will provide a potential candidate compound to control *S. aureus* biofilm formation.

Multi-omics Reveals the *Bifidobacterium* Biofilm Formation Mechanism and Fermentation Regulation

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Bifidobacterium species are the most commonly used probiotics in foods for human consumption, however, it's easy to die during industrial processing. Biofilm is a self-protection model for bacteria to improve environmental resistance. How to promote *Bifidobacterium* biofilm formation has important industrial application value. However, the current biofilm study mainly focused on how to prevent and inactivate the biofilm formation of harmful bacteria, and the biofilm mechanism of bifidobacteria is still unclear. In this study, the bifidobacterial biofilm fermentation system was first established, and then the bifidobacterial biofilm formation mechanism was studied, and finally, different fermentation conditions were used to control the biofilm fermentation.

We found that the biofilm formation ability of *Bifidobacterium* species was different, all *Bifidobacterium bifidum* strains can form strong biofilms, *B. pseudocatenulatum* has strong, weak, and non-biofilm-forming strains, while *Bifidobacterium longum* has only weak and non-biofilm-forming strains. *B. bifidum* mainly regulates biofilm formation through QseC/QseB two-component system (TCS), AI-3/quorum sensing system (QS), and cAMP second messenger system by regulating Tad IV pilin and extracellular substances secretion, among which QseC/QseB is the main regulator. *B. pseudocatenulatum* mainly regulates biofilm formation through c-di-GMP, cAMP, AI-2/QS system by regulating Tad IV pilin and exopolysaccharide secretion, among which c-di-GMP is the main regulator. *B. longum* mainly regulates biofilm formation through AI-2/QS, cAMP, LuxC/LuxE TCS system by regulating Tad IV pilin and extracellular substances secretion, among which AI-2/QS is the main regulator.

Finally, to find the regulatory targets of *Bifidobacterium* biofilm fermentation, we performed

collinearity analysis on the genomes of *B. bifidum*, *B. pseudocatenulatum* and *B. longum*, and weighted gene co-expression network analysis (WGCNA) of transcriptome samples under different glucose, pH, and threonine fermentation conditions. It was found that the Turquoise module containing 414 genes was positively correlated with the bifidobacteria biofilm formation ability. There were 59 genes involved in 11 types of metabolism-related pathways in the Turquoise module, including peptidoglycan biosynthesis; glyoxylate and dicarboxylate metabolism; and histidine metabolism. These results provide targeted substances and genes references for precise regulation of *Bifidobacterium* biofilm fermentation.

Keywords: Biofilm; *Bifidobacterium*; fermentation regulation; Two-component system; Quorum sensing

Biofilm resilience does not rely exclusively on bacterial viability

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Abstract: Most microorganisms on Earth are thought to be sessile, and they can even be found in extreme environments. This ubiquity of biofilms is potentially beneficial but can also cause remarkable problems for public health, medicine, and industry concerns. Therefore, there has been a great deal of research to better understand biofilm development and to identify improved control strategies, taking into account that biofilm cells are extremely tolerant, or even insusceptible, to any biological, chemical, or physical treatment. Microbial surface colonisation and the establishment of a monolayer sessile structure are enough to provide the resident cells with the advantage of tolerating harsh stress conditions. The scenario is highly critical when the biofilm reaches a mature, 3-dimensional, state. Pieces of evidence are demonstrating that biofilm control is not only by killing the bacteria. Their removal from the surface is fundamental, even if much more critical. This presentation will show how our current biofilm control approaches are inadequate. It will further show promising alternative and sustainable strategies for biofilm killing and dispersal.

A combined study on the antibiotic resistance and biofilm-forming abilities of *Campylobacter jejuni* and *Campylobacter coli* isolates from retail raw chicken samples

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Chicken meat is one of the most attractive for consumers kind of meat all over the world, mainly due to its low price, absence of cultural or religious obstacles, and high-quality protein content. Nevertheless, this may also be a source and reservoir for *Campylobacters*, which include microaerophilic bacterial pathogens. Given that the disease provoked (i.e., campylobacteriosis) is the leading cause of bacterial foodborne gastrointestinal infections worldwide, susceptibility testing to clinically relevant antibiotics is crucial not only to guide therapy but for the epidemiological monitoring of resistance. Nevertheless, *Campylobacters* are fragile and fastidious in their growth requirements, as well as sensitive to various environmental stressors, such as oxidative stress. Attachment to surfaces and biofilm formation is suggested to play a key role in the survival, transmittance, and wide prevalence of these bacteria throughout the food chain. In this work, sixty samples (n = 60) of raw chicken meat products sold in retail stores of a Greek island town (Myrina, Lemnos) were initially screened for the prevalence of *Campylobacter* spp. following the established ISO 10272-1:2017 protocol. Recovered isolates (n = 120) were identified by mPCR distinguishing them to the two main species of the genus (turned out to be 65% *C. jejuni* and 35% *C. coli*). All the isolates were tested for resistance against a panel of ten (n = 10) clinically relevant antibiotics, following the agar disk diffusion method (EUCAST protocol), and in parallel for biofilm formation on 96-well polystyrene microtiter plates, under either microaerophilic or aerobic conditions in Muller – Hinton broth supplemented with either 5% chicken juice (MH – CJ), or 5% laked horse blood (MH - HB), at 42 ºC for 48 hours, using the crystal violet and absorbance assay. The results alarmingly showed that 70.0% (84/120) of the recovered isolated were multidrug-resistant (MDR), with 31.7% (38/120) of them being resistant to at least six of the ten tested antibiotics. Concerning the biofilm results, 51.7% (62/120) of the isolates were able to produce biofilm (only in MH-CJ), with 59.7% (37/62) of them being also strong biofilm formers. In general, the biofilm-forming ability was independent of aerobiosis conditions, while *C. coli* isolates showed a stronger such ability compared

to *C. jejuni* ones. No correlation between multidrug resistance and biofilm-forming ability could be here established, with 78.9% (30/38) of MDR *Campylobacter* isolates with resistance to at least six of the tested antibiotics being still unable to form biofilm. Future studies are planned to try to correlate some of the phenotypic properties observed to genetic biomarkers, possibly through whole genome sequencing of some representative isolates.

Keywords: *Campylobacter jejuni*; *Campylobacter coli;* raw chicken meat; food safety; antibiotic resistance; biofilm formation

Acknowledgements: Support for this study was provided by the research program “AGRICA II: AGrifood Research and Innovation Network of ExCellence of the Aegean” (MIS 5046750), ESPA 2014-2020, In the context of the call of the Operational Program "Competitiveness, Entrepreneurship and Innovation", Action "Support of Regional Excellence".

Regulatory mechanism of quorum sensing system and second messenger on biofilm formation in *Listeria monocytogenes*

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Abstract: *Listeria monocytogenes* was studied to analyze the regulation of its biofilm formation by the quorum sensing system of Agr and LuxS and the second messenger signaling molecule cAMP. The quorum sensing system *agrD* and *luxS* genes were knocked out in strain LMB33426. Compared with the WT, the biofilm formation ability of Δ*agrD* and Δ*luxS* mutant strain was decreased. The hydrophobicity of Δ*agrD* strain was significantly decreased and the swimming ability at 37 ℃ was enhanced compared with the WT. Knocking down the c-di-AMP degrading enzyme genes *pdeA* and *pgpH* in strain LMB33426 revealed that the Δ*pdeA* and Δ*pgpH* strains significantly reduced biofilm formation and auto-aggregation ability compared to the WT. RNA-seq showed that Δ*pgpH* resulted in the differential expression of 2357 genes compared to WT. *pgpH* inactivation resulted in the significant downregulation of the cell wall formation-related genes *dltC*, *dltD*, *walK*, and *walR* and the flagellar assembly-related genes *fliG* and *motB*. This research provide a theoretical basis for preventing contamination caused by *L. monocytogenes*.

Keywords: *Listeria monocytogenes*; biofilm; regulatory mechanism; *pgpH*; *agrD*; *luxS.*

The Rcs system in Enterobacteriaceae: envelope stress responses and morphology regulation

Jingyu Chen

Abstract: The bacterial cell envelope is a protective barrier at the frontline of bacterial interaction with the environment, and its integrity is regulated by various stress response systems. The Rcs (regulator of capsule synthesis) system, a non-orthodox two-component regulatory system found in many members of the Enterobacteriaceae family, is one of the envelope stress response pathways. The Rcs system can sense envelope damage or defects and regulate the transcriptome to counteract stress, which affects the morphology and is particularly important for the survival and virulence of pathogenic bacteria. In this study, the role of Rcs system in the regulation of the pathogenic phenotype in *Yersinia enterocolitica* was investigated.

Dysregulated glycogen metabolism in *Candida albicans* impacts innate immune signaling via β-(1→3)-glucan unmasking

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Abstract: The fungal cell wall serves as the interface between the organism and hots environment. Complex carbohydrates are a major component of the *Candida albicans* cell wall, β-(1→3) glucan and plays a key role in fungal structural integrity and immune recognition among the components. Aside from its central role in metabolism, glycogen was shown to be covalently linked to cell wall β-glucan in the model yeast *Saccharomyces cerevisiae*. However, whether cell wall glycogen exists in *C. albicans* cell wall and its potential role in immune recognition are still known. Using a combination of microbiologic, biochemical and CRISPR-Cas9 gene editing approaches, we successfully knocked out *GSY1* (encodes glycogen synthase) and *GLC3* (encodes branching enzyme) in *C. albicans* and confirmed that these genes are essential for glycogen synthesis using iodine staining. Interestingly, demonstrated from 2D NMR spectrum, glycogen was found to be covalently linked to β-(1→3) glucan via the β-(1→6)-linked side chain in cell wall preparations from WT but not in *gsy1Δ/Δ* and *glc3Δ/Δ* strains. Challenge of THP1 macrophages with formalin fixed *gsy1Δ/Δ, glc3Δ/Δ*, or select clinical strains led to exacerbated secretion of IL-1β as compared to WT or revertant strains. While levels of total glucan (aniline blue), phosphomannan (alcian blue), and total mannan (concanavalin A) remained similar, reduced glycogen significantly correlated with enhanced β-glucan display (β-(1→3)-glucan antibody). These findings are the first to validate glycogen synthesis pathway in *C. albicans* and suggests that glycogen synthesis impacts cell-wall glycogen content and further enhances immune cell recognition through β-(1→3)-glucan exposure.

Staphylococcus aureus biofilm cell wall phenotypic changes associated with biofilm age and water stress result in increased disinfectant tolerance

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Abstract: *Staphylococcus aureus* readily forms biofilms that are tolerant to antibiotics and disinfectants. The bacterial cell wall, principally composed of peptidoglycan, is important for cell survival and is a key target for antimicrobial agents. The effect of culture conditions on cell wall ultrastructural changes, peptidoglycan production and sensitivity to sodium dichloroisocyanurate (SDIC) disinfectant was studied in *S. aureus* grown as planktonic organisms, 3-day hydrated biofilm, 12-day hydrated biofilm and 12-day dry surface biofilm (DSB). Bacterial cell wall width was measured using transmission electron microscopy (TEM). A silkworm larva plasma detection system was used to quantify peptidoglycan components inside the cell wall. Bacterial cell wall width and peptidoglycan production increased with culture duration and dehydration. DSB cells were only 70% of the size of one-day planktonic cells ($P<0.05$) while the hydrated biofilm cells were of similar size to the planktonic cells. *S. aureus* cell walls became significantly thicker as the culture aged for both planktonic (1-day 22.95 ± 2.25 nm vs 3-day 33.67 ± 3.69 nm) and hydrated biofilm cells (3-day 38.66 ± 3.58 nm vs 12-days 55 ± 5.85 nm) ($P<0.001$). Subjecting *S. aureus* to water stress significantly increased the cell wall thickness (DSB 66.51 ± 5.23 nm) compared to 12-day hydrated biofilm ($P=0.002$). 12-day hydrated biofilm and DSB had significantly more peptidoglycan than 3-day hydrated biofilm and planktonic bacteria ($P<0.001$) while the 3-day hydrated biofilm had significantly more peptidoglycan than the 1-day planktonic culture ($P=0.003$).

The disinfectant testing showed a significantly higher concentration of disinfectant required to kill older hydrated biofilm and DSB than it needs to kill 3-day biofilms and planktonic bacteria.

The growth conditions and the age of culture have an impact on cell wall synthesis, and biofilm contains bacteria that have thicker cell walls which may be one of the key determinants for biocide resistance.

Interspecies interactions during bacterial biofilm formation

Liang Yang

Abstract: Biofilm formation contributes to structure development and stabilization of microbial communities and thus enables bacteria to survive in detrimental stress conditions and develop into drug resistant chronic infections. Numerous interspecies communication and interaction mechanisms are evolved in mixed-species biofilm development. In this talk, I will introduce our newly developed third generation sequencing-based tool for high resolution examination of microbial community profiles from the complex infection samples. Then I will provide evidence about the c-di-GMP signaling mechanism in promoting *Pseudomonas aeruginosa* as a dominant biofilm former during mixed-species biofilm formation.

Regulation of Pf Phage and Phage Defense in *Pseudomonas aeruginosa* Biofilms

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Abstract: In the opportunistic bacterial pathogen *Pseudomonas aeruginosa*, Pf filamentous bacteriophages are prevalent and they play critical roles in biofilm formation and virulence, but mechanisms governing Pf prophage activation in biofilms are largely unknown. In the past years, we have (i) identified and characterized the excisionase genes *xisF4* and *xisF5* in Pf4 and Pf5, and both of them can significantly promote prophage excision. XisF4 upregulated the phage initiator gene (PA0727); (ii) found that *xisF4* and the neighboring phage repressor c gene *pf4r* are transcribed divergently and their 5′-untranslated regions overlap. XisF4 and Pf4r not only auto-activate their own expression but also repress each other; (iii) showed a type II toxin-antitoxin system PfiT/A inhibits Pf production by inhibiting the expression of *xisF*4 and *PA0727*; (iv) provided evidences that in addition to phage genes, two host H-NS family proteins, MvaT and MvaU, coordinately repress Pf4 production by directly repressing xisF4; (v) found that the phage minor capsid protein pVII (PA0721) inhibits Pf phage adsorption by interacting with PilC and PilJ of T4P. The minor capsid protein pIII provides partial superinfection exclusion and interacts with the PilJ and TolR/TolA proteins. They both provide host protection against pilus-dependent lytic phages. Collectively, we revealed that Pf prophage morons, structural proteins and host essential regulators cooperate in controlling lysogeny and phage defense. These studies suggest that the big iceberg of unknown in phage excision is increasingly clearer and a rethink of the strategy of using lytic phages to treat *P. aeruginosa* biofilm-related infections is needed.

Keywords: Pf phage, regulation, lysogeny, phage defense, biofilm, *Pseudomonas aeruginosa*

The quorum sensing inhibitors from the medicinal and food plants

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Abstract: Increased drug-resistance of *Staphylococcus aureus*, *Pseudomonas aeruginosa, Serratia marcescens, Chromobacterium violaceum*, and *Aeromonas hydrophila* pathogens has enhanced the urge for the development of alternate therapeutics. Quorum sensing (QS) is considered a potential target for the development of newer anti-biofilm agents that do not depend on the utilization of antibiotics. QS inhibitors (QSIs) can inhibit the QS mechanism that forms the major form in the development of bacterial pathogenesis. A diverse array of natural compounds provides a plethora of anti-QS effects. Over the passed decades, more and more quorum sensing inhibitors isolated from the medicinal and food plants, and inhibiting the genes involved in QS. Different QSI studies have been carried out so far for identification of novel natural compounds to help in developing more effective anti-biofilm therapies. In this topic, we tried to review the various natural QSIs in hindering the genes responsible for QS leading to bacterial pathogenesis.

In summary, QSI and anti-biofilm of six G- and G+ bacteria and fungi species were investigated in my group (*S. aureus, C. violaceum, P. aeruginosa, S. marcescens, A. tumefaciens, C. albicans*); There are more than 20 QSIs were identified from TCM in my group. Theses puzzles are still on the way, that is, (1) Does the QS phenomenon popular present in all microbial world? (2) What is the relationship between QS and Biofilm? One-to-one correspondence among QS and BF? (3)The relationship of QS, BF and COVID-19 epidemic?

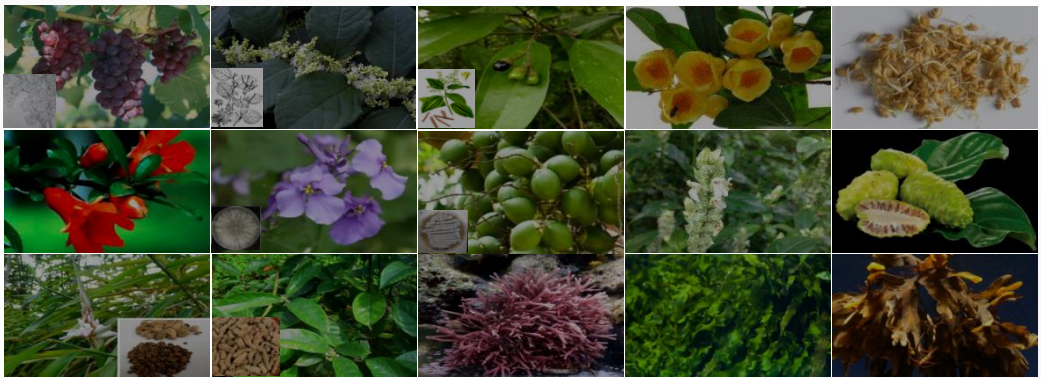
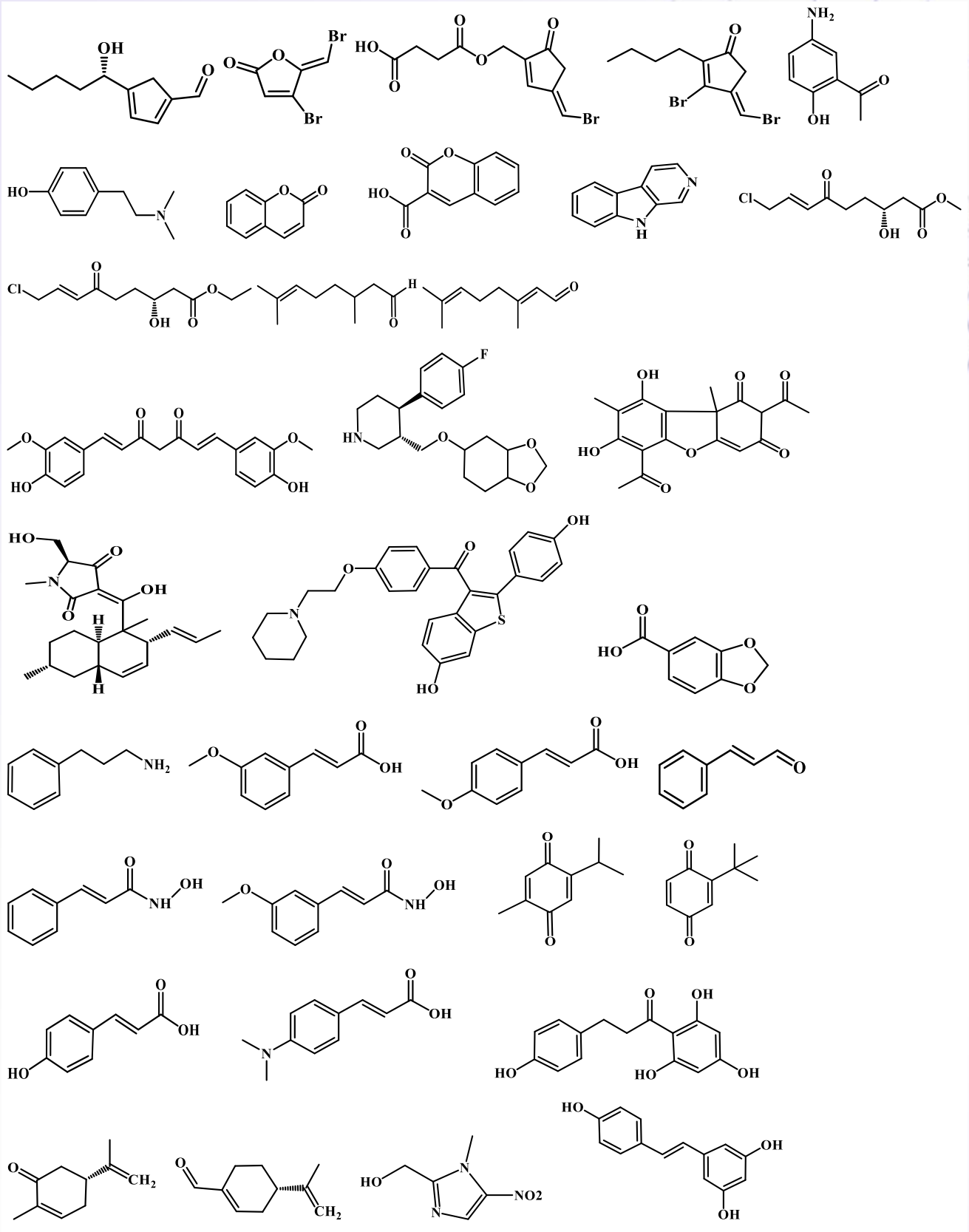


Figure **1**. Some investigated medicinal and food plants



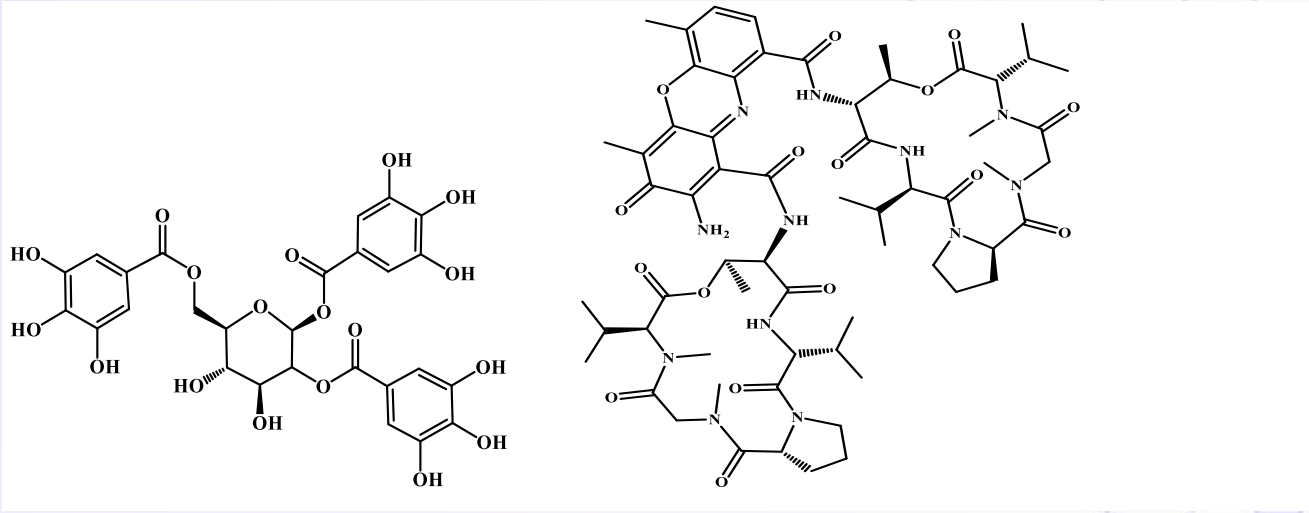


Figure **2**. Some representative QSIs from the medicinal and food plants.

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Antibiotic resistance and pathogenicity assessment of various *Gardnerella* sp. strains in local China

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Abstract: *Gardnerella* overgrowth is the primary cause of bacterial vaginosis (BV), a common vaginal infection with incidences as high as 23% to 29% worldwide. Here, we studied the pathogenicity, drug resistance, and prevalence of varying *Gardnerella* spp. We isolated 20 *Gardnerella* strains from vaginal samples of 31 women in local China. Ten strains were then selected via phylogenetic analysis of *cpn60* and *vly* gene sequences to carry out genome sequencing and comparative genomic analysis. Biofilm-formation, sialidase, and antibiotic resistance activities of the strains were characterized. All strains showed striking heterogeneity in genomic structure, biofilm formation and drug resistance. In particular, seven out of the ten strains exhibited super resistance (≥128 μg/mL) to metronidazole, which is the first line of treatment for BV in China. The bacteria in the biofilm will release dormancy and reactivate after stopping treatment, resulting in recurrence and long-term cure of BV, Therefore, the existence of *Gardnerella vaginalis* biofilm has become the bottleneck of BV treatment. LasA, an elastase from *Pseudomonas aeruginosa*, could more effectively destroy the biofilm of *Gardnerella vaginalis* than antibiotics or lysozyme, which is of great significance in the treatment of BV.

Keywords: *Gardnerella vaginalis*, comparative genomics, antibiotic resistance, biofilm, LasA

Self-organized canals enable long range directed material transport in bacterial communities

Yinlin Wu

Abstract: Long-range material transport is essential to maintain the physiological functions of multicellular organisms such as animals and plants. By contrast, material transport in bacteria is often short-ranged and limited by diffusion. Here we report a unique form of actively regulated long-range directed material transport in structured bacterial communities. Using *Pseudomonas aeruginosa* colonies as a model system, we discover that a large-scale and temporally evolving open channel system spontaneously develops in the colony via shear-induced banding. Fluid flows in the open channels support high-speed (up to 450 μm/s) transport of cells and outer membrane vesicles over centimeters, and help to eradicate colonies of a competing species Staphylococcus aureus. The open channels are reminiscent of human-made canals for cargo transport, and the channel flows are driven by interfacial tension mediated by cell-secreted biosurfactants. The spatial-temporal dynamics of fluid flows in the open channels are qualitatively described by flow profile measurement and mathematical modeling. Our findings demonstrate that mechanochemical coupling between interfacial force and biosurfactant kinetics can coordinate large-scale material transport in primitive life forms, suggesting a new principle to engineer self-organized microbial communities.

Reference: Ye Li, Shiqi Liu, Yingdan Zhang, Zi Jing Seng, Haoran Xu, Liang Yang, Yilin Wu Now published in eLife doi: 10.7554/eLife.79780

Novel drug delivery strategies against biofilm infections

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Abstract: The antibiotics resistance is a serious threat to human health. Recently, The antibiotics resistance caused by biofilms have attracted more and more attention and the strategies to eradicate biofilms, especially novel drug delivery designs were reviewed here. The strategies to eradicate biofilms could be very different due to various microenvironments where biofilms colonized. The comprehensive strategies to eradicate *H. pylori* and *P. aeruginosa* in our lab such as “biofilm-eradicating tetralogy”, “biofilm-eradicating tetralogy plus” including immune activation, killing intracellular bacteria and depressing oxidative stress, and antibiotics-free delivery systems were introduced.

Interactions between live and dead bacterial cells

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Abstract: Natural robust bacterial colonies always contain dead cells. Besides, biocides were widely used in daily life to kill bacteria; however, the interactions between dead and live bacterial cells remains poorly understood. Utilizing a digital holographic microscopy (DHM) and cantilever-modulated atomic force microscopy (CM-AFM), the interactions between *P. aeruginosa* (PAO1) and their dead siblings, as well as dead cells of other species were examined in terms of 3D motions and adhesion forces. Planktonic PAO1 increase in frequency of the 'flick' motion - a sign of chemosensory responses near dead siblings. As a result, the dead siblings on a surface can inhibit the bacterial accumulation and colonization, by activating the adaptive defensive responses of PAO1 in the vicinity. Similar adaptive responses were observed of *P. aeruginosa* near dead probiotics *Lactobacillus rhamnosus* GG (LGG). RNA–seq and metabolites were analyzed reveals movement and biofilm inhibition of PAO1, regulation of chemotaxis and PQS systems accompanied by increased transcriptional levels of the virulence factor related genes.

Inhibiting effect of pH responsive materials on oral biofilm

Lei Cheng

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Abstract: Dental caries caused by oral biofilm is one of the major oral diseases with high prevalence. Although antimicrobial agents for caries prevention have been researched for years, very few reported intelligent anti-caries materials that could respond to the change of oral environment and help keep oral eubiosis. Herein, we developed novel tertiary amines (TAs) with pH-responsive anti-biofilm effect to defend dental caries. the reversible pH-responsive and non-drug-release-type anti-biofilm dental materials ingeniously overcome the defect of the present materials and hold a great promise for clinical application.

A red fluorescent small-molecule for visualization of c-di-GMP tetramer in live bacterial cells and real-time monitoring of biofilm formation on biotic and abiotic surfaces

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Abstract: Cyclic dimeric guanosine monophosphate (c-di-GMP) is an important second messenger in bacteria and regulates a wide range of bacterial functions and behaviors including biofilm formation that causes chronic infections and antibiotic resistance. C-di-GMP being as a signal transducer in bacteria is known to exist in monomer and dimer form. Recent studies also discover that c-di-GMP can form higher-order oligomers such as tetramer and octamer, which may have physiological roles in bacterial cells. Moreover, the tetrameric c-di-GMP structure was reported to link two subunits of a transcription factor (BldD), which controls the progression of multicellular differentiation in sporulating actinomycete bacteria and then mediates the dimerization process. Current understanding on the higher-order oligomers of c-di-GMP is relatively limited compared to its monomer or dimer structures. To probe and visualize the higher-order structures of c-di-GMP and its associated biofunctions in live bacterial cells with fluorescence techniques for mechanistic study and cellular investigation is important. Nonetheless, the sensitive and selective fluorescent probe with a rapid signal response for higher-order oligomers of c-di-GMP is currently lacking. In the present study, a fluorescent probe that preferentially interacted with tetrameric c-di-GMP and generated red fluorescence signal promptly were synthesized and investigated. The interaction mechanism was studied with ^{1}H NMR and molecular docking. In addition, the probe was demonstrated as an excellent molecular fluorescent probe for bioimaging of tetrameric c-di-GMP structure and monitoring of biofilm formation on both biotic and abiotic surfaces with pathogenic bacteria such as *Pseudomonas aeruginosa* PAO1 and *Bacillus subtilis* 168.

EPS from Biofilm: Structure and Functional Relationships

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Abstract: Extracellular polymeric substance (EPS) is the main component of biofilm, accounting for approximately 50%–90%. The composition, architecture, and function of the EPS matrix revealed a very complex, dynamic, and biologically exciting view. Exopolysaccharides as the main component in EPS play a critical role in maintaining the structure of EPS. However, identifying the molecular structure and chemical composition of exopolysaccharides from EPS remains difficult, due to the diversity in sugar monomers, linkages, Mw, etc. Our talk focuses on the strategies for structural characterization of exopolysaccharides from EPS, and summarises some previously reported exopolysaccharides from biofilm, aiming with an intent to understand their structural and functional relationships in biofilm.

Joint cavity infection and biofilm treatment

Qingjun Wei

Abstract:

Background：Bacterial biofilms generally contribute to chronic infections and complicate effective treatment outcomes. *Pseudomonas aeruginosa* (P. aeruginosa) is an opportunistic pathogenic bacterium which can lead to septic arthritis. We established a rabbit model of septic arthritis caused by P. aeruginosa to determine whether it leads to biofilm formation in the knee joint cavity. In addition, we explored the role of cyclic di-GMP (c-di-GMP) concentrations in biofilm formation in rabbit models. Meanwhile, it is known to all that antibiotic resistance of P. aeruginosa leads to the decline of a clinical curative effect. Therefore, we found a natural compound which called Daphnetin (DAP) to investigated the effect of DAP on bacterial motility, biofilm inhibition and eradication.

Methods: First, twenty rabbits were randomly assigned to five groups: PAO1, PAO1ΔwspF, PAO1/plac-yhjH, Luria–Bertani (LB) broth, and magnesium tetrasilicate (talc) control groups. Inoculation in the rabbit knee of P. aeruginosa or with the same volume of sterile LB and talc in suspension. Rabbits were euthanized after 7 days, and pathological examination of synovial membrane was performed. The biofilms on the surface of the synovial membrane were observed by scanning electron microscopy, while the biofilms' fiber deposition was discriminated using peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH).

Second, the MIC of DAP against P. aeruginosa was determined using microdilution method and pyocyanin production was measured by performing a quantitative chemical assay by spectrophotometer. The antibiofilm activity of DAP against P. aeruginosa was determined using the 96-well plate to evaluating the crystal violet (CV) staining and CFU enumeration. The biofilms on the surface of the carriers were observed by scanning electron microscopy. The effect of DAP on P. aeruginosa motility were detected using the swimming, swarming and twitching agar plates to measuring the diameter of the concentric area.

Results: First, A rabbit model for knee septic arthritis induced by P. aeruginosa was successfully established. Scanning electron microscopy revealed that PAO1 strains were surrounded in a self-produced extracellular matrix on the surface of synovial membrane and showed biofilm structures. The PNA-FISH assay revealed that the red fluorescence size in the PAO1DwspF group was greater than in PAO1 and PAO1/plac-yhjH groups.

Second, we demonstrated DAP can effectively inhibit biofilm formation and eradicate biofilm of P. aeruginosa. The concentrations of DAP (0.445 to 1.781 mg/mL) could inhibit the formation of P. aeruginosa biofilms and DAP at concentrations (0.89 and 1.781 mg/mL) could eradicate biofilm. We

showed that DAP can reduce pyocyanin production and inhibit bacterial motility (swimming, swarming, and twitching). The minimal inhibitory concentration (MIC) of DAP on P. aeruginosa was 0.890 mg/mL.

Conclusions: This is the first study to showed that P. aeruginosa forms biofilms in a rabbit model for septic knee arthritis. Furthermore, c-di-GMP is a key signaling molecule which impacts on biofilm formation in rabbit models of knee septic arthritis. Meanwhile, DAP can effectively eradicate the formed biofilm, inhibit biofilm formation, bacterial motility and pyocyanin production of P. aeruginosa and may represent a potential anti-biofilm natural product for therapeutic intervention.

Self-produced dextranase prevents Streptococcus mutans biofilm and dental caries

Nan Liu

Abstract: Dental caries is the most common biofilm mediated disease of the oral cavity caused by some cariogenic bacteria and remains the most prevalent chronic disease in both children and adults. Billions of people suffer dental caries every year in spite of the effort to reduce the prevalence over the past few decades. Streptococcus mutans is the leading member of a specific group of cariogenic bacteria that cause dental caries. S. mutans forms biofilm which is highly resistant to harsh environment, host immunity and antimicrobial treatments. We found that S. mutans biofilm is highly resistant to both antimicrobial agents and lysozyme. DexA is an essential protein in the synthesis machinery of EPS, a key biofilm matrix exopolysaccharide in S. mutans. DexA70, the truncated form of DexA, prevents S. mutans biofilm formation and disassembles existing biofilms within minutes at nanomolar concentrations when supplied exogenously. The co-administration of DexA70 and lysozyme has greater effects in bactericidal and biofilm degradation than when lysozyme or dextranase were tested alone and can inhibit the progress of dental caries in rats. DexA70 treatment markedly enhances biofilm sensitivity to Chlorhexidine and Cetylpyridinium Chloride, indicating its great potential in combating biofilm-related dental caries.

Pseudomonas aeruginosa Quorum Sensing Systems and Iron Homeostasis as Drug Discovery Targets

Pinghua Sun

Abstract: At present, the design of antimicrobial drugs against the mechanism of bacterial resistance has become a research hotspot in this field. Formation of bacterial biofilm is one of the most important bacterial drug resistance mechanisms. Many chronic drug-resistant infections that are permanently unrestrained are closely related to the formation of bacterial biofilms. However, there is no clinical application of biofilm inhibitors. Therefore, it is particularly important to develop new bacterial biofilm inhibitors as antibiotic synergists to overcome bacterial biofilm resistance. In our group, a series of compounds were synthesized for screening as *Pseudomonas aeruginosa* biofilm inhibitors. Many potent biofilm inhibitor (lead compounds) with excellent in vitro biofilm inhibitory effect was identified. The mechanism studies revealed that lead compounds can act not only on the iron homeostasis to cause iron deficiency in bacteria, but also on the QS system to inhibit the secretion of virulence factors (Elastase, Rhamnolipid, Pyocyanin) in *P. aeruginosa*. Further we systematically explored the effects of combining different classes of antibiotics with anti-biofilm compound can effectively enhance the susceptibility of antibiotics in multidrug resistant *P. aeruginosa*. Therefore, it is very likely to be used as antibiotic synergists candidate to be used clinically to overcome *P. aeruginosa* biofilm resistance in future.

Nanoparticle-stabilized encapsulation of borneol and citral: Physicochemical characteristics, storage stability, and enhanced antibacterial activities

Wang Wen, Jianyu Su*

Abstract: Combinations of phytochemical(s) and engineered nanoparticles have attracted immense research interest due to their superior antimicrobial effects against contaminations. Herein, a Pickering emulsion is developed with capsulized phytochemicals including borneol and citral (BC-Cap) stabilized by hydrophilic amine-functionalized silica nanoparticles (SiO2NH2 NPs). The droplet sizes of Pickering emulsion were 5.2 +/- 1.4 mu m under the condition that the concentrations of SiO2NH2 NPs ranged from 0.6 to 1.2 wt.%, and the emulsion showed desirable stability during storage at 40 degrees C for 365 days. In addition, the antibacterial and antibiofilm activities of the Pickering emulsion were investigated. The antibacterial effect of BC-Cap increased by two- to fourfold compared with citral or borneol alone. Treatment of BC/BC-Cap for 4 h eliminated the formation of biofilms generated by *Listeria monocytogenes* (at 5/1.25 mg/ml; 2 x MIC concentration) and *Pseudomonas aeruginosa* (at 5/2.5 mg/ml; 2 x MIC concentration). Further mechanistic studies revealed that the antibiofilm effects of BC-Cap were attributed to its ability to increase the porosity and lytic effects on the cell membrane of bacteria. Findings from the current study support the antibacterial and antibiofilm effects of BC-Cap Pickering emulsion as a promising food additive. Practical Application The Pickering emulsion has potential applications as bacteriostatic agent in packaging materials and general surface disinfectant. The combination of borneol and citral is stabilized by hydrophilic amine-functionalized silica nanoparticles (SiO2NH2 NPs). With the synergistic effects of borneol and citral, the Pickering emulsion shows a promising elimination effect against the formation of biofilms produced by *Listeria monocytogenes* and *Pseudomonas aeruginosa.*

Growth, biofilms and virulence factors of *Pseudomonas aeruginosa* suppressed by the synergistic interaction between bioactive plant extract and antibiotics

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Abstract: Combinational approaches to combat infections caused by multidrug-resistant pathogens is an accepted approach in chemotherapy. Targeting quorum sensing regulated virulence factors and biofilms of *Pseudomonas aeruginosa* may provide a promising anti-infective approach. The synergistic combinations of bioactive plant extract and antibiotics may help to develop new formulations to combat drug-resistant pathogens. In this study, four bioactive plant extracts namely ethyl acetate fraction of *Acacia nilotica* (ANE), *Cinnamom umverum* (CVE), *Punica granatum* (PGE) and hexane fraction of *Syzygium aromaticum* (SAH) were evaluated along with common antibiotics against *Pseudomonas aeruginosa* PAO1. The minimum inhibitory concentration (MIC) values of the plant extracts and antibiotics were first determined by the broth microdilution method. Screening of synergistic interaction between bioactive plant extract with selected antibiotics was carried out using the checkerboard assay. The effect of the most active synergistic combinations was further tested using a time-kill assay. The antibiofilm activity of the most synergistic combinations was determined by crystal violet staining, and biofilm cell viability was determined by MTT assay and confocal laser scanning microscopy analysis. Further, the effect of the most active combination on the virulence factors of *P. aeruginosa* PAO1 and membrane potential was assessed by spectroscopic and CV uptake assay, respectively. *P. aeruginosa* PAO1 was resistant to conventional antibiotics and the MIC value for ANE was 1 mg/ml while for other extracts it was 0.5 mg/ml. In synergy studies, the most active interaction in terms of FIC index was CVE with Cefepime (0.156), CVE with Ceftazidime (0.25) and ANE with Cefepime (0.375). In time kill studies, synergy was observed in most of the combinations tested. Particularly, a combination of CVE+ Cefepime reduced the viable cell number by ~5logs for the first 5 hours and no viable count was observed at 24 hours indicating the bactericidal mode of action. The biofilm formation was drastically inhibited ≥80% by all the above three combinations. The metabolic activity of established biofilms cells treated with CVE+ Cefepime at 2×FIC was reduced up to 35%. The results are also corroborated by live/dead staining of the biofilms where more dead cells were observed in the biofilm matrix treated with the combination compared to the dense biofilm matrix in the control. The QS-regulated virulence factors, pyocyanin and pyoverdine were reduced up to 82% and 78%, respectively by the combination of CVE+ Cefepime. Uptake of crystal violet by *P.*

aeruginosa PAO1 was in the range of 49-60% compared to untreated control (18%). The study demonstrated that plant bioactives can serve as an alternative therapeutic regimen to combat AMR problems when used in combination with antibiotics. Further, mechanisms of synergy and efficacy in infection animal models need to be evaluated to uncover therapeutic potential against biofilm associated infection caused by PAO1 and other drug-resistant bacteria.

Keywords: Biofilms, *Pseudomonas aeruginosa*, Quorum sensing, Antibiotics, Bioactive plant extracts, Synergy

Why do we have to apply engineered biofilms to ecosystems and the environment?

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Abstract: Industrial and green revolutions with the scientific and technological advancement started to cause a chemical (stoichiometric) imbalance in nature, which has led to biochemical and biodiversity imbalances. This in turn has caused to an ecosystem imbalance due to degradation of complex network interactions, eventually collapsing sustainability of managed ecosystems, in particular. It has now been shown however that, biofilm-exuded biochemicals (BFBCs) developed in vitro can reverse these imbalances, thus achieving ecosystem balance and reinstated sustainability via re-established stoichiometric balance, and hence biodiversity balance. This applies for managed and natural ecosystems, including human body ecosystem for better health.The mechanism behind this process is that,degraded biochemical cycles in all living organisms of the degraded ecosystems and the ecosystems per se are triggered by the BFBCs which supply ecologically important, diverse biochemicals with reactive sites(functional groups) that were lost with the human interventions. By now, this theory has been proven in over 150,000 hectares of agroecosystems, paddy in particular during the last three years in Sri Lanka. Similar research on human body ecosystems has already been started.

Keywords: Biofilms, Biochemicals, Biodiversity, Ecosystems, Sustainability

Crude oil degrading microbial biofilms: a synthesis

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Abstract: Biofilms are promising biological systems in which, the increased metabolic activity allows them to thrive in adverse environments. Crude oil is a complex mixture of hydrocarbons which forms a hydrophobic layer on water. Microbial biofilms play a major role in degradation of crude oil in the environment, by forming biofilm structures on oil-water interface, which degrade the oil. Even though themicrobial degradation of crude oil has been studied over decades in laboratory scale experiments, the dependency of the initial concentration of crude oil and the experimental temperature on the rate of degradation has not been properly addressed. Therefore, in this study, the influence of the independent variables, temperature and initial crude oil concentration, on crude oil biodegradation rate was investigated. Data were collected through a literature survey (34 observations in 21 publications) and analysed using SPSS statistical package and MATLAB software. According to the regression analysis, the dependence of the rate of biodegradation of crude oil on its concentration showed a positive 2nd order polynomial relationship ($P < 0.01$), while temperature did not display relationships ($P > 0.01$) with the degradation rate. The effect of the interaction of two independent variables on the rate of degradation was investigated using multiple regression analysis. The predicted model explained ~40% of the observed data ($R2 = 0.37$; $P = 0.003$). The response surface of the model was plotted. According to the response surface, rate of degradation of crude oil is increased with temperature increase. This clearly shows that in tropical region, crude oil degradation rate is higher than that in the temperate region. However, there is a control of the crude oil concentration in water on the degradation rate with respect to temperature. The highest rates of degradation are observed when the initial crude oil concentration is around 1%. In conclusion, it seems that there is a potential of optimizing crude oil degradation by manipulating crude oil concentration in natural settings.

Keywords: Biofilm, Crude oil, Degradation, Temperature

Biofilmed Azorhizobial biofertilizer to replace 50% urea requirement for rice (*Oryza sativa*)

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Abstract: Nitrogen is termed the *sine quo none* or the absolute necessity in rice cultivation. Rice being the staple for more than half of the world population, ensuring the achievement of target yields to feed the population is essential. Nitrogen has a proven and a dependable positive correlation with growth and yield of rice. Hence excess quantity of N-fertilizers is commonly used by the rice farmers. Excessive use of N-fertilizers has led to many environmental hazards and socio-economic complexities. The main objective of this research was to find out a promising, yet a simple alternative for inorganic nitrogen fertilizers applied in rice cultivations. The nitrogen-fixing *Azorhizobium caulinodans* was the organism used in this research. This diazotroph possess several unique characterises that are extremely beneficial for non-legume nitrogen fixation.

First, a series of experiments were conducted to find the best method to inoculate the bacterium, *A. caulinodans* in to the rice plant roots. For easy and accurate detection of the bacterium in and around the rice roots, prior to inoculation, the bacterium was labelled with a green fluorescent protein marker (GFP). Three main types of data were gathered, namely, colonization intensity of the gfp-labelled bacterium measured through epifluorescence intensities, nitrogenase enzyme action through acetylene reduction assay, and the extent of endophytic colonization of the bacterium. When *A. caulinodans* was inoculated to the rice roots as a biofilmed biofertilizer in the presence of naringenin, all three measured parameters provided significantly higher values compared to the other methods used. Second and third steps comprised of the testing the ability of the developed biofertilizer (BF+Naringenin) to fix atmospheric nitrogen and determination of the percent replacement of the inorganic urea fertilizer usage by the biofertilizer in ~~the~~ rice cultivation respectively. A series of pot and field experiments revealed that the developed biofertilizer can replace up to 50% urea fertilizer requirement for rice. When the biofertilizer was added with 50% Urea, the rice plant growth and yield was either similar or better than the addition of 100% urea fertilizer requirement. Also a substantial amount of nitrogen has been fixed by the plant shoot and the grain, as revealed through ^{15}N isotopic data analysis.

Therefore, this method of inoculating *Azorhizobium caulinodans* as a biofilm to rice roots in the presence of flavonoid naringenin with only 50% of the recommended urea fertilizers is an excellent alternative for prevailing detrimental effects arising due to the application of recommended amount of/high amounts of inorganic nitrogen fertilizers. This is a simple, eco-friendly and cost-effective way

of reducing 50% urea fertilizer usage in rice, leading to reduction of the 50% of the problems associated.

Synthetic cyanobacteria/heterotroph communities: engineering in characteristics from biofilming species towards improved consortia robustness

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Abstract: Work in the Ducat lab emphasizes the development and study of cyanobacterial strains engineered as alternative carbohydrate feedstock through the introduction of sugar-export pathways. In strains of model cyanobacteria engineered for the secretion of sucrose, carbohydrate production rates are sufficient to support co-cultured microbes that can convert the sugar into a range of higher-value products. However, for such synthetic microbial communities to be viable for scaled applications, it will be necessary to increase the robustness of the engineered communities against external environmental factors, such as biotic and abiotic stresses. Here, we will briefly review recent developments in the design of mixed cyanobacteria/heterotroph communities and discuss the introduction of quorum-sensing and cell attachment properties with the goal of improving consortia performance.

Keywords: Cyanobacteria communities, Engineering microbial consortia, Biofilms

Cellulolytic activity of fungal-bacterial biofilms developed from brown rot fungi and soil bacteria

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Abstract: Although, cellulolytic microorganisms are responsible for cellulose degradation, recalcitrant nature of the cellulose is a major hurdle that must be overcome to enable the conversion process economical. Many fungi including brown rot fungi (BRF) are capable of degrading cellulose. However, a few of them produce significant quantities of enzyme fractions which hydrolyze cellulose to simple sugars. Biofilms have been recognized for their essential role in the degradation of cellulose in nature through the production of high concentration of enzymes. Thus, this study was aimed at evaluating the cellulolytic activity of individual and combinations of microbial cultures with BRF. Microbial isolations were carried out using coir retting water and leaf litter samples and were inoculated on Congo Red Agar with Carboxy Methyl Cellulose to screen the most effective cellulolytic BRF and bacteria. Fungal-bacterial biofilms (FBB) were developed from the selected microorganisms and evaluated their cellulolytic activities and compared with individual microorganisms in terms of the production of reducing sugar through 3,5-Dinitro Salicylic acid after treating with cellulose powder. FTIR characterization was performed for the best FBB. Molecular identification was performed to identify the individuals of the best performing FBB. Two BRF (F3 and F4) and three bacterial isolates (B3, B4 and B6) were selected as the best cellulolytic microorganisms. Out of them, F4 (*Phellinus noxius* LC066633.1) and B3 (*Bacillus subtilis* -KX711616.1) showed significantly the highest cellulolytic activities ($P < 0.05$). All the biofilm combinations showed significantly higher sugar yield over the monocultures. The highest mean sugar level (2854 ppm) and the highest sugar formation rate were observed in the biofilm of *Phellinus noxius* and *Bacillus subtilis* after eight days of incubation. FTIR characterization of the best performing biofilm confirmed the presence of functional groups available in the cellulolytic enzymes. Thus, the selected FBB combination can be used to enhance the hydrolysis efficiency of cellulose for different industrial applications.

Keywords: Biofilms, Cellulolytic enzymes, Cellulose

Biofilmed Azorhizobial biofertilizer to replace 50%urea requirement for rice (*Oryza sativa*)

Thilini A Perera[1]*, T L Shamala Tirimanne[1]*, G Seneviratne[2], S A Kulasooriya[2]

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Abstract: Nitrogen is termed the *sine quo none* or the absolute necessity in rice cultivation. Rice being the staple food for more than half of the world population, ensuring the achievement of target yields to feed the population is essential. Nitrogen has a proven and a dependable positive correlation with growth and yield of rice. Hence excess quantity of N-fertilizers is commonly used by the rice farmers. Excessive use of N-fertilizers hasled to many environmental hazards and socio economic complexities. The main objective of this research was to find out a promising, yet a simple alternative for inorganic nitrogen fertilizers applied in rice cultivations. The nitrogenfixing *Azorhizobium caulinodans* was the organism used in this research. This diazotroph possesses several unique characterises that are extremely beneficial for non-legume nitrogen fixation. First, a series of experiments were conducted to find the best method to inoculate the bacterium, *A. caulinodans* in to the rice plant roots. For easy and accurate detection of the bacterium in and around the rice roots, prior to inoculation, the bacterium was labelled with a green fluorescent protein marker (GFP). Three main types of data were gathered, namely, colonization intensity of the gfp-labelled bacterium measured through epifluorescence intensities, nitrogenase enzyme activity through acetylene reduction assay, and the extent Of endophytic colonization of the bacterium. When *A. caulinodans* was inoculated to the rice roots as a biofilmed biofertilizer in the presence of naringenin, all three measured parameters provided significantly higher values compared to the other methods used. Second and third steps comprisedof the testing the ability of the developed biofertilizer (BF+Naringenin) to fix atmospheric nitrogen and determination of the percent replacement of the inorganic urea fertilizer usage by the biofertilizer in rice cultivation, respectively. A series of pot and field experiments revealed that the developed biofertilizer can replace upto 50% urea fertilizer requirement for rice. When the biofertilizer was added with 50% urea, the rice plant growth and yield was either similar or better than the addition of 100% urea fertilizer requirement. Also a substantial amount of nitrogen was fixed by the plant shoot and the grain, as revealed through ^{15}N isotopic data analysis. Therefore, this method of inoculating *A. caulinodans* as a biofilm to rice roots in the presence of flavonoid naringenin with only 50% of the recommended urea fertilizers is an excellent alternative for prevailing detrimental effects arising due to the application of recommended amount or high amounts of inorganic nitrogen fertilizers. This is a

simple, eco-friendly and cost-effective way of reducing 50% urea fertilizer usage in rice, leading to reduction of the 50% of the problems associated.

Keywords: Biofilmed biofertilizer, Rice, Urea, *Azorhizobium caulinodans*

Microbial biofilms can shape gut microbiota better than diet-based interventions

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Abstract: Anthropogenic impacts have led to loss of microbial diversity in both managed and natural ecosystems. In fact, excessive use of chemical inputs depletes microbial diversity in agroecosystems whereas modern lifestyle and dietary habits lead do similar consequences in gut microbiota of human body ecosystem. Therefore, restoring the lost microbial diversity is vital to ensure the better functioning of any ecosystem. Our previous studies showed that microbial biofilms developed *in-vitro* are capable enough to restore the lost microbial diversity by secreting a blend of diverse biochemicals that reinstate the microbial diversity and their network interactions in the soil-plant-microbial system. Thus, we hypothesized that the same intervention could also be used to restore altered gut microbiota as gut also contains a subset of microbes originated from the soil through the evolutionary pathway. The present study was designed to examine the ability of biofilm exudates (BFEx, biochemicals secreted by a developed fungalbacterial biofilm) in shaping altered gut microbiota under different dietary patterns i.e. low and high levels of carbohydrate, protein, lipid, and fiber in a simulated gut setting. Four commonly found soil based probiotic gut bacteria *viz.*, *Bacillus clausii*, *Lactobacillus sporogenes*, *Lactobacillus reuteri*, *Bacillus subtilis*, and a fungus, *Aspergillus niger* were used as test microbes. The microbes were grown in mono and mixed culture modes with and without BFEx application. Live microbial cell concentrations of the cultures were measured at 24 and 48 hours after the inoculation using a bacterial viability kit. Furthermore, BFEx was tested for its cytotoxic activity using brine shrimp lethality assay. Results showed that the BFEx produced higher live microbial cell concentrations in all dietary patterns when compared to the treatment without BFEx. However, this result was detected only in the mixed cultures, suggesting the need of microbial interactions to trigger the action of BFEx. Further, the BFEx showed no toxicity to brine shrimp nauplii, instead it supported their survival by providing them with a food source. In conclusion, this biofilm-based method can be used as a promising tool in shaping gut microbiota instead of using diet-based interventions that restrict people from deciding their dietary preferences.

Keywords: Biofilm biochemicals, Fungal-bacterial biofilms, Gut microbiota, Microbial diversity

Development of biofertilizers for Strawberries: a microbial biofilm approach

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Abstract: The usage of heavy doses of chemical fertilizers for crops like strawberry is associated with many environmental and health problems. Thus, the current study focused on developing a biofertilizer for strawberries by using the microbes originating in the strawberry and wild strawberry rhizosphere. Cultivated and wild strawberry rhizosphere associated fungi and bacteria were isolated and screened for strawberry growth promotion. Fungal-bacterial biofilms (FBBs) were developed using those microbes. The potential use of developed FBBs as biofilm biofertilizers (BFBFs) was evaluated with reduced ratesfrom the recommended dosage of chemical fertilizers for strawberry, in glasshouse and field experiments. A simple FBB developed from strawberry growth promoting *Enterobacter* spp. and *Aspergillus* spp. together with 50% of the recommended chemical fertilizers significantly increased the strawberry yield in the glasshouse experiment. The same treatment improved the quality of fruits over the 100% chemical fertilizer application in the field trial. In terms of fruit yield, FBB with 50% of the recommended chemical fertilizers was 152% more profitable than 100% chemical fertilizers treatment in the glasshouse experiment. It was 31% more profitable in the field experiment. Thus, the biofilm developed from *Enterobacter* spp. and *Aspergillus* spp. wasfound to be a potentialmicrobial formulation in strawberry biofertilizations. Further fieldexperiments are needed to investigate the crop response to this biofilm under different soil and climatic conditions.

Keywords: Biofertilizer, Biofilms, Strawberry, Yield

Fungal-bacterial biofilms: promises, progress and prospects

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Abstract: Fungal-bacterial biofilms (FBBs) have created their unique place in the arena of biofilms, mainly through bacterial colonisation on biotic fungal surfaces. Formation of FBBs has shown the biofilm mode to possess enhanced metabolic activities compared to monoculture mode of the microbial life. FBBs have seen promising applications in environmental and agricultural settings as well as emerging avenues of enzyme technology and drug discovery. This has been amply demonstrated in the rhizosphere of plants, where applying biofilmed inocula produced by incorporating a N2-fixing strain to the FBB, shows improved nitrogen fixation in N-deficient agricultural settings. When inoculated directly to soil they also improved N and phosphorus availabilities. FBBs of beneficial endophytes also produced higher levels of acidity and plant growth promoting hormones than in the absence of FBBs. The bioremediation capabilities of FBBs have been further displayed by the successful rhizoremediation of heavy metal-contaminated soils. The successes in using FBBs in biofilmed biofertilizers have shown enhanced soil fertility and plant growth in numerous crops and ornamental plant species. This progress further resulted in the manufacturing of a successful commercial biofertilizer. From the promising technology this posed to be 2 decades ago, to the progress it has made over the years to the prospects of harnessing further multifaceted benefits in the future, FBBs no doubt mark its place in the sphere of biofilm based advances.

Keywords: Fungal-bacterial biofilms, Biofertilizers, Bioremediation, Rhizoremediation

Prospecting cyanobacterium-based biofilms as climate-smart options under elevated CO_2 environments

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Abstract: Photosynthetic prokaryotes, particularly, cyanobacteria exhibit biofilm formation and represent dominant members of several environmental biofilms; however, their utility as inoculants is less studied. In the global climate change scenario, the use of photosynthetic microbes can be of critical significance for sequestering the rising environmental CO_2 and improving the carbon budget in the agricultural landscape. The ecological ubiquity and adaptive nature of cyanobacteria and their specialized Carbon Concentrating mechanisms (CCMs) makes them the ideal choice. Our investigation was aimed towards comparing the C-N metabolism of *in vitro* developed biofilms of cyanobacterium (*Anabaena torulosa*) used as a matrix with *Trichoderma viride* – An Tr, when grown under elevated CO_2 conditions (e CO_2; 650-700 ppm) and ambient (aCO2; 350-400 ppm CO_2). Evaluation of various growth and biochemical attributes revealed significant increase in terms of N fixation, biomass, along with modulation in pigments, proteins, total sugars, under e CO_2. Enzyme activities related to C-N metabolism were significantly influenced and profiles of fatty acid methyl esters (FAME) analyzed showed significant changes under e CO_2 condition. The performance of *Anabaena torulosa-Trichoderma viride* biofilm (An-Tr) and its partners as inoculants in tomato crop grown under elevated and ambient CO_2.was investigated. A distinct enhancement in soil nutrient availability, leaf pigments, with 45-50% increase in the enzyme activities related to carbon and nitrogen assimilation, along with better yields, with An-Tr biofilm or An inoculation, were recorded, particularly under e CO_2 conditions. Fruits from An-Tr treatments under e CO_2 exhibited a higher titrable acidity, along with more ascorbic acid, carotenoids and lycopene content. Multivariate analyses revealed significant ($p \leq 0.05$) interactions, illustrating that cyanobacterial inoculation can be advocated as a strategy to gainfully sequester CO_2. Our investigations reveal the promise of these biofilms as novel organic options for the eco-sustainable management of C, which can also significantly improve yield and fruit quality along with N savings.

Keywords: Cyanobacteria, Biofilms, Elevated carbon dioxide, *Anabaena torulosa, Trichoderma viride*

Application of *Cunninghamella elegans* biofilms in drug metabolite production and pollution removal

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Abstract: The filamentous fungus *Cunninghamella elegans* has long been recognised as a microbial model of mammalian xenobiotic metabolism. The fungus can biotransform drugs, dyes and other xenobiotics via phase I (oxidative) and phase II (conjugative) metabolism yielding the equivalent metabolites to those found in mammals. The standard experiment for biotransformation is to incubate the suspended fungal cultures with the xenobiotic and extract the products which can be chemically determined. We discovered that by adding a steel spring into the culture flask, the fungus grew almost exclusively as a biofilm, and this enabled repeated use of the biomass as a biocatalyst for the production of drug metabolites such as 4'-hydroxyflurbiprofen and 4'-hydroxydiclofenac. By manipulating the incubation time and rejuvenation regime it was possible to improve productivity four-fold and extend the effective biofilm lifetime. In a separate study, the biofilm simultaneously removed the dye malachite green and the toxic metal Cr (VI) from water, in a batch system. Complete removal of the dye and metal was observed for 19 repeated additions to the same biofilm. The mechanism of biofilm formation is largely unknown, although it was observed that 3- hydroxytyrosol is produced by the planktonic cultures in much higher quantities (>10-fold) than in biofilms. Exogenously added 3-hydroxytyrosol inhibits only biofilm growth, suggesting that this molecule has some regulatory function controlling fungal morphology.

Keywords: Biofilms, *Cunninghamella elegans*, Drug metabolites production

Verification of fermentation time of Kombucha 'Tea Fungus'

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Abstract: Kombucha fermentation is traditionally carried out by inoculating a previously grown culture into a freshly prepared tea decoction and incubated under aerobic conditions for 7 – 10 days. The changes in antioxidant activities of Kombucha (which is naturally in biofilm mode) for more than one week of fermentation have not been investigated. Thus, a study was conducted to evaluate the changes in antioxidant and qualitative properties of Kombucha 'Tea Fungus' which has undergone fermentation for 2 months. Physical and chemical parameters were evaluated prior to inoculation of tea with the tea fungus, 1 day after the fermentation, followed by weekly analysis for 2 months. The pH value and TSS of Kombucha biofilm were evaluated as physical parameters. Antioxidant activity parameters of total phenolics content (TPC), Oxygen radical absorbance capacity (ORAC) assay and DPPH radical scavenging assays were determined. All data were presented as means (±standard deviation) of at least three independent experiments ($n \geq 3$). For comparisons between samples, data was analyzed by ANOVA. TSS and pH decreased at a slower rate during the 8 weeks of fermentation. This may have been due to the uptake of soluble solids of the extract by the Kombucha biofilm during the fungal mat development. TPC had not significantly increased with the fermentation time. This was being observed for the first time, since many of the previous studies focused on 7 – 10 days of fermentation. The ORAC values and DPPH scavenging properties of fermented samples had significantly decreased with the fermentation time. Deviations were related to the metabolic rate of culture broth. Overall, the Kombucha samples displayed a decrease in the antioxidant activity during the 2 months of fermentation which was suggestive that the functional properties of the beverage had decreased.

[1] ***Keywords:*** Antioxidants, Fermentation, Tea fungus, Biofilm

Metabolic engineering *Corynebacterium glutamicum* co-culture system to utilize lignocellulose hydrolysate for efficient production of α-carotene

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Abstract: Single microorganism biofilm, as well as microbial consortia biofilm, have been widely used in lignocellulose degradation. Lignocellulose hydrolysate, mainly consist of glucose and xylose, is a potential cheap carbon source for microbial cell factory. However, consuming glucose and xylose could cause to carbon catabolite repression in microorganism, leading to inefficient yield of target product. Herein, we engineered the industrial host *Corynebacterium glutamicum* to utilize glucose and xylose as sole carbon source, respectively. These two strains were co-cultured to utilized lignocellulose hydrolysate for synthesis of high-value product α-carotene. Metabolic engineering single strain and optimizing co-culture system of dual strains increased α-carotene titer by 1.2 fold. In fed-batch fermentation, 2105 mg/L of α-carotene was achieved, which is the highest ever reported in microorganisms.

Novel antimicrobial strategies for bioceramic-based bone substitute biomaterials

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Abstract: Orthopaedic device related infections (ODRIs) are a major source of concern to governments and healthcare institutes around the world. They cause significant impairment to patient health and often lead to implant failure and surgical interventions. Although, Gram-positive Staphylococcus species are the primary causes of ODRIs, Gram-negative bacteria such as *E.coli* can also cause these infections. The traditional strategies to combat ODRIs have relied heavily on antibiotics, which are becoming ineffectual due to the increasing prevalence of antibiotic-resistant bacteria. There is an urgent need to explore alternative antimicrobial agents and develop strategies for utilising them to combat ODRIs.

Antimicrobial peptides (AMPs) and their mimics have emerged as exciting alternatives to traditional antibiotics due to their broad-spectrum activity and the difficulty bacteria have in becoming resistant to them. AMPs are usually cationic, amphiphilic molecules that kill bacteria by disrupting their cell membranes. Mimics of AMPs are designed to replicate AMPs' antibacterial properties but with improved pharmacological and therapeutic properties.

Our research is exploring the activity of AMPs and AMP mimics (peptidomimetics and polymers) as antibacterial coatings for hydroxyapatite (HA) surfaces. HA is a calcium phosphate ceramic used widely in orthopaedic biomaterials research due to its biocompatibility and compositional similarity with the inorganic phase of bone. Four different antibacterial entities, two AMPs, one small molecule peptidomimetic, and a polymer-based AMP mimic, have been successfully coated onto these materials via physical and covalent attachment techniques. For covalent attachment, plasma immersion ion implantation and deposition (PIIID) was utilised. All the coated HA surfaces have exhibited significant antibacterial activity with at least two-log reduction in viability of both Gram-positive and Gram-negative bacteria compared to uncoated control surfaces. The viability of human foetal osteoblast cells on the physically coated surfaces was reduced by 5-40% compared to uncoated controls.

Soil carbon sequestration in lowland paddy cultivation: a Biofilm biofertilizer approach

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Abstract: The amount of carbon (C) added to the atmosphere due to anthropogenic activities is enormous. Consequently, greenhouse effect and climate change have now reached its tipping point. As a global village, even if we are tiring to sequester C with different C farming methods, they have some negative impacts on crop yields. While satisfying food demand to the increasing global population, it is crucial to finding a proper way to remove C from the atmosphere without compromising arable lands and their productivity. In this context, evaluating the potential of paddy soil C sequestration using Biofilm biofertilizer (BFBF) practice and the farmers' conventional practice was the main objective of this study. Paddy soil C sequestration and paddy yields were evaluated during three consecutive seasons. There were significant differences ($p<0.05$) between those two practices in every season, with average paddy yield and soil C sequestration increases by 23% and 16%, respectively in the BFBF practice over the farmers' conventional practice. Thus, the BFBF application in lowland paddy cultivation can be viewed as an innovative way to store more soil C, and it is suggested as a potential approach contributing to reduce global warming while securing food for the future.

Keywords: Biofilm biofertilizer, Mitigation climate change, Paddy Soil carbon sequestration, Sustainable paddy farming

Homologous genes shared between probiotics and pathogens affect the adhesion of probiotics by changing membrane proteins

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Abstract: Clarifying mechanisms underlying the selective adhesion of probiotics and competitive exclusion of pathogens in the intestine is a central theme for host health. Under experimental manipulation of probiotic strain (i.e., *Lactobacillus pentosus* HC-2) adhesion to the shrimp mucus, this study tested the core hypothesis that homologous genes shared between probiotic and pathogen would affect the adhesion of probiotics and exclusion of pathogens by regulating the membrane proteins of probiotics. The results indicated that the reduction of FtsH protease activity, which was correlated with the increase of membrane proteins, could enhance the adhesion ability of *L. pentosus* HC-2 to the mucus. These membrane proteins mainly involved in transporter activity (glycine betaine/carnitine/choline ABC transporter *choS*), transport (ABC transporter, ATP synthase subunit α *atpB*, amino acid permease) and regulation of cellular processes (histidine kinase). These membrane protein genes were significantly ($p < 0.05$) up-regulated except those encoding ABC transporters and histidine kinases in *L. pentosus* HC-2 when it was co-cultured with *Vibrio parahaemolyticus* E1, indicating that these genes could help *L. pentosus* HC-2 to competitively exclude pathogens. This study advances our mechanistic understanding of the selective adhesion of probiotics and competitive exclusion of pathogens in the intestine, and has important implications for screening and applying new probiotics for maintaining gut stability and host health.

Keywords: *Lactobacillus pentosus*, Probiotics, Mucus, Adhesion**,** Competitive exclusion.

LncRNA: A Potential Target for Host-Directed Therapy of *Candida*

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Infection

Abstract: Despite various drugs work against Candida, candidiasis represents clinical management challenges worldwide due to the rising incidence and recurrence rate, as well as epidemics, of new drug-resistant pathogens. Recent insights into interactions between Candida and hosts contribute to exploring novel therapeutic strategies, termed host-directed therapies (HDTs). HDTs are viable adjuncts with good efficacy for the existing standard antifungal regimens. However, HDTs induce other response unintendedly, thus requiring molecular targets with highly specificity. Long noncoding RNAs (lncRNAs) with highly specific expression patterns could affect biological processes, including the immune response. Herein, this review will summarize recent advances of HDTs based on the Candida–host interaction. Especially, the findings and application strategies of lncRNAs related to the host response are emphasized. We propose it is feasible to target lncRNAs to modulate the host defense during Candida infection, which provides a new perspective in identifying options of HDTs for candidiasis.

A Bifunctional Zwitterion-modified Porphyrin for Photodynamic Nondestructive Tooth Whitening and Biofilm Eradication

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Abstract: Tooth staining and biofilm formation are major challenges of dental healthcare around the world. Herein, we designed a bifunctional photodynamic dental therapy (PDDT) strategy based on zwitterion-modified porphyrin (ZMP) prepared by the conjugation of protoporphyrin IX (PP) and superhydrophilic zwitterion moiety for tooth whitening and biofilm eradication. The electron donor-acceptor structure and water solubility of ZMP increased its reactive oxygen species (ROS) output to ~8 times compared with PP under purple light irradiation. Results showed that this new strategy could not only nondestructively whiten tooth by degrading chromogen, but also eradicate ~95% biofilm by disintegrating the extracellular polymeric substances. This study highlighted the superiority of ZMP as a photosensitizer and opened new avenues in exploring PDDT for clinical dentistry to whiten tooth and treat biofilm-induced dental diseases.

Keywords: protoporphyrin IX, zwitterion, photodynamic therapy, tooth whitening, biofilm

The Antimicrobial Peptide AMP-17 Derived from Musca domestica Inhibits Biofilm Formation and Eradiates Mature Biofilm in *Candida Albicans*

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Abstract: The biofilm formation of *C.albicans* represents a major virulence factor during candidiasis. Biofilm-mediated drug-resistance has necessitated search for new anti-fungal treatment strategy. In our previous study, a novel antimicrobial peptide named AMP-17 derived from *Musca domestica* was confirmed to have significant antifungal activity and suppressed hyphal growth greatly in *C.albicans*. In the current work, we aimed to investigate the antibiofilm of AMP-17 in *C.albicans* and explored the underlying mechanism. Antifungal susceptibility assay showed AMP-17 exerted a strong inhibitory efficacy on both biofilm formation and preformed biofilms in *C.albicans*. Furthermore, AMP-17 was found to block the yeast-to-hypha transition and inhibit adhesion of biofilm cells with reduction of cellular surface hydrophobicity. Morphological analysis presented AMP-17 indeed suppressed typical biofilm formation and damaged the structures of preformed biofilm. RNA-Seq identified total of 3054 differentially expressed genes (DEGs) in biofilm forming phrase, 826 DEGs in the preformed biofilm phrase. Gene Ontology analysis and Kyoto Encyclopedia of Gene and Geomes (KEGG) analysis revealed showed that MAPK pathway, biosynthesis of antibiotics and essential components of cell were mainly enriched in the biofilm forming stage, while citrate cycle (TCA cycle), phenylamine metabolism, propanoate metabolism enriched after biofilm mature. Moreover, the co-expressed DEGs in the two pairwise highlighted the terms of transmembrane transporter activity, regulation of filamentation and biofilm formation acted an important role in the antibiofilm effect of AMP-17. Additionally, qRT-PCR confirmed AMP-17 treated biofilms resulted in marked reduction in the levels

of expressions of genes involved cell adhesion (ALS3, HWP1, and SAP5), filamentous growth (ECE1, UME6, EFG1, RFG1, TEC1), MAPK (HOG1, CEK1, CPH1), biofilm matrix (IFD6, CSH1, ALG11), cells dispersal (PES1, SET3), while increased in that of negative regulator in the above terms. In conclusion, our data demonstrated AMP-17 exerted antibiofilm effect against the formation of *C.albicans* biofilms by inhibition of adhesion and yeast-to-hyphae transition mediated probably by MAPK pathway, and against mature biofilms by suppression of extracellular matrix production and cell dispersal besides hyphae repression. Overall, our fundings reveal the underlying antibiofilm mechanisms of AMPs in *C.albicans*, providing an interesting perspective for development of effective antifungal agents with antibiofilm efficacy in *Candida*.spp.

Keywords: AMP-17, *C.albicans*, antibiofilm, adhesion, hyphae, RNA-seq.

Fabrication of patterned calcium carbonate materials through template-assisted microbially induced calcium carbonate precipitation

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Abstract： Patterned calcium carbonate materials with controlled morphologies have broad applications in both environmental and engineering fields. However, how to fabricate such materials through environmental-friendly methods under ambient conditions is still challenging. Here, we report a green approach for fabricating patterned calcium carbonate materials. This eco-friendly approach is based on template-assisted microbially induced calcium carbonate precipitation. As a proof of concept, by varying the templates and optimizing fabrication parameters, different patterned calcium carbonate materials were obtained. The optimized parameters include $C_{Ca^{2+}}$ = 80 mM, T_i = 15°C, and templates made of small-sized $CaCO_3$ particles with a concentration of 1.5 mg/mL, under which better and more sharpen patterns were obtained. Materials with periodic patterns were also fabricated through a periodic template, showing a good scalability of this approach. The results of this study have a great potential in applications where spatially controlled calcium carbonate depositions with user-designed patterns are needed.

Keywords: biomineralization, microbially induced calcium carbonate precipitation (MICP), *Sporosarcina pasteurii*, template, patterning

Dual-species Biofilm Formation of Meat-derived *Escherichia coli* and *Pseudomonas*

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Abstract: The aim of this study was to investigate the dual-species biofilm formation (BF) of meat-derived *Escherichia coli* and *Pseudomonas*. *E. coli* strain $D_{4\text{-}18}$ and *Pseudomonas* strain $Y_{2\text{-}2}10$, respectively isolated from fresh pork and spoilage pork, were co-cultured in sterile glass box containing TSB and stainless-steel sheets (SS) at 25℃, and single-species biofilm on SS surface was prepared as control. The results of CFU counts showed that the interaction changed during the dual-species BF and inhibition was observed more often for *E. coli*. The changes of CFU counts was further confirmed by SEM and CLSM. Meanwhile, the secretion of protein and polysaccharide in L-EPS and B-EPS was inhibited. In addition, absolute quantitative method of reverse transcription-real time PCR found that there was a difference in the expression of related-genes (*papC, fimH, csgA, cbrA, phoR*) between single- and dual-species biofilm, and *csgA* of *E. coli* played a key role in the dual-species BF. This study has increased our understanding of the behavior of multi-species biofilms formed by dominant spoilage bacteria, which will provide a scientific basis for meat safety.

Regulation of the T3SS and quorum sensing systems by a CspA family protein CspC in response to host environment in *Pseudomonas aeruginosa*.

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Abstract: The ability to fine tune global gene expression in response to host environment is critical for the virulence of pathogenic bacteria. However, the mechanisms employed by the *Pseudomonas aeruginosa* to response to host body temperature and nutrients remain to be explored. CspA family proteins are RNA chaperones that modulate gene expression. Here we explored the functions of *P. aeruginosa* CspA family proteins and found that CspC (PA0456) controls the bacterial virulence. Combining transcriptomic analyses, RNA-immunoprecipitation and high-throughput sequencing (RIP-Seq), we demonstrated that CspC represses the type III secretion system (T3SS) by binding to the 5' untranslated region of the mRNA of *exsA*, which encodes the T3SS master regulatory protein. We further demonstrated that acetylation at K41 of the CspC reduces its affinity to nucleic acids. Shifting the culture temperature from 25 ºC to 37 ºC or infection of mouse lung increased the CspC acetylation, which derepressed the expression of the T3SS genes, resulting in elevated virulence. In addition, we found that CspC regulates the quorum sensing (QS) systems by repressing the translation of a QS negative regulatory gene *rsaL*. CspC binds to the 5' untranslated region of the *rsaL* mRNA. Comparing to glucose, itaconate (a metabolite generated by macrophages during infection) reduces the acetylation of CspC, which increases the affinity between CspC and the *rsaL* mRNA, leading to upregulation of the QS systems. Overall, our results revealed novel regulatory mechanisms of the T3SS and QS systems in response to host environment.

Crude extract of Panax quinquefolius has growth inhibitory effect on Mycobacterium abscessus and its biofilm

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Abstract: As an opportunistic pathogen, the prevalence and drug resistance of *Mycobacterium abscessus* are increasing in clinic. Its biofilm can hinder the action of antibiotics on planktonic bacteria, so that antibiotic-sensitive planktonic bacteria increase their resistance, leading to biofilm infections, which requires physical removal. The Panax quinquefolius and its active ingredient infections have certain effects in antipathogenic infection. In this study, the growth of standard *M. abscessus* planktonic was inhibited by crude extract of American ginseng, but the growth inhibition of clinical strains was slightly weaker. The results showed that 50mg/ml PQE had a significant inhibitory effect on the growth of *M. abscessus*, while 25mg/ml PQE had a slightly weaker inhibitory effect, and the inhibitory effect was dose dependent. At the same time, the extract can reduce the number, thickness and biomass of *M. abscessus* biofilm formation, and change the morphology and spatial distribution of *M. abscessus*. Crystal violet staining of strains treated with 25mg/ml of PQE revealed almost no biofilm formation in the wells. In the SEM images, the number of bacteria on glass coverslips and the degree of biofilm aggregation after PQE treatment decreased, which was consistent with the results of crystal violet staining. The Panax quinquefolius extract is expected to be a new antimicrobial agent for the prevention or treatment of *Mycobacterium abscessus* infection and its biofilm infections.

Keywords: Panax quinquefolius; *Mycobacterium abscessus*; Biofilm

Interplay between Persisters in biofilm and chronic mucosa diseases of the gastrointestinal tract

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Abstract: Biofilms are dominant life modes of the microbiomes in both the environment and within the human body. Biofilm formation frequently contributes to increased drug resistance and environmental tolerance. Aside from active signal transduction, secretion, and invasiveness, biofilms contain a subset of persisters that enter a dormant state to withstand environmental stress and antibiotics. Persisters can be reactive without genetic change when the hazards are weakened which performs as a catalyst for chronic infection. Biofilms in the gastrointestinal tract are made up of a diverse range of microorganisms, making it difficult to eliminate pathogenic bacteria effectively. With the help of the multi-strain biofilm exopolymer matrix, persisters can evade antibiotic treatment and immune surveillance. Studies have shown the relationship between biofilm formation (*Escherichia coli*, *Helicobacter pylori*, *Clostridium difficile*) and chronic mucosa diseases including peptic ulcer and inflammatory bowel disease (IBD). In this review, we target the formation of the persisters in biofilms and illustrate their role in resulting chronic infection and inflammation. At the same time, we believe that monitoring intestinal biofilm alternation, particularly the re-emergence of persisters, could be a novel strategy for determining the tipping point in the progression of chronic mucosa diseases. As a result, we reach a conclusion about the dual role of persisters in chronic disease.

Keywords: biofilm; persisters; chronic disease; inflammatory bowel disease (IBD)