Asia-Pacific Biofilms 2024

Scientific Program

November 12-17, 2024 | Guangzhou, China

On behalf of the Organizing Committee, you are cordially invited to attend the 5th International Conference on Biofilms (Asia-Pacific Biofilms 2024), held on November 12-17 of 2024, in Guangzhou of China.

This conference aims to bring together leading academic scientists, engineers, and clinicians globally, primarily from the Asia-Pacific area including China, Japan and Singapore from East or Southeast Asia, India, Sri Lanka and Israel from South or West Asia, Kazakhstan and Uzbekistan from Central 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 2024 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 (EuroBiofilms) and the United States (ASM Biofilms). This year, APB will still be organizing the signature program. The signature program for APB 2024 is Biofilms in Australia supported 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 bacteria**
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
Junyan Liu
The Organizing Committee

Organization

Organizers

South China University of Technology

Southern University of Science and Technology

Singapore Centre for Environmental Life Sciences Engineering

Supporting parties

China Society for Microbiology

ESCMID Study Group for Biofilms

American Society for Microbiology

Australian Society for Microbiology

Overseas Chinese Society for Microbiology (SinoMicro)

Global Chinese Association of Clinical Microbiology and Infectious Diseases (GCACMID)

Center for Biofilm Engineering

Costerton Biofilm Center

National Biofilms Innovation Centre

Biofilm Engineering Lab

ELSEVIER

Biofilm

Mark Shirtliff Biofilm Foundation

Organizing Committee

Founder and Honorable President

Mark Shirtliff

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Enrico Marsili, University of Nottingham

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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 the Asia-Pacific Biofilms 2021/2022 and China Biofilms 2017/2019 series conferences. 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 2024 a successful follow-up to the Asia-Pacific Biofilms 2021/2022 and China Biofilms 2017/2019 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. With a focus on research and encouraging innovation, the entrepreneurial spirit that shines in Shenzhen sees them grow in a multidisciplinary approach that sees substantial collaborations with government and industry. With degree programs across nine schools and 33 departments.

Singapore Center for Environmental Life Sciences Engineering

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.”

China Society for Microbiology

中国微生物学会
Chinese Society for Microbiology

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.

ESCMID Study Group for Biofilms

ESCMID

The objective of ESCMID Study Group for Biofilms (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.

American Society for Microbiology

The American Society for Microbiology is a professional life science organization composed of more than 32,000 scientists, educators and health professionals who are dedicated to promoting and advancing microbial sciences around the world.They know that microbiology has the power to impact lives, and we are uniquely positioned to bring together key stakeholders to institute life-saving programs, advocate for science funding, encourage the next generation of microbiologists and contribute to the resolution of the most pressing global health challenges.They strive to make the microbial sciences the most diverse field in STEM and to be a homebase where microbiologists from every part of our world can come together, connect, learn and recharge for the future.

Australian Society for Microbiology

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.

Overseas Chinese Society for Microbiology (SinoMicro)

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.

Global Chinese Association of Clinical Microbiology and Infectious Diseases (GCACMID)

JGAR is an official journal of and owned by the International Society of Antimicrobial Chemotherapy (ISAC), the Global Chinese Association of Clinical Microbiology and Infectious Diseases (GCACMID), and the Asia-Pacific Society of Clinical Microbiology and Infection (APSCMI).The Journal of Global Antimicrobial Resistance (JGAR) is a quarterly online Open Access journal run by an international Editorial Board that focuses on the global spread of antibiotic-resistant microbes.

Center for Biofilm Engineering

The Center for Biofilm Engineering (CBE) at Montana State University focuses on biofilm-related research with significant industrial, environmental, and health applications. Their research spans various scales, from molecular to field-level studies, and involves collaborations across different disciplines. CBE research addresses challenges in biofilm control, environmental technologies, health, and industrial systems. Some of their applied research areas include antimicrobial efficacy, bioremediation, chronic wound healing, and water treatment systems. CBE also develops standardized

methods for biofilm study and regulation, benefiting industries like healthcare and water management.

Costerton Biofilm Center

The Costerton Biofilm Center is a unique interdisciplinary research center established to explore the field of chronic infections caused by bacteria.

The Center provides a forum for scientists and clinicians and encourages research into the microbial aetiology of biofilms. By integrating translational and clinically relevant research, the Center takes lead in improved prevention and development of new treatments of diseases caused by biofilms. The research aims at explaining the riddle as to why biofilm-bacteria gain the upper hand in the fight against our immune system, and hopefully lead to new and innovative strategies for early diagnosis, treatment and prevention of chronic diseases for the benefit of public health.

National Biofilms Innovation Centre

The National Biofilms Innovation Centre (NBIC) is an Innovation Knowledge Centre (IKC) jointly funded by the BBSRC and Innovate UK. By bringing together the UK's strength in biofilm research, and combining it with the expertise of industrialists, NBIC aims to deliver breakthrough technologies that will have an impact on day-to-day lives.

Led by the University of Southampton, in partnership with the Universities of Liverpool, Nottingham and Edinburgh, the National Biofilms Innovation Centre (NBIC) is a consortium of 63 academic partner institutions across the UK – it is the central hub where academia, industry, government and public policy come together to tackle the grand challenges biofilms present. NBIC's mission is to establish a network of research and innovation capacity to catalyse partnerships with

industry to achieve breakthrough innovations and impact.

Biofilm Engineering Lab

Biofilm Engineering Lab (BEL) is mainly focused on biofilms science and engineering, combining a diversified and unique expertise in microbial processe Swith cellular and molecular interface approaches.

ELSEVIER

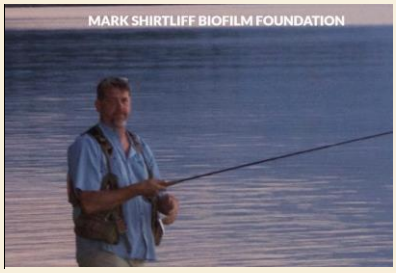
Elsevier as a global leader in information and analytics, Elsevier helps researchers and healthcare professionals advance science and improve health outcomes for the benefit of society. We do this by facilitating insights and critical decision-making for customers across the global research and health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

Biofilm

Biofilm is a multidisciplinary, gold open access journal focused on hypothesis- or discovery-driven studies on microbial biofilms (i.e. multicellular communities, including surface-attached biofilms and suspended aggregates). The journal will cover biofilms in all (micro)environments,

including clinical and industrial settings and the natural environment. We accept articles that describe the basic biology of single or mixed-species biofilms (irrespective of the organism), manuscripts dealing with applied/translational aspects of biofilms, as well as manuscripts describing innovative biofilm methods. Biofilm aims to bring together different disciplines to significantly advance the knowledge of microbial communities, and encourages exploring the interfaces between these disciplines.

Mark Shirtliff Biofilm Foundation



The Mark Shirtliff Biofilm foundation strives to expand the boundaries of knowledge of biofilms through a world-wide exchange of ideas and research.

With a focus on Early Career Researchers, its goal is to support students, educators, and researchers in furthering not only scientific discover, but inclusive scientific community. The work as a foundation supports the following:

- Support for Early Career Researchers via mentoring and education
- Engaging in community and scientific activities for the advancement of knowledge in the field of biofilms
- Raising funds for education and travel for activities including research visits and conference presentations

Agenda

Time and date shown here refers to China Standard Time (GMT+8).
Asia: GMT+9 for JST, GMT+7 for WIT, GMT+5:30 for IST
Oceania: GMT+11 for AEST, GMT+13 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

Nov 12th Registration	
16:00-18:00	**Registration and meeting platform test**
Nov 13th Workshop **Venue: Nanyue Hall**	
9:00-10:30	**Standardization in Biofilm Methods** **Key aspects of spatial structure in the understanding of multispecies biofilms: concepts and methods** Nuno Azevedo, University of Porto, Porto **Assessing the limit of detection for biofilm methods** Albert Parker, Montana State University, Bozeman (Online)
10:30-10:45	Coffee break
10:45-12:15	**Dry surface biofilm study: models and methods** Honghua Hu, Zhejiang University, Jinhua Zhenbo Xu, South China University of Technology, Guangzhou Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou Yu Li, Qiqihar Medical University, Qiqihar Liping Guo, South China University of Technology, Guangzhou
12:15-14:30	Lunch

14:30-16:00	**Methods to Study Biofilms** **Studying the roles of matrix proteins** Boo Shan Tseng, University of Nevada, Las Vegas **Using isothermal microcalorimetry to develop better biofilm models** Kasper Kragh, Symcel, Stockholm (Online)
16:00-16:15	Coffee break
16:15-17:45	**Standardization on anti-biofilm and *in vivo* studies: touching upon fundamental questions** Su Ma & Yulong Tan, Shandong Univ. & Qingdao Agri. Univ., Qingdao Ke Wang, First Affiliated Hospital of Guangxi Medical University, Nanning Zhenbo Xu, South China University of Technology, Guangzhou Yao Sun, Wenzhou Medical University, Wenzhou Xiaomei Lin, South China University of Technology, Guangzhou
17:45-19:00	Dinner & Networking
19:00-20:15	**Getting your article published in biofilm** Tom Coenye, Ghent University, Ghent (Online) Birthe Kjellerup, University of Maryland, College Park (Online)
20:15-20:30	Meet the editors

Nov 14th Medical Microbiology

Venue: Nanhua Hall

Session 1	
Chair	**Garth Ehrlich, Drexel University, Philadelphia** **Zhenbo Xu, South China University of Technology, Guangzhou**
8:50-9:00	**Opening ceremony** **Birthe Kjellerup & Zhenbo Xu**
9:00-9:25	**Biofilm metabolism: the interplay among the stringent response, virulence factor production, and quorum sensing** **Garth Ehrlich, Drexel University, Philadelphia**
9:25-9:50	**Environmental surveillance of infectious diseases for informed risk assessment and public health measures** **Chuanwu Xi, Eastern Institute of Technology, Ningbo**
9:50-10:05	**Co-culture of *Pseudomonas aeruginosa* and *Staphylococcus aureus* triggers *S. aureus* fermentative metabolism in an *in vitro* biofilm flow reactor** **Janette Harro, University of Maryland, Baltimore (Online)**
10:05-10:20	**Host-directed therapies in the fight against antimicrobial-resistant infections** **Nathan Archer, Johns Hopkins University, Baltimore (Online)**
10:20-10:35	**Coffee break**
Session 2	
Chair	**Chuanwu Xi, Eastern Institute of Technology, Ningbo** **Liang Yang, Southern University of Science and Technology, Shenzhen**
10:35-11:00	**How *Pseudomonas aeruginosa* senses surfaces** **Matthew Parsek, University of Washington, Seattle (Online)**
11:00-11:25	**Molecular mapping of the biofilm matrix** **Courtney Reichhardt, Washington University in St Louis, St Louis**
11:25-11:50	**Bacterial biofilm formation: beyond *in vitro* Models** **Rajendar Deora, The Ohio State University, Columbus**
11:50-12:15	**Extracellular aminopeptidase regulates exopolysaccharide production of *Pseudomonas aeruginosa* via quorum sensing** **Luyan Ma, The Institute of Microbiology of Chinese Academy of Sciences, Beijing**
12:15-12:30	**Iron oxide nanoparticles in the prevention and treatment of dental caries and apical periodontitis** **Lei Cheng, Sichuan University, Chengdu**
12:30-13:45	**Lunch**

Session 3	
Chair	**Luyan Ma, The Institute of Microbiology of Chinese Academy of Sciences, Beijing** **Jintao Liu, Tsinghua University, Beijing**
13:45-14:10	**Gut Biofilms, Microbiota, and Pathobionts** **Po-Ren Hsueh, National Taiwan University Hospital, Taipei (Online)**
14:10-14:35	**The role of the infectious microenvironment in chronic infections** **Thomas Bjarnsholt, University of Copenhagen, Copenhagen (Online)**
14:35-15:00	**Essential phage component induces resistance of bacterial community** **Jintao Liu, Tsinghua University, Beijing**
15:00-15:25	**Supportive treatment with S100A8/A9 and hyperbaric oxygen therapy of chronic wounds - experimental studies** **Claus Moser, University of Copenhagen, Copenhagen (Online)**
15:25-15:40	**Diagnosis and treatment of urinary biofilm infections** **Zhijun Song, IRS - Esbjerg and Grindsted Hospital, Esbjerg (Online)**
15:40-15:55	**Coffee break**
Session 4	
Chair	**Rajendar Deora, The Ohio State University, Columbus** **Xin Deng, City University of Hong Kong, Hong Kong**
15:55-16:20	**One step closer to uncertainty - diabetic foot ulcers, biofilms, antimicrobials and fungi?** **Gordon Ramage, University of Glasgow, Glasgow (Online)**
16:20-16:45	**Horizontally transferred cyclic GMP-AMP signaling network in *Escherichia coli* ECOR31 and physiological consequences** **Ute Römling, Karolinska Institute, Stockholm (Online)**
16:45-17:10	**Global regulatory network in *Pseudomonas*** **Xin Deng, City University of Hong Kong, Hong Kong**
17:10-17:35	**Interspecific interactions alter functionality and promote the key-stone species in a synthetic four-species community** **Mette Burmølle, University of Copenhagen, Copenhagen (Online)**
17:35-17:50	**G-quadruplexes and extracellular RNA co-exist in *Pseudomonas* biofilm matrices** **Thomas Seviour, Aarhus University, Aarhus (Online)**
17:50-20:00	**Dinner & Networking**

Nov 15th Biofilms in Australia

Venue: Dongtang Hall

Session 1

Chair Yue Qu, Monash University, Melbourne

9:00-9:30	Uropathogenic *E. coli* biofilms Mark Schembri, University of Queensland, Brisbane
9:30-10:00	Advanced approaches for management of bacterial biofilm wound infections Zlatko Kopecki, University of South Australia, Adelaide
10:00-10:30	New weapons against superbugs Katharina Richter, The University of Adelaide, Adelaide (Online)
10:30-10:45	Coffee break

Session 2

Chair Xenia Kostoulias, Monash University, Melbourne

10:45-11:15	Control of ocular bacterial biofilms by antimicrobial peptides Mark Willcox, The University of New South Wales, Sydney
11:15-11:40	Manipulating bacteria-material interactions with complex surfaces Peter Kingshott, Swinburne University of Technology, Melbourne (Online)
11:40-12:00	Power of plasma-activated water: a novel anti-biofilm lool Heema Vyas, The University of Adelaide, Adelaide
12:00-12:25	Bovine lactoferrin enhances antibiotic killing of *Staphylococcus aureus* biofilms Simon Swift, University of Auckland, Auckland (Online)
12:25-14:00	Lunch

Session 3	
Chair	**Heema Vyas, The University of Adelaide, Adelaide**
14:00-14:30	**Biofilms in ventricular assist device driveline infections: current understanding and perspective** **Anton Peleg, Monash University, Melbourne**
14:30-15:00	**Our biofilm journey in RVVC** **Yue Qu, Monash University, Melbourne**
15:00-15:30	**Biofilms in chronic wounds and the use of non-medicated wound dressings** **Michael Radzieta, Western Sydney University, Sydney**
15:30-16:00	**A new model of endotracheal tube biofilm for basic research and antimicrobial drug discovery** **Freya Harrison, The University of Warwick, Coventry (Online)**
16:00-16:15	**Coffee break**
Nov 15th Special Symposium on Phage Therapy **Venue: Nanyue Hall**	
Chair	**Liang Yang, Southern University of Science and Technology, Shenzhen** **Yanrui Ye, South China University of Technology, Guangzhou**
14:30-15:00	**Phage synthetic biology and phage therapy** **Yingfei Ma, Shenzhen Institutes of Advanced Technology, Shenzhen**
15:00-15:30	**Adaptive evolution of bacterial pathogen *Pseudomonas aeruginosa* against bacterial phages** **Liang Yang, Southern University of Science and Technology, Shenzhen**
15:30-16:00	**Host-phage interaction mediated by prophage-encoded toxin/antitoxin systems** **Xiaoxue Wang & Yunxue Guo, South China Sea Institute of Oceanology, Guangzhou**
16:00-16:30	**A dual-functional bacteriophage protein Dap1 regulates bacterial biofilm and evades Lon protease-mediated anti-phage immunity** **Haihua Liang, Southern University of Science and Technology, Shenzhen**
16:30-16:45	**Genome editing and synthesis of *Pseudomonas aeruginosa* phages** **Yanrui Ye, South China University of Technology, Guangzhou**
16:45-17:00	**Bacteriophage activity in synovial fluid and against synovial fluid induced bacterial aggregates** **James Doub & Guangchao Yu, University of Maryland, Baltimore**
17:00-17:45	**Clinical application of phage therapy** **Anton Peleg, Monash University, Melbourne**
17:45-20:00	**Dinner & Networking**

Nov 15th Early Career Researchers and Students

Venue: Nanyue Hall

Chair	Courtney Reichhardt, Washington University in St Louis, St Louis Heema Vyas, The University of Adelaide, Adelaide
19:00-19:08	Low cell metabolism as a central antimicrobial-resistance mechanism & therapeutic target of staphylococcal biofilms in ventricular assistant device driveline infections Yao Sun, Wenzhou Medical University, Wenzhou
19:08-19:16	Carbon uptake and metabolic characteristics of enriched polyphosphate accumulating organisms from municipal wastewater treatment plants Liping Chen, South China University of Technology, Guangzhou
19:16-19:24	Unraveling the hidden functions of benthic biofilms in drinking water reservoirs through FTICR-MS Analysis Tahir Mehmood, Guangdong Technion-Israel Institute of Technology, Shantou
19:24-19:32	Investigation of quality and microbial dynamics of aged citri reticulatae pericarpium (pericarps of *Citrus reticulata* 'Chachi') during storage Peirong Yu, Guangdong Technion-Israel Institute of Technology, Shantou
19:32-19:40	SPR detection on microbial biofilms: an initial study Haoyue Xue, South China University of Technology, Guangzhou
19:40-19:48	Discovery of *metR* as a regulator of biofilm formation and pathogenicity in *Burkholderia thailandensis* Kaizhong Xu, Hainan University, Haikou
19:48-19:56	Controlling the physicochemical properties of γ-polyglutamic acid in engineered *Bacillus subtilis* PB5760 via redox potential modulation Sunday Oguntomi, University of Nottingham, Ningbo
19:56-20:04	Antimicrobial resistance and biofilm formation in *Candida* strains Xueting Fu, South China University of Technology, Guangzhou
20:04-20:12	The regulatory mechanism of LuxS on the formation of VBNC cells in the biofilm of beer-spoilage *Lactiplantibacillus plantarum* Zhenqing Li, Qingdao Agricultural University, Qingdao
20:12-20:20	Enrichment of salt-tolerant nitrifiers and analysis of their salt-tolerance potential on genomic characteristics Yunyao Liang, South China University of Technology, Guangzhou
20:20-20:28	Development of methods for biofilm analysis: quantification of biofilm viability and amount Biagio Delvecchio, University of Nottingham, Ningbo
20:28-20:36	A study on antibacterial activity and mechanism of carvacrol Ziling Zhi, Guangzhou Medical University, Guangzhou
20:36-20:44	Mechanisms of formation and safety control of different depths of dormant states in foodborne pathogens Yuguo Wang, South China University of Technology, Guangzhou
20:44-20:52	An effective strategy to combat MRSA: the synergistic sensitization with natural compounds Sisi Chen, Guangzhou Medical University, Guangzhou
20:52-21:00	Research on the Adsorption Properties of EPS towards Heavy Metals and Its Mediation in the Synthesis of MeS QDs Liyao Chen, Guangdong University of Technology, Guangzhou

Nov 16th Food Microbiology (Venue 1)

Venue: Nanyue Hall

Session 1	
Chair	**Qingli Dong, University of Shanghai for Science and Technology, Shanghai** **Zhenbo Xu, South China University of Technology, Guangzhou**
9:00-9:25	**Modulation of multispecies biofilms employing antisense oligonucleotides** **Nuno Azevedo, University of Porto, Porto**
9:25-9:40	**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**
9:40-9:55	**Effects of lactic acid bacteria as quorum sensing inhibitors on biofilms of foodborne pathogens** **Qingping Zhong, South China Agricultural University, Guangzhou**
9:55-10:10	**Progress of *Listeria monocytogenes* biofilm risk** **Qingli Dong, University of Shanghai for Science and Technology, Shanghai**
10:10-10:25	**Identification of molecular targets of JX08806 as antibiofilm against *Staphylococcus aureus*** **Chunlei Shi, Shanghai Jiaotong University, Shanghai**
10:25-10:40	**Characterization of *Pseudomonas* spp. contamination and in situ spoilage potential in pasteurized milk production process** **Xin Wang, Northwest Agriculture and Forestry University, Xianyang**
10:40-10:55	**Coffee break**
Session 2	
Chair	**Biao Suo, Henan Agricultural University, Zhengzhou** **Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou**
10:55-11:10	**Dry surface biofilm: an underestimated concern for microbial contamination** **Honghua Hu, Zhejiang University, Jinhua**
11:10-11:25	**High-throughput, rapid and non-destructive detection of common foodborne pathogens via HSI coupled with deep neural networks and support vector machines** **Yu Ding, Jinan University, Guangzhou**
11:25-11:40	**Viable but nonculturable (VBNC) state: an underestimated microbial survival strategy** **Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou**
11:40-11:55	**Modification of cationic antimicrobial peptides and mechanism of antibacterial action at the single-molecule level** **Mingming Guo, Zhejiang University, Hangzhou**
11:55-12:10	**Role of bpfA in adhesion and biofilm formation of *Shewanella putrefaciens* CN32 under cold stress: a comprehensive transcriptomic analysis** **Jun Yan, Shanghai Ocean University, Shanghai**
12:10-12:25	**Study on inhibitory mechanism of linalool against *Listeria monocytogenes*** **Rongrong He, Hainan University, Haikou**
12:25-14:00	**Lunch**

Session 3	
Chair	**Moutong Chen, Guangdong Institute of Microbiology, Guangzhou** **Xiaodong Xia, Dalian Polytechnic University, Dalian**
14:00-14:25	**How chemicals of emerging concern are affecting microbial communities** **Manuel Simões, University of Porto, Porto (Online)**
14:25-14:40	**The role of *rcpA* gene in regulating biofilm formation and virulence in *Vibrio parahaemolyticus*** **Xiaodong Xia, Dalian Polytechnic University, Dalian**
14:40-14:55	**Investigating the potential of L$^{(+)}$-lactic acid as a green inhibitor and eradicator of a dual-species *Campylobacter* spp. biofilm formed on food processing model surfaces** **Efstathios Giaouris, University of the Aegean, Mytilini (Online)**
14:55-15:10	**Platinum-based fluorescent nanozyme-driven "loong frolic pearls" multifunctional nanoplatform for ultrasensitive detection and synergistic sterilization of *B. gladioli*** **Yingwang Ye, Hefei University of Technology, Hefei**
15:10-15:25	**Regulation of non-coding small RNA named *SaaS* in biofilm formation and virulence of *Salmonella*** **Huhu Wang, Nanjing Agricultural University, Nanjing**
15:25-15:40	**Valorization of Soy Whey through Synthetic Biology** **Xiudong Xia, Jiangsu University, Zhenjiang**
15:40-15:55	**Coffee break**
Session 4	
Chair	**Yulong Tan, Qingdao Agricultural University, Qingdao** **Lei Yuan, Yangzhou University, Yangzhou**
15:55-16:20	**Unlocking the potential of biofilm properties in beneficial microbes for one health advancements** **Romain Briandet, University of Paris-Saclay, Paris (Online)**
16:20-16:35	**Role of stringent response factors in response to environmental stress in *Yersinia enterocolitica*** **Jingyu Chen, China Agricultural University, Beijing**
16:35-16:50	**Research and application of key technologies for enhancing biological reaction processes based on cell aggregation effects** **Yong Chen, Nanjing Tech University, Nanjing**
16:50-17:05	**Screening of foodborne active components based on quorum sensing and its effect on *Streptococcus mutans* biofilm and its mechanism** **Su Ma & Yulong Tan, Shandong Univ. & Qingdao Agricultural Univ., Qingdao**
17:05-17:20	**Combating biofilms of foodborne pathogens with bacteriocins by lactic acid bacteria in the food industry** **Xinyi Pang, Nanjing University of Finance and Economics, Nanjing**
17:20-17:35	**Strategies of developing food contact materials with antibiofilm function** **Yue Ma, University of Shanghai for Science and Technology, Shanghai**
17:35-17:50	**From raw ingredients to product - *Salmonella* survival during chocolate production** **Danielle Duanis-Assaf, Volcani Center, Rishon LeZion (Online)**
17:50-20:00	**Dinner & Networking**

Nov 16th Basic Microbiology and Anti-Biofilms (Venue 2)

Venue: Nanhua Hall

Session 1	
Chair	**Wei Hu, Shandong University, Jinan** **Liang Yang, Southern University of Science and Technology, Shenzhen**
8:45-9:10	**Acquisition of biofilm-producing capability made *Yersinia pestis* a flea-transmitted pathogen** **Ruifu Yang, Beijing Institute of Microbiology and Epidemiology, Beijing**
9:10-9:35	**Revealing the heterogeneity of *Pseudomonas aeruginosa* biofilms using single-cell probe-based RNA-sequencing** **Boo Shan Tseng, University of Nevada, Las Vegas**
9:35-9:50	**Extracellular DNA: A multifunctional biofilm component** **Rikke Meyer, Aarhus University, Aarhus (Online)**
9:50-10:05	**Antibiofilm coating and its evaluation methods by ISO.** **Hideyuki Kanematsu, National Institute of Technology, Tokyo (Online)**
10:05-10:20	**LasA from *Pseudomonas aeruginosa* selectively disrupts *Gardnerella vaginalis* biofilm** **Lichuan Gu & Kundi Zhang, Shandong University, Qingdao**
10:20-10:35	**Coffee break**
Session 2	
Chair	**Boo Shan Tseng, University of Nevada, Las Vegas**
10:35-11:00	**Large-scale mechanical spiral waves in bacterial communities** **Yilin Wu, Chinese University of Hong Kong, Hong Kong**
11:00-11:25	**Go with the flow: how shear stress and quorum sensing shape enterococcal virulence in infective endocarditis** **Kimberly Kline, University of Geneva, Geneva (Online)**
11:25-11:40	**Exploring unique aggregate mechanisms in a chronic infection model** **Sophie Darch, University of South Florida, Tampa (Online)**
11:40-11:55	**Novel tetrameric PilZ protein stabilizes stator ring in complex flagellar motor** **Beile Gao, South China Sea Institute of Oceanology, CAS, Guangzhou**
11:55-12:10	**Collective fountain-like flow and fractal wrinkling drive bacterial community morphogenesis** **Boyang Qin, Shanghai Jiao Tong University, Shanghai**
12:10-12:25	**Anti-biofilm enzymes strategy** **Xinjiong Fan, Anhui Medical University, Hefei**
12:25-13:45	**Lunch**

Session 3	**Quorum Sensing in Biofilms**
Chair	**Haihua Liang, Southern University of Science and Technology, Shenzhen** **Yinyue Deng, Sun Yat-sen University, Guangzhou**
13:45-14:10	**Virulence programing and reprogramming in bacterial pathogens** **Lianhui Zhang, South China Agricultural University, Guangzhou (Online)**
14:10-14:35	**Tn-Seq based identification of genes that play a role in antibiotic tolerance of *Pseudomonas aeruginosa* aggregates** **Tim Tolker-Nielsen, University of Copenhagen, Copenhagen (Online)**
14:35-15:00	**Bacterial language: from quorum sensing signal to nucleotide second messenger** **Yinyue Deng, Sun Yat-sen University, Guangzhou**
15:00-15:15	**Bacterial quorum sensing and the strategies of seafood preservation** **Zunying Liu, Ocean University of China, Qingdao**
15:15-15:30	**The mechanism of quorum sensing signaling deterrence of *B. cenocepacia* by rhododendrol and other endophytic metabolites of *A. catechu* L. derived endophytes** **Aiqun Jia, Hainan University, Haikou**
15:30-15:45	**Study on the synergistic mechanism of bacterial inhibition by ITC flavouring substances and essential oils in wasabi** **Gongliang Zhang, Dalian Polytechnic University, Dalian**
15:45-16:00	**Coffee break**
Session 4	**Anti-Biofilms**
Chair	**Haiyan Hu, Sun Yat-Sen University, Guangzhou** **Ning Sun, Guangzhou 11th People's Hospital, Guangzhou**
16:00-16:25	**Novel approaches and tools to predict antimicrobial susceptibility in biofilms** **Tom Coenye, Ghent University, Ghent (Online)**
16:25-16:40	**A multifaceted approach to combating biofilms: computational modeling and novel nanocoatings** **Jinju (Vicky) Chen, Loughborough University, Loughborough (Online)**
16:40-16:55	**Tailored multilayer nanoparticle against resistant *P. aeruginosa* by disrupting the stubborn triad of thickened mucus, dense biofilm and hyperinflammation** **Haiyan Hu, Sun Yat-Sen University, Guangzhou**
16:55-17:05	**The *Staphylococcus aureus* arlS kinase inhibitor tilmicosin has potent anti-biofilm activity in both static and flow conditions** **Yang Wu, Fudan University, Shanghai**
17:05-17:20	**Evolution of antimicrobial resistance in biofilms** **Oana Ciofu, University of Copenhagen, Copenhagen (Online)**
17:20-17:35	**The mechanism of Agr mutation causing persistent *Staphylococcus aureus* infection** **Lei He, Shanghai Jiaotong University, Shanghai**
17:35-17:50	**An antibiofilm peptide AMP-17 inhibits hyphal development in *Candida albicans* exerting antibiofilm effect** **Chaoqin Sun, Guizhou Medical University, Guiyang**
17:50-20:00	**Dinner & Networking**

Nov 16th Early Career Researchers and Students

Venue: Nanyue Hall

Chair Boo Shan Tseng, University of Nevada, Las Vegas
Michael Radzieta, Western Sydney University, Sydney

19:00-19:08	Unveiling the role of fungi in cancers via the metagenomics Yu Li, Qiqihar Medical University, Qiqihar
19:08-19:16	A sex hormone catalyzes biological nitrogen fixation Mahesh Premarathna, South China University of Technology, Guangzhou
19:16-19:24	The influence of dead bacteria on the 3D motion and adhesion of live bacteria Weixiong Zhang, Jimei University, Xiamen
19:24-19:32	Construction of cinnamaldehyde-loaded chitosan nanoparticles functionalized with DNase-I and their anti-biofilm activity against *Listeria monocytogenes* Xueying Du, Nanjing University of Finance and Economics, Nanjing
19:32-19:40	Assessment of eggshell waste as a soil amendment in biosolarization Chunyu Li, Guangdong Technion-Israel Institute of Technology, Shantou
19:40-19:48	Pairwise encounters boost bacterial motion by transient velocity spikes Pu Feng, South China University of Technology, Guangzhou
19:48-19:56	YtnP: one novel quorum quenching enzyme from *Bacillus amyloliquefaciens* W11 inhibits biofilms and spoilage of white radish by Serratia marcescens Zhiwen Ding, Hainan University, Haikou
19:56-20:04	Rapid amperometric determination of bacteria embedded in biocoatings Opeyemi Otemoye, University of Nottingham, Ningbo
20:04-20:12	Effect of sub-MiC of antibiotics on *Staphylococcus aureus* biofilm formation Yaqin Li, South China University of Technology, Guangzhou
20:12-20:20	Preparation of chitosan/sodium carboxymethyl cellulose film loaded with halloysite nanotubes-zingerone and its impact on fish preservation effects. Yanqing Li, Qingdao Agricultural University, Qingdao
20:20-20:28	Pathogenesis and biofilm formation inclinical *Klebsiella pneumoniae* strains Feifeng Zhong, South China University of Technology, Guangzhou
20:28-20:36	Valorization of bioactive compounds extracted or fermented from tea waste using lonic liquids Yuying Zeng, Guangdong Technion-Israel Institute of Technology, Shantou
20:36-20:44	Effect of obstacle size effect on the 3D motion behavior and biofilm formation Xiaolong Zhu, South China University of Technology, Guangzhou
20:44-20:52	Viable but nonculturable state formation and control of pathogenic and spoilage bacteria in rice and flour products Yanling Zhu, Zhongkai University of Agriculture and Engineering, Guangzhou
20:52-21:00	Detection of biofilm in hypervirulent *Klebsiella pneumoniae* isolated from hospital Yuzhu Mao, University of Maryland, Baltimore (Online)

Nov 17th Environmental Microbiology

Venue: Nanhua Hall

Session 1	
Chair	**Zhenbo Xu, South China University of Technology, Guangzhou** **Guanglei Qiu, South China University of Technology, Guangzhou**
9:00-9:25	**Energy efficient wastewater treatment-past to future** **Yan Zhou, Nanyang Technological University, Singapore**
9:25-9:50	**Physical, chemical and microbiological features of saline lakes in Europe** **Tamas Felfoldi, Institute of Aquatic Ecology, HUN-REN Centre for Ecological Research, Budapest, Hungary**
9:50-10:05	**Fungal biofilms: beneficial, harmful, and mysterious frontiers** **Erika Espinosa-Ortiz, Montana State University, Bozeman (Online)**
10:05-10:20	**Quorum quenching driven biofouling control in membrane bioreactor for high-strength wastewater treatment** **Faqian Sun, Zhejiang Normal University, Jinhua**
10:20-10:35	**After the biofilm: bacterial transfer, infections and hand hygiene in a healthcare environment** **Albert Parker, Montana State University, Bozeman (Online)**
10:35-10:50	**Coffee break**
Session 2	
Chair	**Olivier Habimana, Guangdong Technion-Israel Institute of Technology, Shantou** **Jinping Cheng, The Education University of Hong Kong, Hong Kong**
10:50-11:15	**Impacts of silver nanoparticles on freshwater biofilms** **Olivier Habimana, Guangdong Technion-Israel Institute of Technology, Shantou**
11:15-11:40	**Selective succession and enrichment of pollutants in (Micro) plastic biofilms and environmental risks** **Jinping Cheng, The Education University of Hong Kong, Hong Kong**
11:40-11:55	**Advanced genomic sequencing-enhanced wastewater-based epidemiology for monitoring viruses and antibiotic-resistant pathogens** **Xiaoqing Xu, Hong Kong University, China**
11:55-12:10	**Stopping the decay of *Geobacter* electroactive biofilm** **Xing Liu, Fujian Agriculture and Forestry University, Fuzhou**
12:10-12:25	**Lifestyle of marine biofilm bacteria and antimicrobial resource mining** **Wei Ding, Ocean University of China, Qingdao**
12:25-14:00	**Lunch**

Session 3	
Chair	**Yan Zhou, Nanyang Technological University, Singapore** **Jialiang Kuang, South China University of Technology, Guangzhou**
14:00-14:25	**Extracellular DNA and RNA in the drinking water microbiome: quantification, sequencing analyses, and implications** **Bin Cao (Sakcham Bairoliya), Nanyang Technological University, Singapore**
14:25-14:50	**Cabon-emission characteristics of wastewater treatment plants in the Beijing-Tianjin-Hebei region** **Liang Duan, Chinese Research Academy of Environmental Sciences, Beijing (Online)**
14:50-15:05	**Effect of microplastics and antibiotics on the microbiome and resistomes on activated sludge in wastewater treatment process** **Yanping Mao, Shenzhen University, Shenzhen (Online)**
15:05-15:20	**Microalgal-bacterial granular sludge: a novel low-carbon wastewater treatment process sustained by natural light** **Bin Ji, Hunan University of Technology, Zhuzhou**
15:20-15:35	**Rapid recognition of potential microbial resources for bioremediation of organochlorine pesticides and flame retardants** **Jialiang Kuang, South China University of Technology, Guangzhou**
15:35-15:50	**Coffee break**
Session 4	
Chair	**Fangang Meng, Sun Yat-Sen University, Guangzhou** **Le Han, Chongqing University, Chongqing**
15:50-16:15	**The development of membrane bio-contactors for improving nitrogen removal** **Fangang Meng (Zhong Yu), Sun Yat-Sen University, Guangzhou**
16:15-16:30	**Engineering 'trap then release' biofilms for microplastics removal** **Song Lin Chua, Hong Kong Polytechnic University, Hong Kong (Online)**
16:30-16:45	**Hybrid of benthic bioturbation and membrane aerated biofilm ecologically in-situ eliminates overloaded nitrogen in sediments of freshwater system** **Le Han, Chongqing University, Chongqing**
16:45-17:00	**Genomic characterization of *Ca.* Accumulibacter-related polyphosphate-accumulating organisms** **Guanglei Qiu, South China University of Technology, Guangzhou**
17:00-17:15	**Enhanced nitrogen removal in anammox coupled with heterotrophic denitrification processes via directly doing waste activated sludge** **Liang Zhang, Sun Yat-Sen University, Guangzhou**
17:15-17:40	**Intensifying wastewater treatment with sulfur bacterial biofilms** **Di Wu, Ghent University, Ghent (Online)**
17:40-17:45	**Closing ceremony**

Nov 17th Applied Microbiology (Venue 2)

Venue: Nanyue Hall

Session 1	
Chair	**Gamini Seneviratne, National Institute of Fundamental Studies, Kandy** **Junyan Liu, Zhongkai University of Agriculture and Engineering, Guangzhou**
8:30-8:55	**Molecular biology - opportunities and challenges** **Herbert Schellhorn, McMaster University, Hamilton**
8:55-9:20	**Soil biofilm induction to increase crop production and bioremediation: a novel approach** **Gamini Seneviratne, National Institute of Fundamental Studies, Kandy**
9:20-9:35	**Microfluidic investigation on the role of flow dynamics, surface roughness, and bacterial motility on biofilm formation** **Judy Yang, University of Minnesota, Twin Cities (Online)**
9:35-9:50	**Bacteria interactions in soil biofilms: emerging insights from microfluidic techniques** **Peng Cai, Huazhong Agricultural University, Wuhan**
9:50-10:05	**Screening and inhibition mechanism of natural active ingredients on biofilm** **Zaixiang Lou, Jiangnan University, Wuxi**
10:05-10:20	**Biofilm formation in biocontrol *Bacillus* against plant disease** **Yan Li, China Agricultural University, Beijing**
10:20-10:35	**Coffee break**
Session 2	
Chair	**Yigal Achmon, Guangdong Technion-Israel Institute of Technology, Shantou** **Zhenbo Xu, South China University of Technology, Guangzhou**
10:35-11:00	**Diverse functions of the type VI secretion system in complex communities** **Tao Dong, Southern University of Science and Technology, Shenzhen**
11:00-11:25	**Decoding the microbiome volatilome: insights from food waste prevention and valorization** **Yigal Achmon, Guangdong Technion-Israel Institute of Technology, Shantou**
11: 25-11:50	**Biofilm electrochemistry: from characterization to electrofermentation** **Enrico Marsili, University of Nottingham, Ningbo**
11:50-12:05	**Electrochemical biofilm control** **Haluk Beyenal, Washington State University, Pullman (Online)**
12:05-12:20	**Characterization of 3D bacterial adhesion and detachment dynamics** **Xiangjun Gong, South China University of Technology, Guangzhou**
12:20-12:35	**Constructions and applications of biofilm living materials** **Jiaofang Huang, Jiangxi Normal University, China**
12:35-14:00	**Lunch**

Session 3	**Biofilms in Central Asia**
Chair	**Enrico Marsili, University of Nottingham, Ningbo** **Mahesh Premarathna, South China University of Technology, Guangzhou**
13:45-14:10	**Emerging nanotechnologies for targeting pathogenic bacterial biofilms** **Vesselin Paunov, Nazarbayev University, Astana (Online)**
14:10-14:25	**Microbial strategies for enhancing plant stress tolerance in future farming systems** **Dilfuza Egamberdieva, TIAME, National Research University, Tashkent (Online)**
14:25-14:40	**Multi-modal imaging unveils complex biofilm dynamics of probiotic *Lactobacillus* strains from traditional Kazakh dairy** **Almagul Kushugulova, Nazarbayev University, Astana (Online)**
14:40-14:55	**Biofilm mediated bioremediation of heavy metals and organic waste polluted environments** **Iram Liaqat, Government College University, Lahore (Online)**
14:55-15:10	**Cyanobacterial biofilms as a strategy to revitalize and innovate the inoculant technology in agriculture** **Radha Prasanna, ICAR-Indian Agricultural Research Institute, New Delhi (Online)**
15:10-15:25	**Bio solubilization of Eppawala Rock Phosphate (ERP) by fungal-bacterial biofilms and its impact on crop enhancement of potatoes (Solanum tuberosum L)** **Amila Henagamage, Uva Wellassa University, Badulla (Online)**
15:25-15:40	**Coffee break**
Session 4	
Chair	**Yanrui Ye, South China University of Technology, Guangzhou** **Cheng Li, Massachusetts Institute of Technology, Cambridge**
15:40-16:05	**Enhancing industry / academic partnerships** **Darla Goeres, Montana State University, Bozeman (Online)**
15:05-16:20	**Metabolic engineering of non-model microorganisms** **Cheng Li, Massachusetts Institute of Technology, Cambridge**
16:20-16:35	**Accelerating the design of pili-enabled living materials by synergizing bioinformatics, structural biology and synthetic biology** **Yuanyuan Huang, Columbia University, Columbia (Online)**
16:35-16:50	**Heterogeneity of metabolites excreted by fungal, bacterial and fungal - bacterial biofilms** **Darshani Singhalage, Uva Wellassa University, Badulla (Online)**
16:50-17:05	**Biofilm formation and production of EPS by perchlorate reducing microorganisms isolated from serpentine soils in Sri Lanka** **Wajira Balasooriya, Wayamba University of Sri Lanka, Makandura (Online)**
17:05-17:20	**The potential of fungal biofilms in desert soil rehabilitation** **Ishara Manawasinghe, Zhongkai Univ. Agriculture and Engineering, Guangzhou**

Rational development of anti-biofilm drugs with serendipitous results

Garth D. Ehrlich

Drexel University, Philadelphia

Abstract: Our demonstration, nearly a quarter of a century ago, that the recalcitrance of biofilm bacteria to essentially all classes of traditional antibiotics was due to metabolic quiescence (Borreillo et al 2004 & 2006) set the stage for the identification of molecular targets to overcome this metabolic resistance. Subsequently the Collin's lab demonstrated that nearly all antibiotics produce a plethora of reactive oxygen species leading to oxidative stress-based killing (Kohanski et al 2007). Finally, the Singh lab demonstrated the central role for the bacterial RelA protein, which produces the 'magic spot' [(p)ppGpp] trigger for the ancient and universally conserved 'stringent response' in biofilm persister cell formation (Nguyen et al 2011). Their relA knockouts, while still able to form biofilms, were rendered exquisitely sensitive to antibiotics due to their inability to combat oxidative stress. We thus began a quantitative chemical approach taking advantage of the recently published high resolution cryoEM structure of RelA (Brown et al 2015) to screen millions of druggable small molecules to identify potential competitive inhibitors of RelA. From these screens we identified several classes of inhibitors which we have validated in: 1) *in vitro* biochemical studies; 2) *in vivo* microbiological studies; and 3) in both invertebrate and vertebrate model of infection, as being able to serve as co-drugs which re-potentiate traditional antibiotics against biofilm bacteria. Serendipitously, we discovered that these same anti-RelA compounds also inhibited toxin production in both major Gram-negative and Gram-positive pathogens - - at much lower concentrations than required to prevent the stringent response. These observations are due to the fact that bacterial toxin production/virulence factor formation is (often) under control of (p) ppGpp, but is activated at lower levels than are required for triggering the stringent response. Thus, we are exploring our lead compounds as single agents to 'de-fang' pathogens without putting them under the immense selective pressure associated with lethal agents which will hopefully slow the development of resistance.

Environmental surveillance of infectious diseases for informed risk assessment and public health measures

Chuanwu Xi

Eastern Institute of Technology, Ningbo

Abstract: Since the beginning of the pandemic of COVID-19 across the globe, a full knowledge about the SARS-CoV-2 has been lacking and public health policies and prevention approaches have evolved along the way. A limitation public health authorities often face is the non-optimal participation rate in public health measures such as clinical testing and vaccination by the public, which significantly impact the effectiveness of these measures. It is critical to understand the environmental transmission of the SARS-CoV-2 virus and determine the major transmission routes. In addition, tools to determine the overall burden of the spread of the virus prior to outbreaks and clinical interventions have become very valuable. In this talk, we will describe a comprehensive environmental surveillance program of SARS-CoV-2 on a public university campus. We have conducted longitudinal air, surface and wastewater sampling in a wide range of settings on and off-campus to quantify SARS-CoV-2 contamination levels using molecular methods. Air and surface samples were used to evaluate exposure risks and the probability of infection in these settings using quantitative microbial risk assessment (QMRA). Furthermore, wastewater data was used to evaluate the robustness of wastewater-based epidemiology (WBE) for early detection and evaluation of spread of infection in the university community. Our study and others have demonstrated that environmental public health surveillance has the potential to provide insight on real-life environmental exposure risks to infectious respiratory diseases, and WBE can be a valuable early warning alert.

Keywords: SARS-CoV-2, Public Health, Environmental Surveillance, Environmental Transmission, Wastewater-Based Epidemiology (WBE)

Co-culture of *Pseudomonas aeruginosa* and *Staphylococcus aureus* triggers *S. aureus* fermentative metabolism in an *in vitro* biofilm flow reactor

Janette Harro

University of Maryland, Baltimore

Abstract: *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most frequently observed organisms co-infecting the lungs of patients with cystic fibrosis (CF). Until young adulthood, *S. aureus* is the most prevalent bacterial pathogen isolated from CF patients; however, *P. aeruginosa* becomes the dominant organism in most patients. Although the development of new drugs for the treatment of CF has resulted in fewer pulmonary exacerbations and a decrease in bacterial density in sputum, early results from the PROMISE study in the United States have not shown complete eradication of bacteria in most patients. To model the interactions of *P. aeruginosa* and *S. aureus* over time, we used a continuous flow biofilm reactor in which *P. aeruginosa* was introduced to an established *S. aureus* biofilm. Similar to CF patients' airways, *P. aeruginosa* quickly became the dominant species in the biofilm reactor, although *S. aureus* was not completely eliminated. To characterize the interactions between *S. aureus* and *P. aeruginosa*, we performed RNAseq on polymicrobial biofilm samples at 12, 24, and 72 hours after the addition of *P. aeruginosa*. *S. aureus*-only and *P. aeruginosa*-only biofilms served as reference samples. Transcriptomic analysis identified *S. aureus* increased the expression of seven genes at 12 hours, 100 genes at 24 hours, and 44 genes at 72 hours after the addition of *P. aeruginosa*. A single *S. aureus* gene was significantly down-regulated at 12 hours; whereas, 106 genes were down-regulated at 24 hours and 51 at 72 hours after addition of *P. aeruginosa*. *P. aeruginosa* up-regulated 108, 436, and 224 genes at hours 12, 24, and 72 after addition of *P. aeruginosa*, respectively, while down-regulating 268, 446, and 62 genes. At the 24 and 72 hours of interaction, *S. aureus* strongly induced expression of genes required for obtaining iron, including transferrin receptors, transporters, and siderophore synthesis proteins. In contrast, *P. aeruginosa* down-regulated the genes for the iron transport proteins ExbB1 and ExbD1, the heme acquisition protein HasAp, and the iron acquisition protein TonB2. *P. aeruginosa* genes involved in ethanol oxidation and lactate metabolism were up-regulated at 12 and 24 hours, while S. aureus up-regulates alcohol dehydrogenase and lactate dehydrogenase genes. These trends are consistent with previous reports that exposure to *P. aeruginosa* forces *S. aureus* into fermentative metabolism and that *P. aeruginosa* can use *S. aureus* as an iron source.

Keywords: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, flow bioreactor

Host-directed therapies in the fight against antimicrobial-resistant bacteria

Christine Youn[1], Cristina Pontaza[1], Yu Wang[1], Dustin Dikeman[1], Daniel Joyce[1], Martin Alphonse[1], Meng-Jen Wu[1], Sabrina Nolan[1], Mohamed Anany[2], Michael Ahmadi[1], Jeremy Young[1], Aron Tocaj[1], Luis Garza[1], Harald Wajant[2], Nathan Archer[1]*

1Johns Hopkins School of Medicine, USA
2University of Wurzburg, Germany

Abstract: The emergence of antimicrobial-resistant (AMR) bacteria is a serious health burden due to the lack of new antibiotics in development. To overcome this challenge, we investigate the protective immunity against AMR bacteria to develop novel host-directed therapies, as they have the potential to circumvent antimicrobial resistance in bacteria, exhibit broad-spectrum activity by targeting non-specific innate immune mechanisms as well as serve as adjunctive therapies to antibiotics. Of particular interest is the tumor necrosis factor (TNF) receptor, TNFR2, which we discovered to promote neutrophil antimicrobial function and bacterial clearance in the skin. Excitingly, we leveraged these findings into a novel broad-spectrum therapeutic treatment, whereby a TNFR2 agonist was effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* skin infections in a preclinical model. Our work highlights the promise of host-directed therapies as broad-spectrum treatments against AMR bacterial infections in the skin and potentially other infection sites.

Keywords: Skin infections, *Staphylococcus aureus*, Immunotherapy, neutrophils, cytokines

How *Pseudomonas aeruginosa* senses surfaces

Matthew Parsek

University of Washington

Abstract: Surface sensing is a critical step for bacteria to establish colonization on various surfaces. This process elicits diverse responses, such as biofilm formation through c-di-GMP signaling, and pathogenicity and surface-associated twitching through cAMP signaling. These two distinct surface-sensing systems control divergent behaviors. At an air–surface interface, *Pseudomonas aeruginosa* employs the Pil-Chp system and type IV pili to sense the surface, leading to cAMP activation. Conversely, when an aqueous phase is present over the surface, the flagellar apparatus and c-di-GMP-mediated signaling is dominant. These findings emphasize the versatility of *P. aeruginosa* in responding to surfaces and suggest that *P. aeruginosa* tailors different surface behaviors to fit the environment.

Keywords: *Pseudomonas*, c-di-GMP, surface sensing, biofilms

Molecular mapping of the biofilm matrix

Courtney Reichhardt

Washington University in St Louis

Abstract: Most microbes live as multi-cellular communities termed biofilms. This lifestyle protects microbes against harsh conditions including antibiotic treatment and host immune responses. Within biofilms, microbial cells are entangled in a self-secreted extracellular matrix that is rich in biopolymers such as exopolysaccharides and is key to the characteristic properties of biofilms. Detailed analyses of biofilm matrix components, including exopolysaccharides, are essential to our understanding of biofilm development. However, despite the prevalent roles that biofilms play in infections, molecular-level descriptions of the matrix components have been challenging to attain. Biofilms and matrices are neither soluble nor crystalline, which poses challenges to analysis by traditional biochemical techniques. As such, for nearly all biofilm-forming organisms, we still do not have complete or detailed descriptions of what is in the biofilm matrix let alone how the individual components assemble to structure biofilms and protect bacteria. This lack of quantitative understanding means that often we are unable to pinpoint why anti-biofilm therapeutics fail. Solid-state nuclear magnetic resonance (NMR) is uniquely suited to study such complex systems because it provides quantitative information about chemical composition without requiring degradative sample preparation. For example, solid-state NMR has been used to determine the composition of biofilms formed by the human pathogens *Escherichia coli*, Vibrio cholerae, and Aspergillus fumigatus, and the structure of a *Pseudomonas aeruginosa* biofilm matrix exopolysaccharide. *P. aeruginosa* is of particular interest because it is a model for studying biofilms and an important pathogen that causes chronic and difficult to treat infections, including in the lungs of people with cystic fibrosis. Previously, it was determined that up to three structurally distinct exopolysaccharides as well as several proteins and extracellular DNA can contribute to *P. aeruginosa* biofilm matrices. To determine the overall contribution of these components, we used solid-state NMR to investigate biofilms formed by *P. aeruginosa*. Overall, these studies uncovered information about the compositional contributions of the different *P. aeruginosa* exopolysaccharides, which has been inaccessible by other methods, and provide a roadmap for the study of similar complex biological materials.

Keywords: *Pseudomonas aeruginosa*, nuclear magnetic resonance, exopolysaccharides, biofilms, matrix

Bacterial biofilm formation: beyond *in vitro* Models

Rajendar Deora

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Abstract: *In vitro* biofilm models utilizing abiotic surfaces do not always reveal all aspects of host-pathogen interactions since these are heavily dependent on growth media and rely on laboratory conditions. Our laboratory has developed mouse and well-differentiated primary human-epithelial cultures from nose and lungs as models for studying respiratory tract biofilm formation by the obligate human pathogen Bordetella pertussis (Bp). Traditionally, whooping cough or pertussis caused by Bp is described as an acute disease with severe symptoms. However, many individuals who contract pertussis are either asymptomatic or show very mild symptoms and yet can serve as carriers and sources of bacterial transmission. In this presentation, I will talk about how Bp biofilms develop on these biotic systems and the composition of biofilm matrix. I will also discuss the contribution of Bp factors and the differential roles of various pertussis vaccines in influencing biofilm formation in the mammalian respiratory tract.

Extracellular aminopeptidase regulates exopolysaccharide production of *Pseudomonas aeruginosa* via quorum sensing

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Abstract: Biofilm matrix mainly consists of proteins, exopolysaccharides and extracellular DNA (eDNA). *Pseudomonas aeruginosa* aminopeptidase (PaAP) is one of the most abundant matrix proteins in *P. aeruginosa* biofilms and modulates its biofilm development. In a previous study, we have revealed that loss of PaAP enhanced the attachment ability of *P. aeruginosa*. However, it remained unclear how PaAP affects the attachment. In the present study, we have shown that PaAP is the main protein associated with the matrix exopolysaccharide Psl. Loss of PaAP increases the production of Psl, resulting in enhanced attachment of *P. aeruginosa*. Further investigation demonstrated that PaAP represses the transcription of the psl operon through the LasI/LasR quorum sensing (QS) system, rather than other known-psl regulators or cyclic-di-GMP signal molecule. PaAP inhibits the transcription of lasI, thus reducing the biosynthesis of QS signaling molecule, C12-HSL, resulting in a decrease of Psl production. In conclusion, our study demonstrates a novel interplay between two main matrix components via the QS signal, suggesting a mechanism for bacteria to control initial attachment in response to cell densities.

Keywords: *Pseudomonas aeruginosa*, biofilm, aminopeptidase, exopolysaccharide Psl

Iron oxide nanoparticles in the prevention and treatment of dental caries and apical periodontitis

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Abstract: Dental caries is the most prevalent disease globally, and apical periodontitis is one of the most common dental conditions and a leading cause of tooth loss. Dental caries is a prevalent chronic infectious disease causing by dental biofilm that metabolize sugars to produce acid, which, over time, demineralizes hard tissue of the tooth. As dental caries progresses, microorganisms penetrate the hard dental tissue and infect the pulp, leading to pulpitis, which quickly affects the periapical tissues of the tooth, causing apical periodontitis. Therefore, antibacterial biofilms are key to preventing and treating dental caries and apical periodontitis. Iron oxide nanoparticles (IONPs) can inhibit bacterial biofilms through various mechanisms, such as magnetic hyperthermia and reactive oxygen species (ROS), synergistic effects with antibiotics, and have increasingly been applied in recent years for the prevention and treatment of caries and apical periodontitis. In the prevention and treatment of dental caries, IONPs inhibit further microbial infection of dental tissues by sealing dentin tubules. Additionally, under a magnetic field, IONPs penetrate biofilms, where they suppress dental plaque biofilm through synergistic effects with antibiotics and reactive oxygen species (ROS). Furthermore, by modifying the IONP surface, it is possible to create environmentally responsive nanoparticles that selectively inhibit cariogenic bacteria, achieving intelligent and eco-friendly caries prevention. In the prevention and treatment of apical periodontitis, IONPs enhance the antibacterial and sealing effects of root canal disinfectants and sealers within dentin tubules under magnetic forces, effectively blocking microbial infiltration into periapical tissues. They also suppress microorganisms within the root canal system via mechanisms such as magnetic hyperthermia, ROS, and synergistic effects with antibiotics. Notably, IONPs promote osteoblast differentiation while inhibiting osteoclast fusion, reducing the expression of bone-resorptive genes and proteins. This process helps to reduce inflammatory resorption of periapical bone tissue and promotes the healing of resorbed periapical bone.

Keywords: Dental caries, Apical periodontitis, Iron oxide nanoparticles, Anti-biofilm

Gut biofilms, microbiota, and pathobionts

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Abstract: The gut microbiota comprises communities of microorganisms that primarily exist as biofilms. In healthy individuals, these biofilms adhere to the intestinal mucosal surface without contacting the epithelium. Under homeostatic conditions, biofilms are the dominant phenotype of the human gut microbiota, with the relative abundance of their constituents largely influenced by environmental factors. Disruptions in the equilibrium between these biofilms and the host can lead to the emergence of invasive pathobionts from otherwise commensal communities, potentially related to disease pathogenesis. Commensal microorganisms have significant pathogenic potential when, under environmental pressures, they escape the microbiota biofilm and transform into invasive planktonic organisms. Therapeutic strategies aimed at preserving a homeostatic microbiota phenotype could potentially be effective across various genetic backgrounds. These insights offer promising avenues for developing novel therapies targeting microbiota dysbiosis at the phenotype level.

The role of the infectious microenvironment in chronic infections

Thomas Bjarnsholt

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Abstract: The A chronic infection is a complex medical problem. Biofilms have over the last four decades been associated with chronic infections across the health field. It is believed that the bacterial aggregation into biofilms is the cause of the recalcitrance to antibiotic and the host defense in chronic infections. On the other hand, acute infections are supposedly caused by planktonic bacteria. Biofilms are easy to grow in the laboratory and biofilm specific mechanisms have been elucidated. Despite of this we still have difficulties both diagnosing and treating chronic infections, the biofilm explanation does not seem to alleviate the complex problem. A century ago, H. L. Mencken said “For every complex problem there is an answer that is clear, simple, and wrong.”. Maybe the biofilm explanation for chronic infections was too simple and is somewhat wrong? Based on recent publication both aggregated and single cell bacteria are observed in both chronic and acute infections. The difference between acute and chronic infections on the other hand seem to be the metabolic activity of the bacteria. This indicates that the biofilm dogma is wrong, but what is the truth then? In this presentation, I discuss the biofilm dogma related to implant-related infections, wounds and airway infections, both in relation to treatment but also to the immune system. Furthermore, I will discuss the problems and pitfalls regarding diagnosis of these infections. I will discuss, what is a biofilm, do we all know what we talk about *in vitro* vs *in vivo*, and where did we might take the wrong turn? Also, what is the infectious microenvironment of infections, and why do you need to know about this?

Keywords: Biofilms, Microenvironment, aggregated bacteria, single cells

Essential phage component induces resistance of bacterial community

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Abstract: Despite extensive knowledge on phage resistance at bacterium level, the resistance of bacterial communities is still not well-understood. Given its ubiquity, it is essential to understand resistance at the community level. We performed quantitative investigations on the dynamics of phage infection in *Klebsiella pneumoniae* biofilms. We found the biofilms quickly developed resistance and resumed growth. Instead of mutations, the resistance was caused by unassembled phage tail fibers released by the phage-lysed bacteria. The tail fibers degraded the bacterial capsule essential for infection and induced spreading of capsule loss in the biofilm, and tuning tail fiber and capsule levels altered the resistance. Interestingly, latent infections sustained in the biofilm despite resistance, allowing stable phage-bacteria co-existence. Finally, we showed that the resistance exposed vulnerabilities in the biofilm. Our findings indicate that phage lysate plays important roles in shaping phage-biofilm interactions, and open new dimensions for the rational design of strategies to counter bacteria with phage.

Keywords: *Klebsiella pneumoniae*, phage resistance, spatiotemporal dynamics, tail fiber, capsule

Supportive treatment with S100A8/A9 and hyperbaric oxygen therapy of chronic wounds – experimental studies

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Abstract: Non-healing wounds can be defined as wounds without any macroscopic sign of healing within three months despite guideline treatment. Chronic wounds have been found to affect 2-3% of the population in the modern world. Reports suggest involvement of biofilms in 60-80% of the cases of non-healing wounds. Biofilms with their tolerance to antibiotic drugs and the immune system constitutes a special challenge of treatment of patients with chronic wounds. Clinical studies have revealed a suppression of the heterodimeric leukocyte alarmin S100A8/A9 in non-healing wounds as compared to healing wounds. We have confirmed this finding in an animal of chronic wounds with *Pseudomonas aeruginosa* biofilm infection, and we have performed interventions studies with S100A8/A9 showing improved control of pathogens and reduced risk of developing antibiotic resistance. Chronic wounds have also been shown to include anaerobic niches which may impair host responses and antimicrobial effect of antibiotic drugs. Furthermore, it has been shown that hypoxic zones are present deeper in biofilms, even within 50 mm from the biofilm surface. Several bactericidal antibiotics have been shown to include an oxygen dependent killing mechanism by means of intra-bacterial accumulation of reactive oxygen species, in addition to their targeted antibacterial mechanisms. Based on this we have conducted a number of *in vitro* as well as *in vivo* studies on supportive hyperbaric oxygen therapy (HBOT) against chronic wounds with biofilm living *P. aeruginosa*. The studies have been evaluated by means of wound size, quantitative bacteriology, clinical status of the animals and measurement of the host responses. The studies has revealed an antibiotic enhancing effect of HBOT by different mechanisms, promising for clinical implementation. In conclusion, chronic non-healing wounds desperately needs novel supportive treatment candidates to overcome the biofilm tolerance, contributing to the impairment of healing. In this presentation two candidates, feasible to move forward to clinical testing, is advocated.

Keywords: biofilm, chronic wound, S100A8/A9, HBOT, *Pseudomonas aeruginosa*

Diagnosis and treatment of urinary biofilm infections

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Abstract: Urinary tract infections (UTI) are common clinically, especially among the patients, who are carrying permanent medical devices such as various catheters. In the patients carrying permanent urinary catheters, their UTIs in most of the cases are biofilm infections. Highly tolerant to antibiotic treatments and resistant to immune responses are the two major characters of biofilm infections, which become serious challenges globally. Therefore, it becomes crucial to master the correct methods for dealing with biofilm infections. Diagnosis: Biofilm infection is a pathological process of chronic infection accompanied by intermittent acute attacks. The same pathogen can be detected in each acute attack (the bacterial name and drug sensitivity are identical). Treatment: Choose two different types of sensitive antibiotics for combined use; the antibiotic treatment requires as early as possible, high-doses (if renal and hepatic function permit, and matches the bodyweight) and adequate course of treatment. Replace or remove infected catheters at the appropriate time: usually after 2 days of treatment with sensitive antibiotics. Conclusion: Biofilm infection is a complex infection process, which has also its own rules. Understanding the normal occurrence and development of biofilms and their related influencing factors will help us to correctly and effectively manage biofilm infections in clinical practice.

Keywords: Biofilm infection, antibiotic treatment, combination, dosage, catheter

One step closer to uncertainty-diabetic foot ulcers, biofilms, antimicrobials and fungi?

Gordon Ramage

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Abstract: Diabetic foot ulcers (DFUs) are common complication for diabetic patients, often exacerbated by complex polymicrobial biofilm infections. While the majority of DFU studies are bacterial focused, fungi have also been identified. This study aims to investigate the prevalence of fungi in DFUs, as well as their potential role and influence on persistence and wound healing. Consecutive DFU swabs were collected from 128 patients (n=349). Fungal positivity was assessed using enhanced culture and real-time qPCR, and microbiome analysis performed. Meta-data, including antimicrobial management was recorded. Routine microbiology cultures were carried out as part of standard care in the clinics and their results were then compared to our laboratory investigation. Routine and enhanced culture resulted in similar rates of fungal detection (~9%), whereas qPCR resulted in a higher rate of detection (31%). We demonstrated that routine diagnostics methods are sufficient for fungal detection, but enhanced culture methods allow for more precise fungal identification. While fungal presence does not appear to impact patient outcomes in our study, their role within these infections remains poorly understood, and further studies are needed to fully understand their relationship to the microbiome. Microbiome analysis reveals a complex picture, without any clear association with clinical outcome. Moreover, antimicrobial management has little influence in driving the microbial signature of the wound. Together, these data suggest that the wound environment is patient specific and recalcitrant to antibiotic therapies.

Horizontally transferred cyclic GMP-AMP signaling network in *Escherichia coli* ECOR31 and physiological consequences

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Abstract: Cyclic dinucleotides act as intracellular second messengers, modulating bacterial physiology. Cyclic GMP-AMP (cGAMP) synthesized by the dinucleotide cyclase DncV and first been discovered in *Vibrio cholerae* O1 biovar El Tor is a second messenger involved in phage defense. Here I discuss two specific physiological effects of the cGAMP network in *Escherichia coli*. In the animal commensal strain *E. coli* ECOR31 horizontally transferred DncV produces cGAMP to regulate biofilm formation and motility. In contrast to cyclic di-GMP, cGAMP suppresses the rdar biofilm morphotype and cell aggregation. DncV also suppresses swimming and swarming motility posttranscriptional of the class 1 flagellum regulon gene *flhD*. As a second example, overexpression of the patatin-like phospholipase variant $CapV_{Q329R}$, but not wild type CapV, the cGAMP receptor, causes pronounced *sulA*-independent pyridoxine-inhibited cell filamentation in the *E. coli* K-12-derivative MG1655 associated with restriction of flagella production, swimming motility and downregulation of biofilm formation. Conserved amino acids in canonical patatin-like phospholipase A motifs, but not the nucleophilic serine, are required to mediate $CapV_{Q329R}$ phenotypes. Genetically diverse commensal and pathogenic *E. coli* strains and *Salmonella typhimurium* also responded with cell filamentation and modulation in colony morphotype formation to $CapV_{Q329R}$ expression. In conclusion, this work highlights the impact of the substitution of a single conserved amino acid for protein functionality and alteration of host physiology.

Keywords: Cyclic GMP-AMP, *Escherichia coli*, biofilm formation, motility, CapV

Global regulatory network in *Pseudomonas*

Xin Deng

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Abstract: The model Gram-negative pathogen *Pseudomonas* utilises hundreds of transcription factors (TFs) to regulate its functional processes, including virulence, biofilm and metabolic pathways that control its ability to infect host. Although the molecular mechanisms of regulators have been studied for decades, a comprehensive understanding of genome-wide TFs in *Pseudomonas* remains limited. We have mapped the global regulatory network for *P. aeruginosa* and *P. syringae*. This knowledge can advance the development of effective treatment and prevention strategies for infectious diseases caused by *Pseudomonas*.

Keywords: *Pseudomonas*, transcription factor, gene regulation, biofilm, virulence

Interspecific interactions alter functionality and promote the key-stone species in a synthetic four-species community

Mette Burmølle

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Abstract: The Biofilms are highly diverse, harbouring multiple, interacting species. Such interspecies interactions lead to emergent properties unique to the community setting impacting composition, stability and functionality. Here, we I will present recent work demonstrating how interspecific interactions shape community dynamics and functionality of a synthetic four-species biofilm community. We studied four soil isolates, *Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydans* and *P.aenibacillus amylolyticus*, previously shown to interact synergistically in biofilm formation. We used *Arabidopsis thaliana* plants as host to evaluate bacterial impacts. First, the four species were introduced individually and in combination to *Arabidopsis* plants grown in non-sterile conditions. When combined, the four bacterial species protected the seedlings from extended drought, whereas individual strains did not. Also, the introduction of the four species altered the diversity of the indigenous rhizosphere microbiome. Next, the four species were introduced to sterile Arabidopsis roots. We observed enhanced root colonization and plant growth promotion by the four species compared to individual inoculations. *P. amylolyticus* was a weak root colonizer on its own, but in the community it was significantly more abundant. Co-localization analysis identified *P. amylolyticus* as a focal species and pull-out experiments, eliminating *P. amylolyticus* from the community, identified it as a functional keystone species. This shows that interspecific interactions were essential for the keystone species to establish and promote plant growth. In conclusion, bacterial interactions shape community function and should be considered in the design of synthetic communities for biotechnological application.

Uropathogenic *E. coli* biofilms

Mark Schembri

University of Queensland, Brisbane

Abstract: Urinary tract infection (UTI) is one of the most common infectious diseases, with a global annual incidence of ~400M cases. Uropathogenic *Escherichia coli* (UPEC) is the major cause of UTI (>80%) and increasingly associated with rising antibiotic resistance. UPEC form biofilms during infection of the urinary tract, either on the luminal surface of the bladder, intracellularly within bladder superficial epithelial cells, or on the surface of indwelling catheters. This lifestyle of sessile growth promotes enhanced resistance, persistence and increased rates of recurrent UTI. UPEC employ a range of virulence factors to form biofilms, including fimbrial adhesins for attachment and autotransporters to promote cell-to-cell aggregation. In addition, UPEC biofilms are encased in an extracellular matrix comprised of proteins such as curli amyloid fibres and polysaccharides such as cellulose, which form a glue that provides structural support for the biofilm and protects its component cells. This talk will describe our work to understand the key features of UPEC biofilms and their importance for UPEC pathogenesis.

Advanced approaches for management of bacterial biofilm wound infections

Hanif Haidari, Krasimir Vasilev, Zlatko Kopecki

Future Industries Institute, University of South Australia

Abstract: Large areas of open skin provide an inadequate barrier to protect against microbial penetration for patients with chronic non-healing wounds. The success of current treatment is limited, and high cytotoxicity is a major side effect with currently available silver dressing. The aim of this project was to develop a and validate a safe and effective stimuli-responsive silver nanoparticle (AgNP) dressing offering the on-demand release of silver ions triggered by changes in wound microenvironment. Optimization and characterization of the hydrogel delivery system was achieved using cross-linking of N-isopropylacrylamide with acrylic acid and loading with ultrasmall AgNPs. Material characterization, biocompatibility and release studies were undertaken to demonstrate temperature and pH responsive properties and *in-vitro* efficacy against common wound pathogens from EB wounds. Demonstration of *in-vivo* antimicrobial safety and efficacy was achieved using a preclinical murine and porcine models of wound infection and evaluation of early collagen deposition. We demonstrate that the dual-responsive hydrogel is highly sensitive to a typical pH and temperature changes during wound infection development, with restricted release of silver ions at acidic pH (<pH 5.5) and significant release in alkaline conditions (>pH 7.4) (>90% release). The pH dependent release and antimicrobial effect resulted in elimination of 95% of pathogens *in-vitro* at alkaline pH which was confirmed by potent clearing of *S. aureus* infection and significant improvement in healing using preclinical models including faster reepithelization and improved early collagen deposition. This multifunctional hydrogel presents a promising bacteria responsive delivery platform that serves as an on-demand carrier to not only reduce side effects but also boost the antibacterial efficiency based on physiological needs. It offers great potential to improve the way we manage wound infections in patients with chronic wounds, providing a single platform for a long-lasting application in wound management.

New weapons against superbugs

Katharina Richter

The University of Adelaide, Adelaide

Abstract: The rise of antibiotic-resistant bacteria, so called ‘superbugs’, is one of the greatest threats to human health. If we fail to rapidly create new ways to fight superbugs, 10 million people are projected to die every year by 2050. Dr Katharina Richter and her team develop new treatments to join the war on superbugs, pursuing the goal to bring innovations from the lab to real-life applications. Amongst them, shooting lasers and cold plasma technology at bacteria and feeding them a “toxic cocktail”. These innovations may become essential weapons for our arsenal against superbugs and therefore save lives.

Control of ocular bacterial biofilms by antimicrobial peptides

Mark Willcox

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Abstract: Biomaterials are frequently used in the eye for a variety of reasons. For example, contact lenses are used to correct vision, glaucoma stents are used to help reduce intraocular pressure, and intraocular lenses to replace cataractous crystalline lenses. Whilst these are very successful, all are prone to microbial colonisation and biofilm formation. These infections can have a devastating effect on ocular health, leading to blindness in in severe diseases the removal of eyes. My laboratory has been working on ways of minimising microbial colonisation of these medical devices, especially contact lenses. We have produced novel cationic antimicrobial peptides (AMPs) that have broad spectrum antimicrobial action. Their primary mode of action is to disrupt microbial membranes. We have been able to tether these to contact lens materials using a variety of techniques and shown that they retain antimicrobial activity once bound. The compounds not only kill microbes that adhere to them but also prevent the growth of biofilms. Furthermore, our AMPs are active against antibiotic-resistant strains of bacteria. We have taken one of our AMPs, called Mel4, through to Phase III clinical trials as an antimicrobial contact lens. These lenses reduced bacterial adhesion to lenses and reduced the inflammation of the eye associated with wearing lenses contaminated with bacteria. However, we found that the lenses lost activity after 6 days of wear, due to proteolytic degradation. We have therefore produced mimics of our AMPs using several chemical backbones. Our short guanidine-substituted anthranilic amide peptidomimetics and resistant to proteolytic cleavage and we have recently attached these to contact lenses using different covalent linkage strategies. We have found that these mimics have superior anti-adhesion properties to our AMP-coated surfaces. We are now poised to test these in animal models of infection before moving into new clinical trails. I will provide details of this research journey in my talk.

Manipulating bacteria-material interactions with complex surfaces

Peter Kingshott

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Abstract: The demand for advanced, biocompatible surfaces and materials that can prevent both bacterial biofilm formation and enhance cell attachment and growth still exists in the field of biomaterials despite many decades of pioneering research. I will present an overview of the methods we use in our lab to control biointerface interactions including use of colloidal crystals (CCs) to generate micro- and nanostructured surfaces; electrospinning of functional nanofibers; and surface coatings made by cold spray to generate biocompatible control release surfaces. I will show how it is possible to surface engineer new types of colloidal crystal-based patterns and structures on surfaces with a range of colloids of different size and chemistry, including how the colloidal crystals can be used to selectively graft cell adhesive or antimicrobial peptides that enhance both the growth of mammalian cells or kill bacteria, which includes use of CCs as templates to make micro- and nanostructured surfaces for controlling stem cell behaviour. For electrospinning of nanofibers I will present some of our work on making antimicrobial dressings for wound healing applications where we manufacture composite fibers for the release of antimicrobial agents including Ag ions and antimicrobial peptides. Finally, I will discuss some of our research that using porous titanium particles to release antibiotics from particles generated by cold spray processes, and I will also include findings of how a mesoporous silica containing chitosan surface can control the release of eugenol, an essential oil, to prevent the growth a range of bacteria.

Power of plasma-activated water: a novel anti-biofilm tool

Heema Vyas

The University of Adelaide, Australian

Abstract: Plasma-activated water (PAW) demonstrates significant potential in eradicating biofilm and non-biofilm bacteria due to its potent reactive oxygen and nitrogen species (RONS). Here, we examine PAW's anti-biofilm efficacy in treating chronic wound infections and stainless-steel decontamination. PAW pre-treatment markedly enhances the effectiveness of topical antiseptics—polyhexamethylene biguanide, povidone iodine, and MediHoney—against *Escherichia coli* biofilms on human keratinocyte monolayers of an *in vitro* epithelial skin cell model. The RONS in PAW cause rapid damage to the bacterial membrane, thereby potentiating the antiseptics' antibacterial activity. In another study we examined the impact of differing input gases used to generate PAW and its disinfecting capacity. PAW produced with oxygen (PAW-oxygen) was found to be the most effective in eliminating *E. coli* biofilms formed on stainless steel surfaces, outperforming PAW generated from air, nitrogen, and argon. Treatment with PAW-oxygen led to significantly increased intracellular reactive oxygen species (ROS), with the superoxide anion radical identified as crucial for biofilm inactivation and removal. A follow-up study examined the genetic responses of *E. coli* biofilms exposed to sub-lethal PAW-oxygen treatment, with and without superoxide. PAW-oxygen treatment resulted in a 40% variation in gene expression, with 478 genes upregulated and 186 downregulated. Key enriched pathways included sulfur metabolism and oxidative phosphorylation. Knockout mutants of key upregulated genes (*trxC*, *cysP*, and *nuoM*) showed reduced biofilm viability, emphasising the role of superoxide in PAW's antibacterial effects. Together, these studies highlight PAW’s efficacy against biofilms, supporting further research into its diverse applications and real-world utility.

Bovine lactoferrin enhances antibiotic killing of *Staphylococcus aureus* biofilms

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Abstract: Biofilms are associated with around 65% of all infections and 80% of chronic infections. One feature of biofilm infection is tolerance to antibiotics. Tolerance is characterized by the survival of a subpopulation of biofilm bacteria when exposed to high concentrations of antibiotics, where laboratory tests on planktonic cells have indicated susceptibility. An example of tolerance is seen in infections associated with pediatric osteomyelitis in New Zealand, where methicillin susceptible *Staphylococcus aureus* (MSSA) strains predominate, but where treatments with the frontline penicillinase-resistant antibiotic flucloxacillin (FLU) can be ineffective. One approach to this problem may be the inclusion of additional factors to enhance the activity of FLU. We have identified bovine lactoferrin (bLF) as one such factor. *In vitro* CDC-bioreactor grown biofilms of MSSA are 1000-fold more tolerant to FLU than the same MSSA isolates in minimal inhibitory concentration broth assays (MIC). *In vitro* biofilms can be eradicated by FLU at 10´ MIC in the presence of bLF. We have translated this improved activity to the *in vivo* environment in rat models, where delivery of FLU and bLF together is superior to FLU or bLF alone in the treatment of MSSA biofilm infections of the tibia.

Keywords: *Staphylococcus aureus*, biofilm, bone infection, antibiotic tolerance, lactoferrin

Our biofilm journey in RVVC

Yue Qu

Monash University, Melbourne

Abstract: Vulvovaginal candidiasis is a *Candida* infection of the lower female reproductive tract and one of the commonest infectious diseases affecting the morbidity of otherwise healthy women. Emerging experimental evidence suggested that biofilm formation of *Candida species* was involved in the pathogenesis of recurrent vulvovaginal candidiasis (RVVC) and might be the root of treatment failure. Speculation of the roles of *Candida* biofilm formation in RVVC was centered on its impact upon initiation of the infection, and the antifungal resistance of the entire biofilm population. In this presentation, we will discuss important insights in the mechanisms underlying pathogenesis and persistence of human vulvovaginal candidiasis, more specifically, the presence of *Candida* biofilms in the human vagina. This presentation will also shed light on future preventive and treatment strategies for vulvovaginal candidiasis.

Biofilms in chronic wounds and the use of non-medicated wound dressings

Michael Radzieta

Western Sydney University, Sydney

Abstract: The Diabetic related foot ulcerations (DRFUs) remain a common comorbidity in patients with diabetes. Standard of care for treating DRFUs involves the applications of wound dressings, with there being increased adoption of cheaper non-medicated wound dressings which claim to remove bacteria and biofilm from wounds naturally. One example of this is dialkylcarbamoyl chloride coated (DACC) dressings, which bind bacteria and biofilm based on the principle of hydrophobicity which are then claimed to be inactive and can be removed upon removal of the dressing itself. However, there is little clinical evidence demonstrating this in practice. To examine the antimicrobial potential of DACC coated dressings we used a direct contact suspension model to assess the ability of DACC dressings to irreversibly bind bacteria as well as examine it's ability to restrict bacterial metabolic activity, in comparison to silicone and silver containing dressings. Furthermore, DACC coated dressings were applied to a cohort of patients with DRFUs to examine their potential to reduce bioburden within wounds and alter the wound microbiome. Results from this work identified that DACC coated dressings readily bind bacteria in an *in vitro* model, however this binding is not irreversible and does not prevent bacterial growth and metabolism. Furthermore, clinical use of DACC coated dressings again shows that bacteria readily bind to dressings, however this has little to no impact on bacterial load within wounds or in altering the wound microbiome.

A new model of endotracheal tube biofilm for basic research and antimicrobial drug discovery

Freya Harrison

University of Warwick, Coventry

Abstract: The endotracheal tubes (ETTs) used to connect hospital patients to ventilators provide a nidus for biofilm formation; fragments of the biofilm detach and disperse to the lungs, leading to ventilator-associated pneumonia which is costly to treat and often fatal. Treating & preventing ETT biofilm is a major unmet healthcare need. The causal organisms persist despite antimicrobial treatment in 56% of patients; it is hard to predict which antimicrobials might work because the unique *in vivo* environment alters microbial physiology, and antimicrobial sensitivity; and research into antibiofilm ETT coatings has generally failed to translate into useful products. In this talk, I will introduce a novel *in vitro* platform for growing microbial biofilms on sections of ETT in chemically-defined medium that mimics ventilated airway mucus. We have found that our model cues altered biofilm structure, and increased drug tolerance, compared with standard biofilm growth platforms. We have also used the model to assess the ability of matrix-degrading enzymes, and the small molecule gladiolin, to potentiate antibiotic action against ETT biofilms.

Phage synthetic biology and phage therapy

Yingfei Ma

Shenzhen Institutes of Advanced Technology, Shenzhen

Abstract: Bacteriophage has important ecological functions and is a classic model organism in molecular biology research. However, the extreme diversity of bacteriophage in nature makes its cognition very limited. In recent years, focusing on the research goal of analyzing the diversity and function of phages, designing and constructing engineering phages for the prevention and control of bacterial infection, the following work has been carried out: 1) revealing the correlation between phages, bacteria and human health; Based on the culture of intestinal phage, the mechanism of long-term co-existence between intestinal phage and host bacteria was analyzed. 2) Successful clinical application of phage therapy; 3) Developed a general phage genome reduction technique, identified the essential functional gene elements of phage, and obtained the chassis phage. This series of work has expanded the current understanding of phage diversity and function, and laid a foundation for engineering phage research and application. In the future, we plan to further construct efficient and safe engineering phage on this basis, and explore the path of its use in preventing and controlling bacterial infection.

Keywords: Phage synthetic biology, metagenome, engineered phage, phage therapy, antibiotic resistant pathogen

Adaptive evolution of bacterial pathogen *Pseudomonas aeruinosa* against bacterial phages

Liang Yang

Southern University of Science and Technology

Abstract: The opportunistic human bacterial pathogen *Pseudomonas aeruginosa* is able to cause drug-resistant infections, especially in people with underlying immunodeficiencies or inflammatory lung diseases. *P. aeruginosa* has excellent capability to evolve its genomes to adapt to a variety of environmental challengers, which often leads to the emergence and spread of dominant epidemic clones. While most studies are focused on antibiotic resistance and virulence as the driven force for *P. aeruginosa* adaptation and dissemination, the impact of phage defence systems on *P. aeruginosa* adaptation is less understood. In this talk, I will discuss the acquirement of novel DNA methylation elements by *P. aeruginiosa* clinical isolates and illustrate our in-house developed software package Bacmethy-a novel and convenient online tool for investigating bacterial DNA methylation pattern and their transcriptional regulation effects. Next, I will show how acquisition of novel phage defense systems can facilitate adaptation of *P. aeruginiosa* clinical isolates, which might potentially impair the efficacy of phage therapy. Furthermore, I will provide evidence how point mutations of *P. aeruginosa* global regulator *xidF* lead to a change in biofilm fitness in relation to prophage pf4 activation, which might have global impacts on host immune defence and antibiotic resistance.

Keywords: DNA methylation, biofilm, prophage, Phage therapy, *Pseudomonas aeruginosa*

Host-phage interaction mediated by prophage-encoded toxin/antitoxin systems

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Abstract: Filamentous bacteriophages (Pf) play a critical role in biofilm formation and virulence in the opportunistic pathogen *Pseudomonas aeruginosa*. However, mechanisms governing Pf prophage activation in biofilms are largely unknown. Studies of the two filamentous Pf prophage (Pf4 and Pf6) in *P. aeruginosa* biofilms, we revealed novel roles of filamentous encoded toxin-antitoxin (TA) systems. Specifically, a tripartite KKP TA encoded by Pf6 prophage d not only controls virion production of co-resident Pf prophages, but also mediates defense against diverse lytic phages. The balance between kinase and phosphatase expression regulates phage production by controlling the phosphorylation of a host nucleoid binding protein. KKP's also provides defense against lytic phage infection. A conserved lytic phage replication protein inhibits the KKP phosphatase, stimulating toxic kinase activity and blocking lytic phage production. Additionally, we discovered that Pf4 encoded a retron-like TA that functions in phage genome dynamics. The reverse transcriptase and the non-coding RNA PhrD collaborate to edit the Pf4 phage genome to generate superinfective Pf4 variants capable of rapid propagation within biofilms. This genome reduction process preserves genes essential for virion assembly while discarding genes that are not critical for phage propagation. During biofilm formation, mutant cells emerge where intact Pf4 prophages are replaced by these reduced-genome phage variants. These mutants not only facilitate the production of superinfective Pf4 phages but also demonstrate resistance against reinfection by these phages. The discovery of TA role in phage-host interaction expands understanding the versatility of phage biology and its impact on microbial community dynamics within biofilms.

Keywords: Biofilm, host-phage interaction, superinfection, toxin-antitoxin systems, reverse transcriptase

A dual-functional bacteriophage protein Dap1 regulates bacterial biofilm and evades Lon protease-mediated anti-phage immunity

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Abstract: As absolute parasites, bacteriophages (phages) have evolved diverse strategies to overcome bacterial defense mechanisms and redirect the host metabolism to ensure successful phage propagation. Here, we identify a phage protein, named Dap1, from P. aeruginosa phage PaoP5 that exhibits dual functions in modulating host bacterial behavior as well as contributing to phage fitness. Expression of Dap1 in P. aeruginosa reduces bacterial motility and promotes biofilm formation via Dap1 direct binding to and interfering with the function of DipA. Meanwhile, the deletion of *dap1* in PaoP5 significantly impairs phage fitness. Mechanistically, we documented that Dap1 directly binds to phage HNH endonuclease, and protects it from Lon-mediated HNH degradation, thus promoting phage genome packaging. Phage dap1-deletion mutant is outcompeted by wild-type phage and is ineffective in phage therapy. This study highlights a phage protein's remarkable dual functionality to modulate bacterial behaviors by interfering with biological pathways and confer fitness to phage by helping it evade bacterial Lon-mediated anti-phage defense mechanism.

Keywords: Dap1 protein, Lon protease, HNH endonuclease, Phage therapy, *Pseudomonas aeruginosa*

Genome editing and synthesis of *Pseudomonas aeruginosa* phages

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Abstract: Engineering phages as therapies that can address the limitations of traditional antibiotics offers new approaches in the fight against antibiotic-resistant bacteria. Here, we undertook a comprehensive study of bacteriophages targeting *Pseudomonas aeruginosa*, a significant opportunistic pathogen. We developed efficient CRISPR-Cas12a-based genome editing tools for precise manipulation of phage genomes. This enabled us to identify dispensable genes and create recombinant phages with enhanced therapeutic potential. Furthermore, we introduced a novel two-plasmid transposon insertion system for site-specific gene disruption, facilitating a deeper understanding of phage biology. Lastly, we demonstrated the feasibility of synthesizing the complete genome of a headful packaging phage using the yeast TAR system. This approach allows for the rational design and construction of synthetic phages with tailored properties, opening new avenues for phage therapy and biotechnology.

Keywords: *Pseudomonas aeruginosa*, Phages, Genome editing, Genome synthesis

Bacteriophage activity in synovial fluid and against synovial fluid induced bacterial aggregates

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Abstract: Bacteriophage therapy is a promising adjuvant therapy for the treatment of periprosthetic joint infections. However, limited knowledge is available on the activity of bacteriophages in synovial fluid (SF). Therefore, the aims of this study were to evaluate the activity of a bacteriophage in SF as well as the ability of that bacteriophage to prevent the formation of and eradicate bacteria in SF induced aggregates. A *Staphylococcus aureus* clinical isolate and a clinically used bacteriophage (UMB phage 3) were used in this study. Assessment of aggregate formation in different SF concentrations was initially conducted. Afterward, the activity of UMB phage 3 in SF to reduce bacterial concentrations was conducted compared to activity in tryptic soy broth. As well, the ability to prevent and eradicate bacteria in SF induced aggregate was evaluated over a 24-hour period. SF induced aggregates formed rapidly in all SF concentrations (10%-100%) with aggregate sizes that ranged from 10μm-2 mm. There was a statistical significant reduction in bacteriophage activity in SF compared to tryptic soy broth ($p < 0.05$) and UMB phage 3 could not prevent the formation SF aggregates. UMB phage 3 also could not eradicate bacteria in the SF induced aggregates and this was not secondary to resistance. The reduced activity of UMB phage 3 in SF was likely a consequence of the increased viscosity of SF inhibiting the interaction of bacteriophages with bacteria. Likewise, the inability to eradicate bacteria in SF induced aggregates is likely secondary to bacteriophage receptors being masked by interactions with synovial fluid polymers. While these findings show a shortcoming of bacteriophage therapy in periprosthetic joint infections, the knowledge gained should spearhead further research to ultimately devise effective and reproducible bacteriophage therapeutics.

Unraveling the hidden functions of benthic biofilms in drinking water reservoirs through FTICR-MS analysis

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Abstract: Effluent discharge and the geospatial features of catchment areas have multidimensional impacts on the ecosystem and functioning of receiving waters. In essence, the introduction of effluent modifies the components of dissolved organic matter (DOM). It plays a critical role in maintaining the health and functionality of benthic biofilms and provides essential nutrients and energy for microbial communities, enhancing biofilm productivity, but it can hinder disinfection and advanced treatment processes at high concentrations. Since the composition of benthic biofilms and DOMs can serve as a proxy for the pollution profile of a water reservoir, by analyzing the specific organic compounds and microbial communities present in biofilms and DOM, researchers can infer the types and sources of pollutants affecting the water body. Despite the availability of various characterization techniques, a high-resolution technique is deemed necessary for in-depth analysis of DOM. This study pioneers using FTICR-MS analysis to characterize DOMs in benthic biofilms. The exceptional resolution and mass accuracy of FTICR-MS provided unprecedented detail in identifying the intricate mixtures of organic compounds within biofilms. About 48 water and benthic biofilm samples from three different freshwater reservoirs of the Chaoshan region (Guangdong, China) were collected. Samples were carefully transported to the laboratory, processed, and stored appropriately for physiochemical and biological analyses. Total organic carbon (TOC) was 2.69 to 6.13 mg/L across all the reservoirs. The average molecular weight of all detected compounds was higher in benthic samples compared to bulk water samples (irrespective of intensities). Double bond equivalent (DBE) and aromaticity index (AI) were significantly higher in the benthic samples, suggesting the presence of more aromatic compounds. The nominal oxidation state of carbon (NOSC) was higher in benthic samples, indicating a higher degree of oxidized state.On the contrary, the larger positive values of (DBE−O)/C imply a higher degree of unsaturation found in bulk water samples of all three reservoirs. Considering the compound classes, highly unsaturated and phenolics (HuPh) constituted almost half (i.e., 46 – 49%) of the total compounds detected. Despite the slight differences among reservoirs, polycyclic aromatic (PAH) compounds were more in the benthic samples (31%) compared to bulk water (22%). Polyphenols were less in the benthic samples compared to bulk water. Interestingly, unlike N-containing compounds, S-containing compounds were significantly lower in the benthic samples. Most importantly, FI and BIX values were higher in benthic samples, suggesting microbial-derived DOMs. 3D EEM clearly distinguishes molecular classes of fluorescent DOMs in bulk and benthic samples. Proteins were prominent in the bulk water, whereas humic acid-like substances were significantly higher in benthic samples. Detailed microbial analyses revealed a clear distinction of microbial diversity in the bulk water and benthic biofilms. We believe the application of FTICR-MS in studying the interactions between benthic biofilms and bulk water pollutants provided significant insight into understanding the microbial activities and transformation of organic matter in the benthic biofilms. It highlights the composition and dynamics of biofilm-associated pollutants and microbial diversity and considers water quality monitoring and environmental protection implications.

Keywords: Benthic Biofilm, DOM, Freshwater, FTICR-MS, Microbial diversity

Investigation of quality and microbial dynamics of aged citri reticulatae pericarpium (pericarps of *Citrus reticulata* 'Chachi') during storage

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Abstract: Citri Reticulatae Pericarpium (CRP), particularly including the pericarp *of Citrus reticulata* 'Chachi' (GCP), is the sun-dried pericarp derived from *Citrus reticulata* Blanco and its cultivars. CRP has been widely used as a food, a dietary supplement and traditional Chinese medicine (TCM). Since ancient times in China, CRP has been traditionally aged by storing it in a shade and dry place to improve efficacy and reduce side effects. Typically, CRP products are aged for periods ranging from one year to over ten years, with TCM practices asserting that aging enhances the quality of CRP. This enhancement may be scientifically linked to the transformation of components mediated by environmental microorganisms, leading to changes in the content of bioactive compounds, particularly flavonoids and volatile oils over the years. Our study aims to investigate the microbial changes occurring on the surface of CRP and their impact on the quality of aged GCP throughout its shelf life. We simulated different storage environments with controlled temperature and humidity, and then assessed the microbial dynamics and flavonoid levels in GCP during storage. Microbial respiration in GCP was monitored by measuring the production or consumption of oxygen (O_2), carbon dioxide (CO_2) and methane (CH_4) gases throughout the shelf life, with O_2 and CO_2 validated as prospective indicators of microbial activity. Our results indicate that both the duration of storage simulation and the aging periods of GCP significantly affected the contents of key flavonoids such as hesperidin, didymin, nobiletin, tangeretin and 3,5,6,7,8,3',4'-heptamethoxyflavone ($p < 0.05$), which are critical to bioactivity and quality of GCP. By applying next-generation DNA sequencing and bioinformatics analyses, we examined the dynamics of bacterial and fungal communities in GCP during storage, identifying significant interactions between microbial communities and flavonoids levels. Notably, correlation analysis revealed that specific microbial phyla, such as *Basidiomycota*, *Acidobacteriota* and *Cyanobacteria* exhibited a significant positive correlation with hesperidin, the primary flavonoid ($p < 0.05$), suggesting their potential impact on flavonoid metabolism. These findings provide in-depth insights into the microbial-mediated transformation of bioactive compounds in GCP. Overall, this study highlights the significant effect of microbial communities on the quality of aged GCP throughout the aging process.

Keywords: *Citrus reticulata* 'Chachi', Aging process, Flavonoids, Microbial communities, Quality

Discovery of *metR* as a regulator of biofilm formation and pathogenicity in *Burkholderia thailandensis*

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Abstract: Melioidosis is a chronic infection caused by *Burkholderia pseudomallei*, which is particularly prevalent among immunocompromised patients and is associated with high pathogenicity and mortality rates. *B. thailandensis*, a closely related low-virulence laboratory model, is extensively employed to investigate the pathogenic mechanisms of melioidosis. The ability of *B. thailandensis* to form biofilms is known to confer resistance to antibiotics and host immune responses, complicating treatment strategies for chronic infections. However, the mechanisms underlying biofilm formation and regulation in *B. thailandensis* remain poorly understood. This study conducted laboratory evolution experiments using *B. thailandensis* E264. The mutant library was constructed and screened to obtain the transcription regulatory factor MetR related to biofilm formation. Gene knockout experiments confirmed the critical involvement of MetR in biofilm development, with the knockout of *metR* resulting in a significant impairment of biofilm formation capabilities. Further investigations employing RNA-seq, RT-qPCR, and EMSA revealed that biofilm formation is regulated by MetR through the positive modulation of the expression of *N*-acyl homoserine lactone synthase BtaI1, resulting in reduced levels of the signaling molecule C8-HSL. This reduction diminishes the binding of C8-HSL to BtaR1, thereby facilitating biofilm development. Additionally, it was found that MetR influences the production of various virulence factors, including extracellular proteases, rhamnolipids, and exopolysaccharides, and modulates pathogenicity in *Galleria mellonella* larvae. In conclusion, the significant role of MetR in regulating biofilm formation and virulence in *B. thailandensis* is highlighted. The elucidation of the "MetR-BtaI1/BtaR1-biofilm" signaling pathway provides new insights into potential therapeutic strategies for melioidosis.

Keywords: Melioidosis, *Burkholderia thailandensis*, biofilm, transcriptional regulatory factor MetR, quorum sensing

Controlling the physicochemical properties of γ-polyglutamic acid in engineered Bacillus subtilis PB5760 via redox potential modulation

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Abstract: The ability of electroactive microorganisms to couple an extracellular electrode to their metabolic pathway is increasingly being explored for several applications. One of such is electrofermentation, in which metabolic carbon flux can be steered towards a product of interest during biosynthesis, leading to improved yield. However, the effects of electrofermentation on the physicochemical properties of such products has not been reported. In this study, we investigated the effects of controlled application of oxidative electrochemical potential to modulate the metabolism of *B. subtilis* PB5760, genetically modified to overproduce a biotechnologically relevant biopolymer called poly-gamma glutamic acid (γ-PGA) in the presence of isopropyl β-D-1-thiogalactopyranoside. The biofilms were grown on a 2 x 2 x 0.4 cm stainless steel working electrode and conditioned for anode respiration by limiting dissolved oxygen concentration in the fermentation medium and polarizing the electrode at open circuit potential, 0.2 V and 0.4 V. The culture parameters were as follows, pH 8, 30 ℃, agitation 200 rpm, incubation time 48 h. The molecular weight of γ-PGA obtained was characterized by size exclusion chromatography. The mRNA transcript of *pgdS* in the cells grown under different potentials were monitored by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). The results obtained indicates a significant decrease in the molecular weight of γ-PGA in a potential-dependent manner. Steady application of 0.2 and 0.4 V reduced the molecular weight of the biofilm matrix γ-PGA. Similarly, the relative levels of *pgdS* expression observed under in cells growing at various potentials show a positive correlation with the molecular weight data. This study provides a novel approach for producing γ-PGA with a defined range of molecular weight based on controlled application of electrochemical potential.

Keywords: Electrofermentation, electrochemical potential, *B. subtilis* PB5760, γ-PGA, molecular weight

The regulatory mechanism of LuxS on the formation of VBNC cells in the biofilm of beer-spoilage *Lactiplantibacillus plantarum*

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Abstract: *Lactiplantibacillus plantarum* stands as one of the most prevalent culprits in beer-spoilage. During production, it often persists in the guise of biofilm and can transition to a viable but non-culturable (VBNC) state triggered by environmental stressors. This transition has the potential to alter the beer's flavor profile and compromise its biological stability, ultimately leading to significant economic losses. In this study, we zeroed in on *L. plantarum*, a notorious beer-spoiling bacterium. We developed a culture model to simulate the conditions that induce the formation of membrane cells and investigated the intricate rules governing the induction and resuscitation of these cells into the VBNC state, particularly under the stress of high concentrations of isomerized hop extracts. Additionally, we delved into the impact of AI-2 on the biofilm formation capabilities associated with the VBNC state. To further our understanding, we constructed strains with *luxS* gene deletions and overexpression variants. These allowed us to explore the nuances in their biofilm formation abilities when in the VBNC state. At the molecular level, we revealed the intricate regulatory mechanisms of the quorum sensing system in shaping the formation of VBNC biofilms by *L. plantarum*.

Development of methods for biofilm analysis: quantification of biofilm viability and amount

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Abstract: For years, biofilm characterization has been the subject of intensive study by the scientific community. In clinical settings, biofilms affect the interior of surgical prostheses like catheters, stents, and heart valves, increasing the risk of hospital-acquired infections and the emergence of antibiotic resistance. In industrial settings, in addition to causing cleanliness and hygiene issues, biofilms lead to energy loss and blockages in cooling and wastewater pipelines, as well as on ship hulls, thereby increasing the microbial risk due to the release of pathogens into the environment. In the food industry, biofilms contribute to food spoilage and deterioration, leading to substantial economic losses. However, it is worth noting that biofilm formation is currently used for the production of high-value substances, such as polymers, for example in electrofermentation processes. The vast number of biofilm-producing microbial species that exist under various natural and human-made conditions has led to the need for rapid, economical and sustainable methods for characterizing various aspects of biofilms, such as biofilm quantity and viability. Specifically, the most used method for quantifying biofilm amount is the Crystal Violet (CV) assay. This assay uses a dye that can intercalate within the structure of the biofilm, and the amount is detected using a spectrophotometer. This assay is fast, economical, and sensitive even at low concentrations, and does not require special safety precautions, but it cannot distinguish a biofilm composed of live cells from one with dead cells. For cell viability quantification, the widely employed method is the Resazurin (RZ) assay. This uses Resazurin, which can be oxidized to Resorufin, changing color through dehydrogenase activity. This enables cell viability quantification by measuring the fluorescence. However, this method is labor-intensive, requires strict sterility conditions, and loses sensitivity over long durations. The objective of this work is to create a new approach that characterizes biofilm-producing species by combining the values provided by these two assays. The aim is to create a 2D graph where the x- and y-axes display the values from the two assays, resulting in a unique and characteristic position for each species under the applied conditions. After building a calibration curve, knowing the values derived from the simpler and more economical method, CV, will allow the RZ values to be determined by interpolation. This new approach to data interpolation opens the door to a multitude of variations that allow different types of assays or experimental methods to be correlated according to the aspect and conditions of the biofilm being studied. Indeed, the possibility of varying study conditions, such as incubation time, medium composition, pH, and applied potential, expands the level of detail that this type of analysis can provide. For example, it will be possible to simultaneously observe cell respiration and the amount of biofilm produced in preliminary stages or after long incubation times, in nutrient-rich or nutrient-poor media, and in the absence or presence of stress factors. Additionally, this level of detail has the potential to distinguish different cell populations within the biofilm that possess high or low metabolic activity.

Keywords: Biofilm, Crystal Violet, Resazurin, Assay, methodology

Research on the adsorption properties of EPS towards heavy metals and its mediation in the synthesis of MeS QDs

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Abstract: This study investigates the changes in the composition of microbial extracellular polymeric substances (EPS) and their adsorption capacity for heavy metals and mediate the biosynthesis of quantum dots (QDs) under two different stress systems. The effects of ammonium salts and Cu (II) stress on *Desulfovibrio desulfuricans* and *Pseudomonas aeruginosa* were explored, and the key role of EPS in the biosynthesis of PbS and CuS quantum dots (QDs) was elucidated. Under ammonium salt stress, the study showed that the EPS content of *D. desulfuricans* and its ability to adsorb Pb (II) significantly increased, particularly under NH_4Cl stress. The content of acidic amino acids (such as glutamic acid and aspartic acid) in the EPS increased, providing more active sites for Pb (II) adsorption and promoting the rapid nucleation of PbS QDs. Similarly, under Cu (II) stress, especially with $Cu(NO_3)_2$, the EPS production of *P. aeruginosa* and its adsorption capacity for Cu (II) were also significantly enhanced. 3D-EEM, FTIR, and XPS analyses showed that stress increased the content of protein-like substances and key functional groups (such as -COOH, O-C-O, -SH, etc.) in the EPS, which enhanced its ability to adsorb heavy metals and facilitated the biosynthesis of quantum dots. Further analysis of the synthesized quantum dots by XRD, TEM, and BET revealed that the quantum dots not only exhibited excellent fluorescence properties, crystal phase stability, and uniform particle size but also showed a large specific surface area, demonstrating outstanding performance. The study suggests that stress/induction can significantly enhance the ability of microbial EPS to adsorb heavy metals and promote the biosynthesis of quantum dots, providing an important theoretical basis and practical reference for microbial applications in heavy metal wastewater treatment and resource recovery. The research also reveals the differences in the effects of different stress factors on EPS composition, highlighting the diversity of microbial response mechanisms under various stress conditions, which provides valuable research foundations for future environmental management and nanomaterial development.

Keywords: EPS, Adsorption, QDs, Mediated biosynthesis, Metal biorecovery

Key aspects of spatial structure in the understanding of multispecies biofilms: concepts and methods

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Abstract: Biofilms have always been associated with spatial complexity, and a surge in biofilm studies assessing this complexity at the single cell level has appeared over the last years. In terms of concepts, the paradigm of assessing biofilm reproducibility with colony forming units or total cell counts is changing into a reproducibility that takes into account the location of the cells and other biofilm components (1). While statistical methods are still being figured out in order to deal with this novel concept of reproducibility, laboratorial methods are continuously improving and providing a spatial mapping of biofilms at unprecedented detail. For instance, novel labelling methods employing nucleic acids and image analysis can potentially discriminate and locate hundreds of species in multispecies biofilms (2), and spatial transcriptomics have been employed in single-species biofilms to assess the transcriptome of individual cells in situ (3, 4). In here, I will also discuss how these strategies are being integrated in a novel project named e.Biofilm, that is aiming to engineer biofilms at the spatial level, as well as discuss some future paths for research in this area.

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Keywords: Reproducibility in biofilms, Spatial location, Multispecies biofilms, nucleic acids

Scavenging efficacy and mechanism of electrolyzed functional water on biofilms

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Abstract: Biofilms attached to food contact surfaces due to insufficient removal of microorganisms can cause cross-contamination resulting in foodborne disease outbreaks that pose a considerable threat to food safety. The control and elimination against biofilm in food processing is a research focus in the field of food safety. Electrolyzed Functional Water (EFW), which can be categorized as alkaline electrolyzed water (AlEW), acidic electrolyzed water (AcEW), and slightly acidic electrolyzed water (SAEW) depending on pH, has been recognized for its efficient and broad-spectrum abilities to kill pathogens, and has been shown to synergistically enhance disinfection in combination with other processing methods. Our previous studies found that the combination of AcEW and AlEW could result in the enhancement of disinfection effect and eliminate the biofilm effectively on the fruits and vegetables. Based on the objective analysis on the properties of EFW and biofilm as well as the scientific problems which reflected their mutual influencing mechanisms, the changes of physical and chemical parameters of EFW during preparation were clarified by evaluating the properties of EFW by electrochemistry point of view. Combined with the process conditions, the factors influencing the elimination of biofilm were evaluated for SAEW, AcEW and AlEW combinations, EFW combined with other treatments such as ultrasonic treatment, addition of silver ions, and other methods. Moreover, the elimination efficiencies and regulation methods against biofilm on different typical food materials were clarified. Based on the research methods of molecular biology, the study on the elimination mechanisms against biofilm by the EFW and combination of other treated methods were performed. Specifically, the theory of layer-by-layer elimination of biofilms was proposed and demonstrated. The effects of EFW combined with other treatments on the changes of signaling molecules in biofilms were assessed. The molecular regulatory mechanisms of the effects of EFW in combination with other treatments on biofilms were revealed using proteomics and transcriptomics. As a result, the implementation of this project will provide the theoretical and technical support to solve the problem of microbial pollution in food processing.

Keywords: Electrolyzed functional water (EFW), biofilm, scavenging efficacy, mechanism, food safety

Effects of lactic acid bacteria as quorum sensing inhibitors on biofilms of foodborne pathogens

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Abstract: Foodborne pathogens pose serious challenges to food safety and food preservation. *Vibrio parahaemolyticus* and *Listeria monocytogenes* are important foodborne pathogens with strong biofilm-forming abilities. Quorum sensing (QS) is the bacterial communication process that regulates biofilm formation, expressions of virulence factors, and resistances to adverse environments. Interference with bacterial QS does not cause bacterial resistance, it is a new strategy to control food spoilage and food poisoning. Lactic acid bacteria (LAB), recognized for their probiotic properties and safety in food applications, have been proposed as potential quorum sensing inhibitors (QSIs). This report presented the studies on LAB with targeted interferences on QS systems of the two kinds of foodborne pathogens. Hundreds of LAB strains were screened, the inhibitory effects of ethyl acetate extracts from the cell free supernatants of LAB strains on the AI-2 activities and biofilm formations of *Vibrio parahaemolyticus* and *Listeria monocytogenes* were measured. The probiotic potentials of partial LAB strains were characterized, and the LAB-derived QSIs were obtained. The quenching effects of LAB-derived QSIs on the QS systems of *V. parahaemolyticus* and *L. monocytogenes* were studied. The results showed that sub-MICs of LAB-derived QSIs significantly inhibited biofilm formations of *V. parahaemolyticus* and *L. monocytogenes*, effectively removed mature biofilms, reduced the metabolic activities of biofilms, and inhibited the motility abilities, AI-2 activities, extracellular polysaccharide and protein syntheses. The inhibitory effects were also observed by fluorescence microscope, scanning electron microscope and laser confocal microscope. RNA-seq and quantitative PCR (qPCR) revealed that the differentially expressed genes were enriched in biological process, cell component and molecular function, and were concentrated in the KEGG pathways such as ABC transporters, quorum sensing, etc. LAB-derived QSIs effectively inhibited the expressions of flagella synthesis related genes, extracellular polysaccharide and protein synthesis related genes, quorum sensing related genes and hemolysin secretion related genes, etc. The pure QSIs were obtained by ethyl acetate extraction, Sephadex LH-20 chromatography and RP-HPLC. LC-MS/MS analysis identified the amino acid sequences. In conclusion, LAB possess the abilities to effectively inhibit QS and biofilm of foodborne pathogens. Applying LAB as QSIs to control bacterial biofilm formation is an effective and novel biocontrol strategy that could be employed in food preservation and safety control. This study highlights the potential of LAB as QSIs to mitigate biofilm formation of significant foodborne pathogens, it is also of significance to broaden the research and application fields of LAB. Further research is warranted to elucidate the specific mechanisms of interaction between LAB and pathogenic bacteria, and to explore practical applications in food systems to improve safety and shelf-life.

Keywords: foodborne pathogens, biofilms, lactic acid bacteria, quorum sensing, inhibition

Progress of *Listeria monocytogenes* biofilm 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. 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.

Keywords: *Listeria monocytogenes*, biofilm, risk assessment, model, control

Identification of molecular targets of JX08806 as antibiofilm against *Staphylococcus aureus*

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Abstract: *Staphylococcus aureus* is an important food-borne pathogen, which can produce a variety of virulence factors, including staphyloxanthin, biofilm, hemolysin, enterotoxin and so on, threatening public health. Previous studies in our laboratory have found that the antifungal drug naftifine derivative JX08806 has a significant inhibitory effect on the formation of staphyloxanthin and biofilm. In order to further screen and verify the molecular targets of JX08806 against *S. aureus*, virtual screening, prokaryotic expression, surface plasmon resonance, gene knockout and overexpression techniques were used in this study. Through the Glide semi-flexible virtual docking, the screening of protein structure database of *S. aureus* was carried out based on the molecular structure of JX08806, and 2888 molecular docking experiments were completed. Three new potential targets were obtained, namely the efflux pump protein SepA, 1,4-dihydroxy-2-naphthoate octaprenyltransferase MenA, and N-acetylglucosaminyltransferase IcaA. At the same time, the docking of JX08806 and potential target proteins EssB, EssD and HlgB obtained in previous studies was also detected. The verification of these potential targets was carried out through the knockout, overexpression and function characterization of certain target proteins. It turned out that HlgB was one of the main targets of JX08806 against hemolytic virulence. Other potential targets of JX08806 against *S. aureus* virulence were also verified, among which SepA and IcaA may be the targets of JX08806 against biofilm formation. In conclusion, the discovery of these anti-biofilm potential targets further elucidates the inhibitory mechanism of JX08806 on the biofilm and virulence of *S. aureus*.

Keywords: *Staphylococcus aureus*, naftifine derivatives JX08806, antibiofilm, virtual screening, target validation

Characterization of *PseudoXmonas* spp. contamination and in situ spoilage potential in pasteurized milk production process

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Abstract: To investigate the prevalence of *Pseudomonas* in the pasteurized milk production process and its effect on milk quality, 106 strains of *Pseudomonas* were isolated from the pasteurized milk production process of a milk production plant in Shaanxi Province, China. The protease, lipase and biofilm-producing capacities of the 106 *Pseudomonas* strains were evaluated, and the spoilage enzyme activities of their metabolites were assessed by simulating temperature incubation in the refrigerated (7 ℃) and transport environment (25 ℃) segments and thermal treatments of pasteurization (75 ℃, 5 min) and ultra-high temperature sterilization (121 ℃, 15 s). A phylogenetic tree was drawn based on 16S rDNA gene sequencing and the top 5 strains were selected as representative strains to identify their in situ spoilage potential by examining their growth potential and ability to hydrolyze proteins and lipids in milk using growth curves, pH, whiteness, Zeta-potential, lipid oxidation, SDS-PAGE and volatile flavor compounds. The results showed that half and more of the isolated *Pseudomonas* had spoilage enzyme production and biofilm capacity, and the spoilage enzyme activity of metabolites was affected by the culture temperature and sterilization method, but ultra-high temperature sterilization could not completely eliminate the enzyme activity. The growth of *Pseudomonas lundensis* and *Pseudomonas qingdaonensis* was less affected by temperature and time, and the hydrolytic capacity of extracellular protease and lipase secreted by *Pseudomonas lurida* was the strongest, which had the greatest effect on milk quality. Therefore, it is crucial to identify the key contamination links of *Pseudomonas*, the main bacteria responsible for milk spoilage, and the influence of environmental factors on its deterioration.

Keywords: Pasteurized milk, Production process, *Pseudomonas*, Lipase, Protease, Biofilm, Milk quality

Dry surface biofilm study: models and methods

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Abstract: Dry surface biofilms (DSBs) are microbial communities that form on dry surfaces and are significant in medical environments due to their potential risks to public health. Here are some key features of dry surface biofilms:

1. Definition and Characteristics: A dry surface biofilm is a newly identified type of biofilm that is invisible to the naked eye. These biofilms harbor multidrug-resistant microorganisms, showcase resistance to cleaning and disinfection methods, and cannot be detected by standard wet or dry swabs. This resistance may contribute significantly to the persistence of pathogens in medical settings. Compared to wet biofilms, dry biofilms are more prevalent in healthcare environments and demonstrate a higher tolerance to disinfectants.

2. Role in the Medical Environment: Dry surface biofilms are commonly found in hospital settings, which may elucidate how microbial contamination can persist for extended periods in clinical areas, potentially contributing to the spread of hospital-acquired infections. They play a crucial role in the durability of microbial presence on dry surfaces in medical facilities, which can lead to the incidence of infections related to medical procedures.

3. Formation and Growth: Although the mechanisms behind the formation and growth of biofilms on dry surfaces in clinical settings are not well understood, research indicates that conditions in dry and nutrient-poor environments can promote their development. The emergence of dry surface biofilms may be linked to microscopic surface wetness (MSW), which can facilitate the formation of these biofilms.

4. Difficulty in Detection and Removal: Detecting biofilms on dry surfaces is challenging, and such biofilms are resistant to standard cleaning and disinfection techniques. Their inherent characteristics make them difficult to eliminate from surfaces, particularly due to the unique nature of dry surface biofilms.

5. Resistance to Disinfectants: Dry surface biofilms exhibit greater resistance to disinfectants compared to wet surface biofilms and planktonic bacteria. Studies have examined the effectiveness of various disinfectants on dry surface biofilms of *Staphylococcus aureus.*

6. Distribution on Hospital Surfaces: Dry surface biofilms are widely distributed across hospital surfaces, which may help explain why certain pathogens can remain viable on these surfaces for extended periods.

7. Impact on Public Health: Dry surface biofilms can serve as vectors for infection transmission within the hospital environment, increasing the risk of hospital-acquired infections.

8. Impact on the Food Industry: Dry surface biofilms are relevant to food safety in low-humidity food processing (LHF) plants. These biofilms play a significant role in the survival and persistence of pathogens in food processing environments and on the surfaces of agricultural products.

Understanding dry surface biofilms through models and methods is essential for exploring the formation, structure, and function of microbial communities, as well as their interactions with the environment and hosts. These models are invaluable for investigating the mechanisms behind biofilm formation, developing effective removal strategies, and studying their roles in hospital infections and food processing.

This workshop presents various dry surface biofilm models and study methods and discusses their applications, advantages, and disadvantages.

Keywords: dry surface biofilm, hospital environment, food industry, microscopic surface wetness, low-humidity food processing

Dry surface biofilm: an underestimated concern for microbial contamination

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Abstract: Approximately 10% of hospitalized patients develop a nosocomial infection. Our studies demonstrated that dry surface biofilms (DSB) contaminate over 90% of hospital intensive care unit (ICU) surfaces and many contain multidrug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. aureus* was found in 50% of the ICU samples. Our research showed that DSBs are more tolerant of chlorine and heat treatment. It is harder to remove DSB than planktonic organisms and even hydrated biofilm physically. We also showed that *S. aureus* DSB can be transmitted to multiple surfaces by hand and gloves. Our research demonstrated a thickened bacterial cell wall, increased peptidoglycan production, and a thickened extracellular polymeric substance (EPS) in DSB. Proteomic analysis identified proteins significantly upregulated in DSB including phosphotransferase system glucose transporter subunit IIBC (PtaA), UDP-N-acetylmuramate-L-alanine ligase (MurC) and UDP-N-acetylenolpyruvoylglucosamine (MurB) compared to hydrated biofilm. These three proteins are all linked with the peptidoglycan biosynthesis pathway and are responsible for cell wall formation and thicker EPS matrix deposition. Determining the mechanism behind DSB tolerance can lead to the development of better cleaning agents against DSB.

Keywords: dry surface biofilm, resistance, cell wall, peptidoglycan, proteomic analysis

High-throughput, rapid, and non-destructive detection of common foodborne pathogens via Hyperspectral Imaging coupled with deep neural networks and support vector machines

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Abstract: Foodborne pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* are major causes of gastrointestinal diseases worldwide and frequently contaminate dairy products. Compared to nucleic acid detection and MALDI-TOF MS, hyperspectral imaging (HSI) offering advantages such as multiple bands, rapid, minimal damage, non-contact, and non-destructive detection. However, current HSI methods require agar plate cultures, which are time-consuming and labor-intensive. This study is the first to use bacterial broth in a 24-well plate to collect HSI spectra, combined with machine learning for enhanced feature extraction and classification. After data augmentation and dimensionality reduction via principal component analysis (PCA) and linear discriminant analysis (LDA), deep neural networks and support vector machines (DNN-SVM) resulted in prediction accuracies of 100% on the training set, 98.31% on the testing set, and 93.33% on the validation set for classifying *B. cereus*, *E. coli*, and *S. aureus*. As a result, a high-throughput, rapid, and non-destructive detection method was developed, which is expected to detect 24 bacterial broth samples in less than ten minutes. It indicates the potential of HSI to be used as a feasible, robust, and non-destructive solution for real-time monitoring of microbial pathogens in food.

Viable but nonculturable (VBNC) state: an underestimated microbial survival strategy

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Abstract: As a unique microbial response to adverse circumstances, the viable but nonculturable (VBNC) state is characterized by the loss of culturability of microbial cells on/in nutrient media that normally support their growth, while maintaining metabolic activity. These cells can resuscitate to a culturable state under suitable conditions. The ability of VBNC cells to maintain metabolic activity and produce virulent/harmful substances, but fail to detect by culture-based methods poses a significant health risk to humans. In this study, the VBNC state formation and characterization, detection and safety control of enterohemorrhagic *Escherichia coli* O157, lactic acid bacteria, bacteria in rice and flour products were investigated. Firstly, food-borne *E. coli* O157 strains were capable of entering into the VBNC state by incubation under freezing conditions with continuous toxin gene expression. Secondly, 6 species of lactic acid bacteria entered into the VBNC state under beer subculturing or low temperature storage to escape sterilization and routine microbe detection, and lead to beer spoilage within expiration date. Thirdly, in rice and flour products, planktonic and biofilm cells of *E. coli*, *S. enterica*, *S. aureus*, and *L. casei* could partially or all enter into the VBNC state at -20°C. All bacterial virulence genes were continuously expressed during VBNC state formation. Control (changing environmental factors) and detection (PMA-PSR/CPA) methods were further proposed to battle against VBNC state.

Keywords: Food microorganisms, Viable but nonculturable state, Biofilm, Rapid detection

Modification of cationic antimicrobial peptides and mechanism of antibacterial action at the single-molecule level

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Abstract: The objective of this report is to examine modification strategies for cationic antimicrobial peptides (AMPs) and their single-molecule mechanisms of action. Cationic AMPs have garnered considerable attention due to their potent antimicrobial efficacy and low toxicity. However, their practical application is often hindered by issues such as limited stability and suboptimal selectivity. Structural modifications of AMPs, including amino acid substitution and deletion, can further refine their properties, enhancing both antimicrobial activity and selectivity. Initially, the report outlines our research group's strategies and findings on optimizing AMPs. By employing targeted amino acid substitutions and deletions, we successfully engineered a high-activity, low-toxicity AMP, designated FCLAP. This section provides a detailed overview of the rationale behind our design choices, highlighting how specific modifications contributed to improved performance metrics. Following this, the report delves into the application of single-molecule optical tweezers to investigate the interactions between AMPs and bacterial membranes at a molecular level. This technique allows us to capture real-time dynamics and mechanical forces involved in these interactions, thereby elucidating the antimicrobial mechanisms of AMPs. The findings demonstrate that the modified AMP, FCLAP, can effectively interact with bacterial cell membranes and specifically target lipopolysaccharides (LPS). This targeting mechanism is crucial for disrupting the integrity of the bacterial membrane, ultimately leading to cell death. Furthermore, the report utilize molecular dynamics simulations and surface plasmon resonance (SPR) technology to further investigate AMP interactions with membrane proteins. Molecular dynamics simulations provide a computational framework to model and predict the behavior of AMPs at the atomic level, offering insights into their stability and interaction pathways. SPR technology, on the other hand, enables the real-time measurement of binding kinetics between AMPs and membrane proteins, offering a quantitative assessment of their interaction strength and specificity. These investigations not only offer novel insights into the modification and optimization of cationic AMPs but also provide new insight for understanding their antimicrobial mechanisms. By integrating experimental and computational techniques, we can develop a comprehensive model of AMP action, which is critical for rational design and optimization.

Keywords: Cationic antimicrobial peptides, modification, single-molecule, antimicrobial mechanism, bacterial membrane

Role of bpfA in adhesion and biofilm formation of *Shewanella putrefaciens* CN32 under cold stress: a comprehensive transcriptomic analysis

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Abstract: Biofilm-promoting factor A (BpfA) promotes the adhesion and biofilm formation of *Shewanella* spp., but its role in biofilm formation of *S. putrefaciens* under cold stress needs to be further investigated. To better comprehend the impact of BpfA on adhesion and biofilm formation of *S. putrefaciens* under cold stress (4 °C), adhesion, biofilm phenotype, and transcriptomics of *S. putrefaciens* CN32 WT and Δ*bpfA* at 4 °C were analyzed in this study. The results revealed that the deletion of *bpfA* had minimal impact on the growth of *S. putrefaciens* CN32 at 4 °C. However, it did diminish the unicellular adhesion capacity of *S. putrefaciens* CN32 and compromised the stability of the multicellular adhesion layer. The cell membrane of Δ*bpfA* exhibited significant disruption. Moreover, the biomass of the mature biofilm formed of Δ*bpfA* was merely around 50% of that observed in the mature biofilm of *S. putrefaciens* CN32 WT, the average thickness and volume of the biofilm decreased by 3% and 8%, respectively, and the composition of the biofilm changed. Transcriptome analysis demonstrated that the deletion of *bpfA* led to differential expression of genes involved in metabolic pathways such as bacterial chemotaxis, two-component system, tyrosine metabolism, drug metabolism-other enzymes and biofilm formation-*Vibrio cholerae*, which in turn influenced bacterial adhesion and biofilm formation. Those results advance our acknowledge of the character of BpfA on adhesion and biofilm formation of *S. putrefaciens* CN32, which contributes to understand bacterial adhesion and the control of biofilm formation.

Keywords: *Shewanella putrefaciens*, cold stress, BpfA, adhesion, biofilm, transcriptomics

Study on inhibitory mechanism of linalool against *Listeria monocytogenes*

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Abstract: This experiment was conducted to evaluate the inhibitory activity of linalool against *L. monocytogenes*. The main results are as follows:(1) The inhibitory activity of linalool against *L. monocytogenes* was evaluated and the results showed that linalool has a strong inhibitory effect on *L. monocytogenes* with a MIC of 1.5 mL/L. Particle size distribution, field emission scanning electron microscopy and transmission electron microscopy confirmed the ability of linalool to cause cell aggregation, adhesion, rupture, and induce protoplasmic folds and loss of border sensation. The cell membrane permeability was detected using fluorescent probe combined with spectroscopic techniques, and the results indicated that the addition of linalool significantly increased the permeability of the cell membrane thereby increasing the uptake of PI and crystalline violet dyes, resulting in depolarization of the cell membrane and a decrease in the cellular zeta potential. Multiple components, including ions, nucleic acids and proteins, were leaked to the outside of the cell. The changes of cell membrane conformation and biofilm formation ability were also monitored under the effect of linalool. The data obtained supported that linalool significantly changed the conformation of *L. monocytogenes* cell membrane and reduced its biofilm formation ability. (2) The metabolic capacity of *L. monocytogenes* under linalool stress was quantified, and the results revealed that the addition of linalool significantly weakened the intracellular metabolism level. Then, the content of coenzyme I and ATP, and their enzyme activities were measured, and it was found that the insufficient energy phenomenon appeared in the presence of linalool. Subsequently, we examined the activities of various enzymes involved in respiratory and energy pathways. Multiple intracellular enzyme activities showed a significant decrease under linalool, indicating that linalool can cause energy depletion by inhibiting the activities of enzymes involved in energy supply pathways. The intracellular redox status was also evaluated, and it was found that the intracellular levels of ROS, MDA, H_2O_2, O_2^- and GSSG tended to increase in the presence of linalool, while the levels of GSH and -SH decreased significantly. These data indicated that linalool caused oxidative damage to *L. monocytogenes*. Subsequently, enzyme activities involved in antioxidant and defense systems were measured and the results confirmed that the addition of linalool significantly inhibited the intracellular free radical scavenging capacity. (3) It was found that the presence of linalool caused differential expression of the metabolites of the test organisms. There were 107 differential metabolites were identified in the ESI+ model, of which 44 were up-regulated and 63 were down-regulated; A total of 99 differential metabolites were identified in the ESI-, of which 35 were up-regulated and 64 were down-regulated. The most involved differential metabolites were amino acids, and most of them showed a down-regulation trend, indicating that amino acid limitation phenomenon occurred in bacteria in the presence of linalool. Combined with the results of enzyme activity assays, it was revealed that the presence of linalool induced a deficit in energy levels and thus increased amino acid consumption. In particular, intracellular lipid and nucleic acid metabolism was also disturbed.

Keywords: Essential oil, Linalool, *Listeria monocytogenes*, antibacterial mechanism, Preservation

How chemicals of emerging concern are affecting microbial communities

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Abstract: Contaminants of emerging concern (CECs), including pharmaceuticals and personal care products (PPCPs), are increasingly being detected at residual levels (< µg/L) in water bodies worldwide. This can be explained by the societal dependence on pharmaceuticals and PPCPs and the inadequacy of the existing wastewater treatment processes to remove these chemically diverse contaminants. There is concern that these contaminants may impact aquatic life [1]. In particular, musk fragrances are used in a wide variety of PPCPs and house-care products, providing pleasant scent products of routine use and the resulting contaminants can have critical consequences for the environment and human health, due to their bioaccumulation in the environment and the associated endocrine disruption effects. However, it remains unclear if and how the microorganisms can be affected by exposure to CECs, particularly to these non-antibiotics. Based on the principles of the “One Health” initiative, I will present the available evidence on how non-antibiotic CECs may have an important impact on microbial communities, particularly on the tolerance of biofilms to disinfection and susceptibility to antibiotics.

The role of *rcpA* gene in regulating biofilm formation and virulence in *Vibrio parahaemolyticus*

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Abstract: *Vibrio parahaemolyticus* (*V. parahaemolyticus*) is a common seafood-borne pathogen that can colonize the intestine of host and cause gastroenteritis. Biofilm formation by *V. parahaemolyticus* enhances its persistence in various environments, which poses a series of threats to food safety. This work aims to investigate the function of *rcpA* gene in biofilm formation and virulence of *V. parahaemolyticus*. Deletion of *rcpA* deletion significantly reduced motility, biofilm biomass, and extracellular polymeric substances, and inhibited biofilm formation on a variety of food and food contact surfaces. In mice infection model, mice infected with Δ*rcpA* strain exhibited a decreased rate of pathogen colonization, a lower level of inflammatory cytokines, and less tissue damage when compared to mice infected with wild type strain. RNA-seq analysis revealed that 374 genes were differentially expressed in the *rcpA* deletion mutant, which include genes related to quorum sensing, flagellar system, ribosome, type VI secretion system, biotin metabolism and transcriptional regulation. In conclusion, *rcpA* plays a role in determining biofilm formation and virulence of *V. parahaemolyticus* and further research is necessitated to fully understand its function in *V. parahaemolyticus.*

Keywords: *V. parahaemolyticus,* biofilm, *rcpA*, RNA-seq, virulence

Investigating the potential of L (+)-lactic acid as a green inhibitor and eradicator of a dual-species *Campylobacter* spp. biofilm formed on food processing model surfaces

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Abstract: *Campylobacter* spp. are prevalent zoonotic foodborne bacterial enteric pathogens. Their inclusion in biofilms on abiotic surfaces is considered a strategy that facilitates their extraintestinal survival. Organic acid (OA) treatments could be used in a green approach to decontaminate various such and other surfaces. This work aimed to evaluate the inhibitory and eradicative effects of L (+)-lactic acid (LA), a naturally occurring OA, on a dual-species biofilm formed on two food processing model surfaces (polystyrene and stainless steel) by three selected foodborne *Campylobacter* spp. isolates (two *C. jejuni* and one *C. coli*). To maximize biofilm formation, the bacteria were grown under mixed-culture conditions in a standard laboratory broth supplemented with chicken juice for 48 h at 42 °C, under microaerophilic conditions, and the biofilm inhibitory and destructive (disinfectant) actions of the acid were determined through agar plating against both the strains' consortium and separately against each strain. Mixed-culture biofilm formation was also tested under aerobic (and carbon dioxide-enriched) conditions to see if this could influence biofilm resilience against LA dis-infection. To check for any relationship between planktonic growth and biofilm-forming ability, the growth dynamics of each strain grown planktonically under monoculture conditions were followed, while the surrounding free-swimming bacteria were also counted (for each strain and in total) at the end of the biofilm formation procedure (48 h). In parallel, the predominant metabolites contained in the planktonic media of biofilm monocultures and mixed-culture were comparatively analysed by an untargeted metabolomics approach. Results revealed that LA inhibited mixed-culture biofilm formation by more than 2 logs (99%) on both surfaces when this was applied at its highest tested concentration (4096 μg/mL; 0.34% v/v). However, all the preformed mixed-culture biofilms (ca. 10^{6-7} CFU/cm^2) could not be eradicated even when the acid was used at concentrations exceeding 5% v/v, denoting their extremely high recalcitrance which was still influenced by the abiotic substratum, and the biofilm-forming aerobiosis conditions. The metabolic analysis revealed a strain-specific metabolite production which might also be related to the strain-specific biofilm-forming and resistance behaviors and resulted in the distinct clustering of the different samples. Overall, the current findings provide important information on the effectiveness of LA against biofilm campylobacteria and may assist in mitigating their risk in the food chain.

Platinum-based fluorescent nanozyme-driven “loong frolic pearls” multifunctional nanoplatform for ultrasensitive detection and synergistic sterilization of *Burkholderia gladioli*

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Abstract: *Burkholderia gladioli* is a food-borne pathogen associated with human infections. Rapid and accurate detection of B. gladioli and effective sterilization are crucial for ensuring food safety. Hence, a novel "loong frolic pearls"platform based on platinum-based fluorescent nanozymes (Pt-OCDs) and strand exchange amplification (SEA) was reported. Magnetic nanoparticles were modified on primer SEA-F, while Pt-OCDs were covalently coupled with primer SEA-R. The highly efficient amplification capability of SEA permitted the accumulation of a large number of double-labeled amplicons. After magnetic adsorption, the supernatant was detected in reverse direction to collect colorimetric-fluorescence-photothermal signal values, enabling ultra-precise detection within 1 h. Furthermore, the Pt-based multifunctional nanoplatform generated abundant •OH and 1O2, which synergistically attacked B. gladioli and its biofilm, resulting in significant bactericidal efficacy within 30 min. This "triple-detection and double-sterilization" platform has been successfully applied in the field of food analysis with good recovery rates and immediate control over B. gladioli, thus demonstrating promising prospects for broad applications.

Keywords: Platinum based orange carbon dots, Sequence exchange amplification, Tri-modal detection, Biofilm, *Burkholderia gladioli*

Regulation of non-coding small RNA named SaaS in biofilm formation and virulence of *Salmonella*

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Abstract: *Salmonella* has emerged as a major public health hazard worldwide. *Salmonella* biofilm formation on the solid-contacted surfaces would be prone to exacerbate cross-contamination, and directly cause large-scale outbreaks. Revealing the molecular mechanism of biofilm formation is a prerequisite for developing effective prevention and control technologies. Non-coding small RNA is a novel post-transcriptional regulatory factor discovered in recent years. In our previous study of biofilm formation by *Salmonella*, a novel sRNA has firstly been identified, namely SaaS. This report analyzed detailly the action mode and process of sRNA SaaS to regulate the biofilm formation in *Salmonella*, and further resolved the regulatory mechanisms of sRNA SaaS on the virulence of *Salmonella* through cells and animal models. Specifically, SaaS inhibits biofilm formation by repressing the adhesion potential and the secretion of EPS components. Integration of transcriptomics and proteomics analysis revealed that SaaS strengthened the expression of the flagellar synthesis system and downregulated the expression of curli amyloid fibers. Furthermore, RNA-protein pull-down interactome datasets indicated that SaaS binds to Hfq uniquely among 193 candidate proteins, and promoter–reporter β-galactosidase activity assay confirmed target mRNAs including *hilD*, *cheA*, and *csgA*. Besides, by employing ELISA and western blot analysis, we demonstrated that SaaS regulated intestinal inflammation through sequential activation P38-JNK-ERK MAPK signaling pathway, which enabled immune escape at primary infection stage but strengthened pathogenesis at later stage, respectively. These findings suggest that SaaS plays an essential role in the virulence of *S.* Enteritidis and reveals its biological role in intestinal pathogenesis. Collectively, SaaS inhibits the properties of bacterial mobility, perturbs the secretion of EPS, and contributes to the inhibition of biofilm formation and the promotion of virulence by interacting with target mRNA through the Hfq-mediated pathway, and our findings provide the foundation for fully extending the global regulatory network of biofilm formation and virulence invasion in *Salmonella*.

Keywords: *Salmonella*, small RNA, regulation, biofilm, virulence

Valorization of Soy Whey through Synthetic Biology

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Abstract: Soy whey is a byproduct of the soy food production process, yields 50-70 million tons annually in China. Although rich in nutrients, it is often discarded as wastewater, incurring additional costs and resulting in significant resource waste. To repurpose soy whey, we designed a microbial cell factory to convert soy whey into high-value natural products. This approach includes engineering *Escherichia coli* to express sucrose and α-galactosidase for extracellular utilization, conferring the ability to metabolize sucrose, stachyose, and raffinose, thus exploiting the carbohydrates in soy whey. To circumvent the cytotoxicity and expense associated with IPTG-induced expression systems, we further developed an auto-induction system utilizing galactose from soy whey as an inducer, offering efficient and tightly regulated control. (*S*)-Equol, one of the most bioactive isoflavone metabolites, holds substantial nutritional and pharmaceutical potential. Using this engineered chassis, we integrated the expression of the (*S*)-equol biosynthesis pathway and optimized daidzin transporters, achieving a substrate conversion rate of 96% from soy whey to (S)-equol. Additionally, we focused on medium-chain fatty acids (MCFAs), which are key platform chemicals. Employing the engineered chassis, we expressed a reverse β-oxidation pathway and applied metabolic engineering strategies to balance intracellular redox, achieving an MCFA yield of 16.42 g/L, the highest reported to date, while reducing medium costs by 88% compared to traditional media. This innovative approach offers a promising pathway for expanding soy whey's applications and achieving sustainable production of high-value natural products.

Keywords: Microbial cell factories, Soy whey utilization, (*S*)-equol biosynthesis, Medium-chain fatty acids (MCFAs), Cost-effective production

Unlocking the potential of biofilm properties in beneficial microbes for One Health advancements

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Abstract: The Meeting the increasing demands for animal welfare, sustainability and microbiological safety in livestock production requires innovative control solutions, such as the use of positive biofilms. Applied to building surfaces in animal facilities after cleaning and disinfection, these positive biofilms are introduced prior to the arrival of animals and have the potential to reshape the surface microbial ecology. The anticipated One Health benefits span animal, environmental and human health, making these biofilms a promising component of sustainable agricultural practices. While positive results have been observed in field trials, the scientific rationale for selecting specific microbial strains in these biofilm formulations remains underexplored. Most commercial formulations consist of strain mixtures, but the presence of additive or synergistic effects in terms of colonisation efficiency or antagonistic action against pathogens in the biofilm mode has not been rigorously validated. Theoretical mechanisms such as spatial and nutrient competition and secretion of antagonistic molecules have been proposed to explain these effects, but empirical evidence is limited. Advancing beneficial biofilm applications requires a deeper understanding of their functional properties and the development of reliable tools to assess their efficacy in situ. Research efforts must focus on identifying strains with strong surface colonisation capacity and antagonistic potential, both individually and as synergistic consortia, and characterising the underlying ecological interactions within these biofilms. Understanding how these microbial communities compete, cooperate or interact with each other will be essential for the development of optimised formulations that will perform consistently in agricultural environments. This research paves the way for future innovations in microbial biofilm applications for One Health. By establishing a scientific framework for strain selection and efficacy assessment, we can develop biofilm-based solutions that not only enhance microbial safety, but also promote animal welfare and environmental sustainability. Ultimately, these efforts contribute to a holistic approach to health that addresses interrelated animal, environmental and human health concerns, and represent a major step forward in the sustainable management of microbial communities in agricultural environments.

Keywords: positive biofilm, synthetic communities, bacterial pathogens, confocal imaging, one health

Role of stringent response factors in response to environmental stress in *Yersinia enterocolitica*

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Abstract: Stringent response is a global regulatory mechanism that is widely present in bacteria. It is mediated by two intracellular signaling molecules, guanosine pentaphosphate and guanosine tetraphosphate (collectively referred to as (p)ppGpp) and the co-fator DksA. When the external environment changes, the two molecules help bacteria reintegrate intracellular resources and energy by stopping the synthesis of DNA, stable RNA and membrane components and rapidly synthesizing factors that are important for stress resistance, thereby ensuring bacteria survive in adverse environments. Our study revealed biological functions and potential molecular mechanisms of stringent response in *Yersinia enterocolitica* and underlying the interaction action between stringent response factors (p)ppGpp and DksA. The overall understanding of the physiological regulatory mechanism of (p)ppGpp and DksA in *Y. enterocolitica* was obtained. These results promoted the understanding of stringent response and the cooperation action between its regulatory factors in Enterobacteriaceae family.

Keywords: *Yersinia enterocolitica*, stringent response, environmental response, (p)ppGpp, Dks

Research and application of key technologies for enhancing biological reaction processes based on cell aggregation effects

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Abstract: In recent years, biocatalysis has established its position in sectors such as food, chemistry, and pharmaceuticals, emerging as a leading approach for the green production of biochemical products. Due to its economic and environmental advantages, the use of biocatalysts in industrial processes is increasingly popular. However, the development of viable bioprocesses are still confronted with some challenges. Addressing the prevalent issues of low catalytic efficiency and reuse rate of enzymes during biocatalysis, as well as the difficulties in continuous production and industrial application, we propose a novel integrated continuous catalytic system characterized by enzyme in situ preparation, intelligent assembly, and green catalysis. We focus on constructing chassis cells with a "dual protection system," establishing cell-enzyme composite catalysts based on enzyme surface display, and developing a green continuous catalytic system. Our research reveals the physiological characteristics of enzyme self-assembly, catalysis, and regeneration. We explore the mechanisms of biofilm formation and the establishment of efficient biofilm-forming strategies through the design of intelligent adsorption materials and quorum sensing signaling systems. This work provides a theoretical foundation and implementation strategies for a new continuous catalytic technology system that couples microbial surface display and biofilm cluster effect catalyst technologies, enabling the cyclic process of continuous enzyme catalysis and in situ regeneration. This advancement holds significant importance for promoting the industrial application of high-value biochemical products centered on enzymes in the field of biomanufacturing.

Keywords: biofilm-based fermentation, cell aggregation, surface display, bio-manufacturing

Combating biofilms of foodborne pathogens with bacteriocins by lactic acid bacteria in the food industry

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Abstract: Most foodborne pathogens have biofilm-forming capacity and prefer to grow in the form of biofilms. Presence of biofilms on food contact surfaces can lead to persistence of pathogens and the recurrent cross-contamination of food products, resulting in serious problems associated with food safety and economic losses. Resistance of biofilm cells to conventional sanitizers urges the development of natural alternatives to effectively inhibit biofilm formation and eradicate preformed biofilms. Lactic acid bacteria (LAB) produce bacteriocins which can effectively inhibit biofilm formation in a dose-dependent manner, but are difficult to disrupt preformed biofilms. Synergistic combination with other antimicrobials, incorporation in nanoconjugates and implementation of bioengineering can help to strengthen their antibiofilm activity. Chitosan nanoparticles loaded with nisin and DNase I (DNase-CS-N) was successfully prepared and characterized with smooth surface and nearly spherical shape, high surface positive charge, and good stability, which exhibit antimicrobial activity against *L. monocytogenes*. DNase-CS-N could inhibit biofilm formation of *L. monocytogenes*, with inhibit rate reaching 99% and 99.5% at 1/2 MIC and 1×MIC, respectively. At sub-MICs, DNase-CS-N could reduce cell motility (swimming and swarming) and slime production of *L. monocytogenes*, possibly contributing its antibiofilm activity. In terms of effect on biofilm elimination, DNase-CS-N at the concentration of 4×MIC led to 3-4 log reduction of biofilm cells in preformed *L. monocytogenes* biofilms, performing higher efficiency compared with other treatments. Furthermore, the three-dimensional structure of *L. monocytogenes* biofilms was severely disrupted after DNase-CS-N treatment, with bacterial cells scattered on surface. The morphology of *L. monocytogenes* biofilm cells was also greatly damaged with wrinkled surface, disrupted cell membrane as well as leakage of intracellular nucleic acid and protein. These results indicate the potential applicability of DNase-CS-N for inhibiting and eliminating *L. monocytogenes* biofilms on food contact surfaces.

Keywords: biofilms, foodborne pathogens, bacteriocin, nanoparticles

Strategies of developing food contact materials with antibiofilm function

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Abstract: Foodborne diseases caused by pathogen-contaminated foods have become a significant global challenge afflicting public health. Developing a universally applicable food-contact surface with an effective antibiofilm function is urgently needed. In recent years, various types of antibiofilm materials have been developed through the incorporation of antimicrobial agents, which could inhibit the growth of biofilm via inactivating the pathogens. However, the effectiveness in preventing biofilm formation remains unsatisfactory. This is primarily due to the irreversible chemical consumption of the incorporated antimicrobial agents. In addition, the accumulation of killed bacterial debris on the material surface of antimicrobial materials can obstruct contact between the materials and pathogens, contributing to ongoing contamination. To overcome these challenges, we first clarified that the initial adhesion of the biofoulants was the most critical stage in controlling the biofilm formation via building a boundary model of biofilm development. Therefore, preventing the adhesion of biofilms at the initial stage is a more effective control strategy compared to the removal of already mature biofilms. Based on N-halamine technology and photoinduced antibacterial technology, various antimicrobial materials with regenerable and sustainable antimicrobial functions have been synthesized, ensuring rapid and durable bactericidal effects. For example, we covalently bonded halamine precursors to the polymeric substrates via a controlled radical graft polymerization reaction. Different from the conventional antimicrobial materials, the resulting halamine materials can regenerate biocidal function through a simple chlorination process, which maintained a promising biocidal efficiency of 5 log CFU reductions even after five chlorination-decontamination cycles. Additionally, vitamin K3 as natural photosensitizers was modified onto the substrates, which could continuously generate biocidal reactive oxygen species (ROS) under visible light, ensuring the continuous biocidal function of the photoactive materials. However, these materials lack antibacterial activity in the dark. To address this limitation, we found that a benzophenone structure can store light energy through intermolecular electron rearrangement, allowing for the release of ROS under dark conditions and achieving an "always online" antibacterial function. Furthermore, superhydrophilic zwitterionic molecules were modified onto the obtained antimicrobial materials, embedding them with antifouling functional layers. The obtained bifunctional materials exhibited a functional loop that includes "reduction of initial bacterial adhesion," "efficient and sustained biocidal activity," and "rapid removal of bacterial debris, thereby, controlling the biofilm formation effectively.

Keywords: Antibiofilm, N-halamine, Vitamin K3, Benzophenone, Zwitterion

From raw ingredients to product- *Salmonella* survival during chocolate production

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Abstract: Bacterial contaminations are the predominant factor of food spoilage and, therefore, are the main threat to the safety and quality of food products. Although the food industry has different solutions, such as pasteurization and cleaning processes, several bacterial populations can survive these processes. One of the bacterial survival mechanisms is to adopt a sessile lifestyle and form a biofilm. Biofilm formation is highly dependent on nutrient availability and their chemical composition. *Salmonella* is the leading cause of hospitalization due to food poisoning. *Salmonella* outbreaks in low-water activity food products have increased steadily in recent years. In 2022, two *Salmonella* outbreaks in chocolate manufacturing in Israel and Europe led to a massive recall of many products, resulting in high economic loss. The combination of low water activity and relatively high-fat content in food products such as chocolate reduces the amount of Salmonella that can cause severe illness. Indeed, the outbreak in Europe led to over 400 illness cases in 17 countries, with a 41% hospitalization rate. *Salmonella* contamination can appear at every step during food production: in the field, supply chain, manufacturing, and storage. We aim to elucidate the correlation between chemical composition and bacterial survival mechanisms at the physiologic, metabolic, and molecular levels in chocolate as a model system. We observed a higher decrease in bacterial survival during cocoa powder storage than dry milk powder. The cocoa powder type also affected the cell viability reduction (Dutch vs. Dark). Interestingly, while the survival rate of *Salmonella* was similar in two different dry milk powders (Israel vs. USA) during storage, we observed a significant difference in bacterial survival to the thermal production processes. Our results indicate that *Salmonella*'s survival rate depends on the raw ingredient chemical composition, which might contribute to bacterial adaptation to desiccation and lead to resistance to other abiotic stresses at high temperatures. It has been suggested that dairy components such as milk protein, fat, and sugar may affect bacterial biofilm formation. Therefore, our next aim is to identify whether biofilm process and matrix production serve as physiological barriers and contribute to *Salmonella* survival during thermal treatments.

Keywords: *Salmonella*, Biofilm, Food Microbiology, Chemical composition, Thermal treatment

Evolutionary selection of biofilm-mediated extended phenotypes in *Yersinia pestis* in response to a fluctuating environment

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Abstract: *Yersinia pestis* is transmitted from fleas to rodents when the bacterium develops an extensive biofilm in the foregut of a flea, starving it into a feeding frenzy, or, alternatively, during a brief period directly after feeding on a bacteremic host. These two transmission modes are in a trade-off regulated by the amount of biofilm produced by the bacterium. Here by investigating 446 global isolated *Y. pestis* genomes, including 78 newly sequenced isolates sampled over 40 years from a plague focus in China, we provide evidence for strong selection pressures on the RNA polymerase ω-subunit encoding gene *rpoZ*. We demonstrate that *rpoZ* variants have an increased rate of biofilm production *in vitro*, and that they evolve in the ecosystem during colder and drier periods. Our results support the notion that the bacterium is constantly adapting—through extended phenotype changes in the fleas—in response to climate-driven changes in the niche.

Revealing the heterogeneity of *Pseudomonas aeruginosa* biofilms using single-cell probe-based RNA-sequencing

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Abstract: Within the biofilm, gradients of oxygen, nutrients, and waste products shape gene expression, rendering resident cells distinct not only from their planktonic counterparts but also from other cells in the same biofilm aggregate. This inherent heterogeneity in gene expression contributes significantly to the enhanced tolerance of biofilms against diverse stressors. Despite its profound impact on biofilm physiology, the study of biofilm heterogeneity has been hindered by the lack of appropriate methods. Current state-of-the-art methods for studying biofilm heterogeneity relies on highly specialized FISH-based approaches that enable the simultaneous examination of a limited number of genes to identify subpopulations within a biofilm. Here we present an innovative approach to investigate biofilm transcriptional heterogeneity at a genome-wide scale. Using *Pseudomonas aeruginosa* as a model organism, we employ single-cell, probe-based RNA-sequencing – a powerful tool providing insight into gene expression at the individual cell level with high sequencing depth – to identify biofilm subpopulations. While our methodology involves homogenizing biofilms in order to conduct the single-cell analysis, we plan to regain the spatial information of subpopulations within a biofilm by identifying unique, highly expressed genes within each subpopulation and performing mRNA-FISH. Thus far, our results of ~50K mature biofilm cells have identified 13 subpopulations with gene expression patterns that are indicative of their potential location in the biofilm. For instance, the largest subpopulation has genes encoding the translational machinery upregulated, suggesting that this subpopulation is in the biofilm exterior, since these cells are the most active in the biofilm. Interestingly, our method also identified a small subpopulation upregulating expression of R2-pyocin genes. Consistent with suggestions from the literature, we find that these cells, using a GFP reporter, are at the base of the biofilm where the biofilm under anoxic conditions. Ultimately, our goal is to uncover distinct spatiotemporal subpopulations within biofilms through their gene expression profiles, shedding light on novel genes crucial for biofilm function and enhancing our understanding of biofilm heterogeneity.

Keywords: *Pseudomonas aeruginosa*, transcriptomics, heterogeneity, scRNA-seq, R2-pyocin

Extracellular DNA: A multifunctional biofilm component

Rikke Louise Meyer

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Abstract: The significance of eDNA in biofilms has been known for two decades, and it appears to be the one component that most biofilms have in common. New research reveals that eDNA in the biofilm matrix forms several non-canonical structures that provide previously overlooked functionality to the biofilm. In addition to the canonical B-DNA double helix, eDNA also forms the left-handed Z-DNA, and G's coordinate to form G-quadruplexes. Z-DNA is mechanically strong, and G-quadruplexes provide elasticity, and can form a DNAzyme with peroxidase-like activity when coordinating with iron porphyrins (heme). We are only now starting to understand how the versatile structure and function of eDNA affects the biology of bacteria in biofilms. In this talk, I will give an overview of the origin, form, and function of eDNA in biofilms.

Antibiofilm coating and its evaluation methods by ISO.

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Abstract: Biofilms are viscous water films formed on material (product) surfaces through bacterial action. They consist of bacteria, bacterial polymers (such as extracellular polysaccharides and proteins), and a large amount of water, creating a heterogeneous thin film. Once formed, biofilms can induce corrosion on metal surfaces, cause scaling in pipelines, and produce slime in wet areas like kitchens, bathrooms, and toilets, deteriorating sanitary conditions. When biofilms develop on medical implants or in hospital facilities, antibiotics and drugs often become ineffective at standard concentrations, leading to infections and chronic diseases. Given these negative impacts, effective countermeasures are essential. Precise evaluation is necessary before implementing these measures. In collaboration with the Society of International sustaining growth for Antimicrobial Articles (SIAA), we established an international standard on July 18, 2023. This presentation will provide an overview of the standard, along with examples of anti-biofilm coatings developed for material surfaces.

Keywords: Biofilms, EPS, ISO 4768:2023, bacteria, SIAA, anti-biofilm materials

LasA from *Pseudomonas aeruginosa* selectively disrupts *Gardnerella vaginalis* biofilm

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Abstract: Since its initial report in the 1950s, bacterial vaginosis (BV) has been a global concern among women of reproductive age. BV is a prevalent polymicrobial infection associated with significant health risks, including increased susceptibility to sexually transmitted diseases and adverse obstetric outcomes. *G. vaginalis* biofilms contribute to antimicrobial resistance and disease recurrence, which is widely recognized as the primary causative agent responsible for BV development, hence highlighting an urgent need for novel therapeutic strategies. Our research demonstrates that LasA, an elastase from *Pseudomonas aeruginosa*, selectively targets the protein-rich extracellular matrix of *G. vaginalis* biofilms, effectively disrupting their structure and lysing the cell walls of the bacteria within. *In vitro* experiments revealed minimal adverse effects of LasA on probiotic lactobacilli strains, suggesting a potential for selective antibacterial activity. *In vivo* studies utilizing murine models artificially infected with *G. vaginalis* and naturally susceptible fox models confirmed the efficacy of LasA in eliminating *G. vaginalis* infection rapidly, with concurrent promotion of probiotic lactobacilli recovery and no observed detrimental effects on vaginal epithelial tissue. Comparative analyses indicated that LasA outperforms both antibiotics and lysozyme in disrupting *G. vaginalis* biofilms and lysing bacterial cell walls. Additionally, LasA did not exhibit bactericidal effects on probiotic *Lactobacillus crispatus*, preserving the vaginal microbiota balance. These findings position LasA as a promising candidate for BV treatment, offering a potential solution to the challenges posed by antibiotic resistance and biofilm-associated infections. The study's outcomes support further investigation and development of LasA as a therapeutic agent for BV, with implications for improving patient outcomes and reducing disease recurrence rates.

Keywords: *Gardnerella vaginalis*, biofilm, LasA, lactobacilli, vaginal infection therapy

Large-scale mechanical spiral waves in bacterial communities

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Abstract: Propagating spiral waves have been discovered in various chemical, biological and physical systems. While spiral waves in multicellular organisms are often associated with essential living functions, evidence of spiral wave pattern has been lacking in the bacterial world. Here we report the discovery of a first instance of propagating spiral waves in dense bacterial populations. Specifically, we discovered that type IV pilus activity in bacterial biofilms gives rise to large-scale spatiotemporal regulation of tension force in the form of propagating spiral waves. Theoretical modelling reveals that the spiral tension waves result from nonreciprocity in cell-cell interactions. Our findings reveal a novel collective behavior of type IV pilus motility and may shed light on the emergent mechanics of biofilms and microbiomes.

Keywords: type IV pilus motility, *Pseudomonas aeruginosa*, active matter

Go with the flow: how shear stress and quorum sensing shape enterococcal virulence in infective endocarditis

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Abstract: The transition of bacteria from a planktonic lifestyle to a biofilm is influenced by environmental stimuli, including surface adhesion and fluid flow. However, the effects of these factors in gram-positive bacteria are not well-understood. *Enterococcus faecalis*, which accounts for 5-15% of infective endocarditis (IE) cases, encounters pulsatile blood flow and high shear stress on heart valves. To investigate how fluid flow impacts *E. faecalis* virulence in IE, we used microfluidics to simulate heart valve shear stress and exposed surface-adhered bacteria to pulsatile fluid flow. After 30 minutes, transcriptional analysis revealed significant downregulation of the Fsr quorum sensing (QS) system suggesting that fluid flow interferes with autoinducer accumulation and QS activation during early IE. Consistent with this, *E. faecalis fsr* mutants are able to establish IE as well as the parental wild type strain in a rat model, indicating a limited role for QS in early infection. Surprisingly, *fsr* deletion mutants developed significantly larger cardiac vegetations at later stages of infection, characterized by extensive biofilm coverage and larger biofilm microcolonies resistant to neutrophil infiltration and antibiotic clearance. Clinically, >50% of *E. faecalis* isolates from IE patients contain inactivating deletions in the *fsr* locus, which correlates with prolonged bactermia increased disease severity. One explanation for the increased bacterial burden of *fsr* mutants is the role of gelatinase, a protease that is positively regulated by the Fsr QS system. We found that gelatinase can activate a human pro-inflammatory cytokine, suggesting that QS inactivation–and the resulting reduction of gelatinase-promotes an immunosuppressive microenvironment, enhancing biofilm development and persistence. These findings offer insight into the prevalence of QS mutations in clinical *E. faecalis* isolates, suggesting that while QS may facilitate biofilm formation in certain environments, its loss may confer a selective advantage during mammalian infection.

Exploring unique aggregate mechanisms in a chronic infection model

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Abstract: In the context of disease, bacterial aggregates have been isolated from both chronic and acute infections and can be formed by bacteria, archaea, and fungi. As an opportunistic pathogen, *Pseudomonas aeruginosa (Pa)*, causes disease in those whose immune systems or barrier functions are compromised. This includes those with chronic and acute wounds, medical devices, and chronic infection in the lungs of people with the genetic disease cystic fibrosis (CF). Once chronic *Pa* colonization is established, a large proportion of the infecting bacteria grow within airway sputum as aggregates (~10-1000 cells). Previous studies of *Pa* cells in large, well-mixed flask cultures, (macro-scale biofilm structures) have contributed significantly to our understanding of *Pa* growth, communication systems, and mechanisms *Pa* utilizes to become tolerant to many antibiotics. However, growth in this context does not closely recapitulate that of actual infection-growth as aggregates. This discrepancy signifies a significant gap in translating our knowledge of biofilm biology into meaningful insights for human health. Using a synthetic cystic fibrosis (CF) sputum media (SCFM2), we have identified a subset of genes critical for aggregate formation. Disruption of these genes results in a spectrum of spatial phenotypes that result in micron-scale changes in organization of *Pa* cells within aggregates. We show for the first time a direct relationship between changes in spatial organization and tolerance to antibiotic therapies. These data significantly contribute to our understanding of aggregate physiology during infection, with the potential develop new therapeutic strategies against *Pa* aggregates and other aggregate forming multi -drug resistant pathogens.

Keywords: Aggregate, *Pseudomonas aeruginosa*, cystic fibrosis, spatial organization

Novel tetrameric PilZ protein stabilizes stator ring in complex flagellar motor

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Abstract: Rotation of the bacterial flagellum, the first identified biological rotary machine, is driven by its stator units. Knowledge gained about the function of stator units has increasingly led to studies of rotary complexes in different cellular pathways. Here, we report that a tetrameric PilZ (tPilZ) family protein, FlgX, is a novel structural component underneath the stator units in the flagellar motor of *Campylobacter jejuni*. FlgX forms a stable tetramer that does not bind c-di-GMP, unlike other canonical PilZ domain-containing proteins. Cryo-electron tomography and sub-tomogram averaging of flagellar motors *in situ* provide evidence that FlgX interacts with each stator unit and plays a critical role in stator ring assembly and stability. Furthermore, FlgX is conserved and was most likely present in the common ancestor of the phylum *Campylobacterota*. Overall, FlgX represents a new divergence in function for PilZ superfamily proteins as well as new player in the key stator-rotor interaction of complex flagellar motors.

Keywords: PilZ, stator, flagella, motor evolution, *Campylobacter*

Collective fountain-like flow and fractal wrinkling drive bacterial community morphogenesis

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Abstract: Bacterial biofilms represent a basic form of multicellular organization that confers survival advantages to member cells, including increased resistance to antibiotics, phage invasion and immune clearance. However, it is unclear how founder cells and their progeny assemble biofilms cell-by-cell, a developmental process highly relevant to host infections. In this talk, I will present two discoveries on how bacteria construct biofilms. First, by combining dual-view light-sheet microscopy and intracellular puncta labeling, we traced single-cell trajectories and lineages in developing *Vibrio cholerae* biofilms. Two distinct cell position fates are uncovered: one set of biofilm cells expands ballistically outward, while the other becomes trapped at the substrate. A collective fountain-like flow transports cells to the biofilm front, bypassing substrate-trapped cells. This cooperative behavior enables community expansion into new territory. Coordinated expansion requires the extracellular matrix protein RbmA, without which cells move erratically as uncorrelated individuals. Second, we discovered a fractal wrinkling morphogenesis program, marked by the emergence of a cascade of ever finer wrinkles, that determines *V. cholerae* community morphology. Quorum sensing, the mechanism of cell-to-cell communication, regulates this program, in which community morphology can be controlled by varying the level of a key quorum-sensing regulator, the Qrr small RNA. Together, these findings reveal that individual bacterial cells rely on social interactions and collective dynamics to shape community development.

Keywords: Bacterial Biofilms, Light-sheet microscopy, quorum sensing, *Vibrio cholerae*

Anti-biofilm enzymes strategy

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Abstract: Biofilm has strong resistance to antibiotics, which are known as the leading cause of nosocomial infections. Elimination of biofilm is the key to treating biofilm-associated infections. Enzymes have shown great potential for application in the field of anti-pathogenic bacterial biofilm infections, and their mechanism of action is different from that of antibiotics, which does not lead to the emergence of drug-resistant strains. Our team combines bioinformatics and enzyme engineering techniques to mine mutant enzymes with excellent performance. The mutants showed significantly enhanced thermostability and anti-biofilm activity compared to the wild-type enzyme (Aii810, AidH and AlgL). Based on the enzymatic reaction, multi-targeted therapy (targeting EPS polysaccharides and AHLs mediated quorum sensing) is used to disrupt *Pseudomonas aeruginosa* biofilm. A combined enzyme anti-biofilm strategy (AidH and PslG) was also put forward for the first time to simultaneously prevent biofilm formation and break down preformed biofilms. Interestingly, under the combined-enzyme intervention for *P. aeruginosa* wild-type and clinical strains, no biofilm was observed on the bottom of NEST glass-bottom cell culture dishes. The combination strategy also helped multidrug-resistant clinical strains change from resistant to intermediate or sensitive to many antibiotics commonly used in clinical practice. This treatment was effective in quorum-quenching and biofilm inhibition on infected wounds. Compared with the tobramycin treatment only, simultaneous treatment with the enzymes and antibiotics significantly reduced the severity of tissue damage, decreased the bacterial load, and reduced the expression of the inflammatory indicators. Topical application of the enzymes also reduced the bacterial load and inflammation to some extent. These results indicate that the combined-enzyme approach is a potentially effective treatment for *P. aeruginosa* biofilm infections of burn wounds.

Keywords: biofilm, enzyme, rational design, quorum sensing, *Pseudomonas aeruginosa*

Tn-Seq based identification of genes that play a role in antibiotic tolerance of *Pseudomonas aeruginosa* aggregates

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Abstract: Microbial biofilms cause a variety of persistent infections due to their inherent tolerance to antibiotic treatment. Current knowledge of biofilm-associated antibiotic tolerance is fragmented, and the relative importance of the identified factors is unknown. We developed a Tn-Seq based procedure that enables simultaneous assessment of the relative antibiotic tolerance of hundreds of thousands of individual biofilm aggregates. We used a super-saturated *P. aeruginosa* PA14 transposon mutant library consisting of more than 800,000 unique mutants, to identify mutants with increased susceptibility towards ciprofloxacin. The transposon mutants were grown in agar plates under conditions where each mutant forms an agar-embedded aggregate. Antimicrobial tolerance increased with aggregate age, but even relatively young aggregates displayed tolerance up to 10-fold higher than their planktonic counterparts. Furthermore, the level of c-di-GMP - as measured through a c-di-GMP-monitor - was significantly higher in the aggregated bacteria than in planktonically grown bacteria, confirming that the aggregates represent a biofilm-like growth physiology. For a single experiment, we used large agar plates that combined harbored over 150,000 aggregates. The aggregates were treated with antibiotics, washed, and then disintegrated. The identity and relative number of the mutants present in the pool of antibiotic-treated versus non-treated aggregates was then determined by Tn-Seq analysis. Using this genome-wide Tn-Seq analysis procedure, we found that ciprofloxacin tolerance of *P. aeruginosa* aggregates is not dependent on a specific pathway but relies on numerous functionally diverse genes. The majority of these genes play a role in microbial metabolism.

Keywords: Microbial aggregates, *Pseudomonas aeruginosa*, antibiotic tolerance, Tn-Seq, metabolism

Bacterial language: from quorum sensing signal to nucleotide second messenger

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Abstract: Quorum sensing signals and intracellular nucleotide second messengers are widely employed by bacterial cells to control virulence and biofilm formation. Therefore, interfering or blocking quorum sensing communication and intracellular nucleotide second messenger signaling has become a new antibacterial strategy with broad potential applications. Our research group has been engaged in the study of quorum sensing, intracellular nucleotide messengers, pathogenic mechanisms of pathogens, and the development of new antibacterial drugs. Our main research achievements: (1) For the first time, we identified six bacterial quorum sensing signals and revealed the signaling pathways and regulatory mechanisms of these signals (*PNAS* 2012, 2017; *ISME J* 2008, 2020; *PLoS Pathogens* 2022）;(2) In *Acinetobacter baumannii*, translation elongation factor P (EF-P) was identified to be a novel receptor for cyclic guanosine monophosphate (c-di-GMP) messenger, and the mechanism by which EF-P induced by c-di-GMP to regulate the translation levels of proteins containing polyprolines, ultimately regulating the physiological functions and virulence was elucidated (*PNAS* 2022); (3) Identifying the novel function of the GGDEF domain of *Ralstonia solanacearum* Rsp0334, which catalyzes the conversion of 2',3'-cGMP to 2',3'-c-di-GMP, and revealing the mechanism by which 2',3'-cGMP regulates the physiological function and pathogenicity of *R. solanacearum* by binding to the transcriptional regulator Rsp0980 (*Nature Communications* 2023); (4) Cyclic dinucleotides can regulate type I interferon through interferon gene stimulating factor (STING). Our latest research results reveal a new mechanism of STING cyclic dinucleotide mediated type I interferon regulation by Dual Specificity Protein Phosphatase 4 (DUSP4) (*Cell Death and Differentiation* 2024). Our previous research has shown that RpfR acts as a receptor for the BDSF quorum sensing signal in *Burkholderia cenocepacia*, regulating its important biological functions and virulence. Our recent research has found that the fatty acyl CoA ligase DsfR (BCAM2136) from *B. cenocepacia* not only effectively catalyzes the synthesis of lauroyl CoA and oleoyl CoA from lauric acid and oleic acid *in vitro*, but also serves as a novel transcriptional regulator to regulate the physiological functions and virulence of *B. cenocepacia* by sensing BDSF signals. Our research findings indicate that DsfR is not only a novel class of transcriptional regulatory proteins, but also a new type of signal receptor for DSF-family quorum sensing signals (*Cell Reports* 2024). Together, our results have revealed the signaling pathways of quorum sensing systems and intracellular nucleotide second messengers in various pathogenic bacteria. The research findings will lay a theoretical foundation for the development of new methods of prevention and control of bacterial pathogens.

Keywords: quorum sensing, nucleotide second messenger, bacteria, biofilm, virulence

Bacterial quorum sensing and the strategies of seafood preservation

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Abstract: Microorganism is one of the important factors leading to food spoilage. The recent studies indicated that the quorum sensing (QS) system of bacteria can regulate the spoilage ability of bacteria, thereby affecting the spoilage process of seafood and the products. Biological preservatives based on bacterial quorum sensing target has attracted increasing attention due to its less bacterial resistance, lower toxicity and higher efficiency. Therefore, this article elaborates on the strategies of seafood preservation based on the bacterial AHLs/LuxR or AI-2/LuxS quorum sensing regulation. For the bacterial AHLs/LuxR quorum sensing system, the effects of quorum sensing inhibitors, quorum sensing quenching enzymes, and the biofilm degrading enzymes on seafood spoilage bacteria including Shewanella, Acinetobacter, Aeromonas were evaluated. This studies indicated that Forsythia suspensa water extract (FSE), lotus leaf water extract (LLE), dandelion water extract (DE) and cinnamon water extrac(CE) had strong quorum sensing inhibitory activities against seafood spoilage bacteria. The quorum sensing quenching enzyme AiiA enzyme were obtained from the recombinant *E.coli* BL. The addition of AiiA enzyme to the co-culture system can inhibit the formation of co-culture bacterial biofilms and reduce the secretion of proteases. The combined enzyme of lipase, cellulose and proteinase K could significantly inhibited the biofilm formation and the content, and provided a novel candidate to overcome biofilm's problem of spoilage bacteria and foodborne pathogens in the seafood preservation. For the bacterial AI-2/LuxS quorum sensing system, the screening and antibacterial effect of marine biocontrol lactic acid bacteria, and the regulation of marine biocontrol lactic acid bacteria AI-2/LuxS system on spoilage bacteria in seafood products were elaborated. More 54 biocontrol lactic acid bacteria (LAB) strains with excellent adhesion potential and high antibacterial activity were isolated and purified from marine fish intestine. This study revealed that AI2/LuxS system regulates the nutritional competitiveness of LAB by facilitating membrane transport systems. This research will provide a theoretical basis and basic research data for the development of biocontrol agents and storage technologies based on bacterial quorum sensing targets.

Keywords: Seafood, Biological preservation strategies, Quorum Sensing, AHLs/LuxR, AI-2/LuxS

The mechanism of quorum sensing signaling deterrence of *Burkholderia cenocepacia* by rhododendrol and other endophytic metabolites of *Areca catechu* L. derived endophytes

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Abstract: In recent years, bacterial drug-resistance has become a new global health issue and poses a significant threat to global public health. *Burkholderia cenocepacia* is a drug-resistant bacterium that poses a major threat to cystic fibrosis patients, exhibiting intrinsic high resistance to some traditional antibiotics. Therefore, the development of novel targeted drugs to address the increasingly serious issue of *B. cenocepacia* resistance is of great significance. Quorum sensing (QS) is the cell-cell communication among bacterial cells by signaling molecules, regulating various biological functions including collective movement, which play a crucial role in regulating bacterial pathogenicity and resistance. QS has become a new strategy to combat the escalating problem of antibiotic resistance. Therefore, this study focused on the QS system of *B. cenocepacia* and screened for QSIs from natural products.

First, one isolated clinical strain, *B. cenocepacia* X85, can produce a higher amount of extracellular polysaccharides, proteases, lipases, and siderophore compared to the reference strain *B. cenocepacia* H111. It also exhibited stronger motility and formed more abundant and dense biofilms. It was observed that the clinical strain *B. cenocepacia* X85 rapidly colonized the *Galleria mellonella* and displayed higher virulence and pathogenicity, resulting in a higher mortality rate. Drug susceptibility testing revealed that the clinical strain *B. cenocepacia* X85 was resistant to folate metabolism pathway inhibitors, tetracyclines, carbapenems, aminoglycosides, β-lactams, fluoroquinolones, and quinolones. Subsequently, whole-genome resequencing revealed the activation of quorum sensing systems, two-component systems, and ABC transport systems in the clinical strain *B. cenocepacia* X85.

Secondly, based on the AHL signaling molecular inhibitory activity screening of the reporter strain *C. violaceum* CV026, total 41 compounds were isolated and identified from the endophytic bacteria *B. amyloliquefaciens* RB39 and endophytic actinomycete *S. parvus* RC5. *C. violaceum* CV026 was used to track the QS activity of the 41 compounds, and total 21 compounds were found to have potential QS inhibitory activity.

The physicochemical properties, *in vivo* and *in vitro* safety, thermal and acid-base stability of the active compounds was further evaluated. It was found that rhododendrol derived from endophytic actinomycetes, is a safe and non-toxic quorum sensing inhibitor against *B. cenocepacia* X85. The structure-activity relationship and molecular docking studies were further investigated.

The combination drug indicated that rhododendrol could serve as an adjuvant for tetracycline, sulfamethoxazole, imipenem, meropenem, levofloxacin, and erythromycin, significantly enhancing the antibacterial activity of these antibiotics. Moreover, it demonstrated the best sensitization effect on tetracycline, with an 8-fold recovery of tetracycline sensitivity when the two were used in combination.

This comprehensive approach provides new insights for developing novel therapeutic approaches and offers hope for controlling bacterial infections and reducing antibiotic resistance.

Keywords: (R)-4-(3-hydroxybutyl) phenol, *Burkholderia cenocepacia* X85, Quorum sensing, Quorum sensing inhibitor, Drug-Resistance, Infection control

Study on the synergistic mechanism of bacterial inhibition by ITC flavouring substances and essential oils in wasabi

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Abstract: Isothiocyanates (ITCs) analogues are unique flavour substances and characteristic odours in wasabi. It was found to have good antibacterial activity. Synergistic effects with different types of plant essential oils were also found. To further clarify the mechanism of their synergistic effect on prokaryotic organisms, transcriptomics-based studies were carried out on foodborne pathogenic bacteria and foodborne spoilage bacteria, which were validated by morphological and other epigenetic properties. The transcriptomics results showed that ITCs affect the pathways of protein efflux, secretion system of bacteria, and carotenoid synthesis in *Staphylococcus aureus*. They affect pathways such as pyrimidine metabolism in *Pseudomonas fluorescens* and bacterial chemotaxis. Affects pathways such as aminosugar and ribose metabolism in *Salmonella*. Affect the pathways of *Listeria monocytogenes* such as acetaldehyde and dicarboxylic acid metabolism, fructose metabolism, and glycerol ester metabolism. In addition, the results of phenotyping experiments showed that synergistic treatment of bacterial cells with ITC and plant essential oils resulted in loss of cell membrane integrity, leakage of intracellular substances, exocytosis of intracellular ATP and hyperpolarisation of the cell membrane. The bacterial cell membrane permeability was disrupted, allowing more ITCs to enter the bacterial cells to exert direct bacteriostatic effects. Therefore, all these results directly or indirectly indicate that ITC analogues can affect microbial cell membranes and so on from multiple pathways. In this study, we verified that ITCs and different types of plant essential oils have synergistic inhibitory effects on different bacteria, which provides new ideas for inhibiting the growth of different prokaryotes in food.

Keywords: prokaryotic bacterium, isothiocyanates, synergistic antibacterial effect, antibacterial mechanism, transcriptomics

Novel approaches and tools to predict antimicrobial susceptibility in biofilms

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Abstract: There is a growing awareness that the behaviour of bacteria (whether it be single cells, small agregates or mature biofilms) is to a large extent determined by the microenvironment at the site of the infection. Important microenvironmental factors include oxygen levels, the presence/absence of various carbon and nitrogen sources, as well as the presence/absence of certain host-derived factors. Considering the importance of the microenvironment in determining antimicrobial susceptibility and the outcome of antimicrobial treatments, especially for biofilm-related infections, there is an urgent need for novel tools and approaches that allow us to study susceptibility in conditions that mimick what happens in a patient, before and during treatment. In addition, the use of these *in vivo*-like conditions to grow biofilms, combined with state-of-the-art analytical approaches (including isothermal microcalorimetry) and various machine-learning algorithms could improve the accuracy of predicting biofilm susceptibility.

Keywords: antimicrobial susceptibility, biofilm testing, prediction

A multifaceted approach to combating biofilms: computational modeling and novel nanocoatings

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Abstract: To gain a deeper understanding of biofilm mechanics and detachment, we developed a coupled computational fluid dynamics-discrete element method (CFD-DEM) model. By simulating hydrodynamic fluid flow at varying velocities and loading rates, we have elucidated the mechanisms of biofilm detachment, including erosion and sloughing. Our model, which allows for adjustments in the proportion of different microbial functional groups, enables a detailed investigation of the contribution of extracellular polymeric substances (EPS) to biofilm resistance against fluid shear stress. Our modeling suggests that materials that inhibit EPS production could be promising antibiofilm candidates. To complement this computational approach, we introduce novel slippery omniphobic covalently attached liquid-like nanocoatings. These coatings provide a stable and durable liquid-like surface that effectively resists biofilm formation. Our results demonstrate a remarkable reduction in biofilm formation by three to four orders of magnitude compared to conventional materials under both static and dynamic conditions. Unlike traditional liquid-infused slippery surfaces, our nanocoatings maintain their antibiofilm properties even under challenging environments. By combining computational modeling and innovative nanocoatings, we offer a comprehensive approach to combating biofilms. Our findings pave the way for the development of more effective antibiofilm strategies and materials.

Tailored multilayer nanoparticle against resistant *P. aeruginosa* by disrupting the stubborn triad of thickened mucus, dense biofilm and hyperinflammation

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Abstract: Therapeutic challenges of chronic pulmonary infections caused by multidrug-resistant *Pseudomonas aeruginosa* (MDR-*P. aeruginosa*) biofilms due to significantly enhanced antibiotic resistance. This resistance is driven by reduced outer membrane permeability, biofilm barriers, and excessive secretion of virulence factors. Thickened mucus in the airways exacerbates the problem by impeding antibiotic penetration, providing a breeding ground for biofilms, consequently aggravating infection. Moreover, biofilms recruit numerous immune cells, resulting in chronic inflammation and lung tissue damage. In turn, damaged airway further facilitates bacterial colonization and elevated mucus production. To thoroughly disintegrate the stubborn triad of "thickened mucus & dense biofilm & excessive inflammation" and address drug resistance, tailored multilayer nanoparticles (NPVC/PBIP NPs) were developed. NPVC/PBIP NPs were engineered through self-assembly of vanillin-chitosan amphiphilic polymer loading polymyxin B-linoleic acid ion pairs in. Then polyaspartic acid and N-acetylcysteine-ε-poly-L-lysine were coated by layer-by-layer on the surface of vanillin-chitosan NPs *via* electrostatic interactions. As expected, the NAC units on NPVC/PBIP NPs effectively thinned human clinical sputum and porcine sputum, resulting in rapid sputum penetration followed by biofilm permeation. NPVC/PBIP NPs achieved over 99% eradication of mature biofilms *in vitro*. Furthermore, they effectively inhibited virulence factors production and bacteria re-adhesion (biofilm reformation) while exhibiting superior anti-inflammatory and antioxidant activities. In a chronic pulmonary infection model, NPVC/PBIP NPs remarkably thinned airway mucus, reduced bacterial burden by 99.7%, alleviated inflammatory cell infiltration, and minimized lung tissue damage. In summary, the NPVC/PBIP NPs represent a novel and promising strategy to manage MDR-*P. aeruginosa* biofilms associated infections by disintegrating the stubborn triad of "thickened mucus & dense biofilm & excessive inflammation".

Keywords: MDR-*P. aeruginosa* Biofilms, Vanillin-chitosan, N-acetylcysteine-ε-poly-L-lysine, Tailored multilayer Nanoparticles, Thickened Mucus, Dense Biofilm, Excessive Inflammation

The *Staphylococcus aureus* ArlS kinase inhibitor tilmicosin has potent anti-biofilm activity in both static and flow conditions

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Abstract: *Staphylococcus aureus* can form biofilms on biotic surfaces or implanted materials, leading to biofilm-associated diseases in humans and animals that are refractory to conventional antibiotic treatment. Recent studies indicate that the unique ArlRS regulatory system in *S. aureus* is a promising target for screening inhibitors that may eradicate formed biofilms, retard virulence and break antimicrobial resistance. In this study, by screening in the library of FDA-approved drugs, tilmicosin was found to inhibit ArlS histidine kinase activity (IC50 = 1.09 μM). By constructing a promoter-fluorescence reporter system, we found that tilmicosin at a concentration of 0.75 μM or 1.5 μM displayed strong inhibition on the expression of the ArlRS regulon genes *spx* and *mgrA* in the *S. aureus* USA300 strain. Microplate assay and confocal laser scanning microscopy showed that tilmicosin at a sub-minimal inhibitory concentration (MIC) had a potent inhibitory effect on biofilms formed by multiple *S. aureus* strains and a strong biofilm-forming strain of *S. epidermidis*. In addition, tilmicosin at three-fold of MIC disrupted USA300 mature biofilms and had a strong bactericidal effect on embedded bacteria. Furthermore, in a BioFlux flow biofilm assay, tilmicosin showed potent anti-biofilm activity and synergized with oxacillin against USA300.

Evolution of antimicrobial resistance in biofilms

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Abstract: The Antimicrobial resistance (AMR) is a global problem, known as the "silent pandemic". Evolutionary medicine emphasizes the necessity of shifting the focus from developing new antibiotics to tackling the evolutionary process itself and identifying strategies to prevent it. The *in vitro* and *in vivo* pathways of AMR evolution in *P. aeruginosa* biofilms will be presented as well as the potential of antioxidants as anti-mutagenic drugs.

The mechanism of Agr mutation causing persistent *Staphylococcus aureus* infection

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Abstract: Quorum cheating, a socio-microbiological process that is based on mutations in cell density-sensing (quorum-sensing) systems, has emerged as an important contributor to biofilm-associated infection in the leading human pathogen *Staphylococcus aureus*. This is because inactivation of the staphylococcal Agr quorum-sensing system leads to pronounced biofilm formation, increasing resistance to antibiotics and immune defense mechanisms. Since biofilm infections in the clinic usually progress under antibiotic treatment, we here investigated whether such treatment promotes biofilm infection via the promotion of quorum cheating. Quorum cheater development was stimulated by several antibiotics used in the treatment of staphylococcal biofilm infections more strongly in biofilm than in the planktonic mode of growth. Sub-inhibitory concentrations of levofloxacin and vancomycin were investigated for their impact on biofilm-associated (subcutaneous catheter-associated and prosthetic joint-associated infection), where in contrast to a non-biofilm-associated subcutaneous skin infection model, a significant increase of the bacterial load and development of Agr mutants was observed. Our results directly demonstrate the development of Agr dysfunctionality in animal biofilm-associated infection models and reveal that inappropriate antibiotic treatment can be counterproductive for such infections as it promotes quorum cheating and the associated development of biofilms.

An antibiofilm peptide AMP-17 inhibits hyphal development in *Candida albicans* exerting antibiofilm effect

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Abstract: The persistence of *Candida* infection is due to its ability to form biofilms that enable to resist antifungals and host immune systems. Hence, inhibitions of the biofilm formation and virulence characteristics of *Candida* ap. offers a promising approach to combat these infections. High-efficiency and low-toxic antimicrobial peptides (AMPs) are anticipated to be the future candidates to solve the increasingly prominent problems of *Candida albicans* infection and drug resistance. AMP-17, an antifungal peptide from *Musca domestica* found in our lab, is a *Candida*-selective antimicrobial peptide capable of preferentially killing *Candida* spp. Previous studies have demonstrated that AMP-17 effectively inhibits adhesion and filamentous development of *C. albicans*, leading to biofilm formation *in vitro*. Significantly, AMP-17 protects *Galleria mellonella* and mice from systemic candidiasis. Furthermore, AMP-17 also obviously inhibited the formation of *C. albicans* biofilms within subcutaneous catheters. Further research demonstrated that AMP-17 primarily regulates filamentation and inhibits biofilm formation through the Cek-MAPK pathway by RNA-seq analysis. While the nature of hypha-related gene expression required for fungal virulence has been extensively researched, potential morphotype-dependent activity of metabolic pathways remained unclear. Here, we combined global transcriptome and metabolome analyses to identify the metabolic adaption that accompany the filamentation process when *C. albican*s is challenged under the press of AMP-17. We identified *PYC2*, a gene encoding pyruvate carboxylase, played an important role in adhesion, yeast-to-hyphae transition, and biofilm formation. Most strikingly, we found deletion of *PYC2* decreased the sensibility of AMP-17 against *C. albicans* in rather than in planktonic cells. Additionally, deletion of *PYC2* led to a minor reduction on the expression levels of adhesion and filamentation-specific genes (ALS1 and HWP1) of *C. albicans* after AMP-17 treatment. Additionally, the inhibitory effect of AMP-17 on the hyphal development in *PYC2* mutant strain was significantly reduced, and the downregulation level of the key genes (*CEK1*, *CPH1*, and *UME6)* expression within Cek-MAPK signaling pathway was also markedly decreased. Our findings emphasize the activity of AMP-17 in inhibiting *C. albicans* filamentation and anti-biofilm properties, which involves *PYC2*-mediated Cek-MAPK regulation of *C. albicans* filamentation. Thus, this study demonstrated that pyc2 is a potential target of AMP-17 for developing antifungal agents.

Keywords: antibiofilm peptide, AMP-17, *Candida albicans*, filamentation, *PYC2*

A sex hormone catalyzes biological nitrogen fixation

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Abstract: Biological nitrogen fixation (BNF) involves the conversion of atmospheric nitrogen gas (N_2) into ammonia (NH_3), which plants and some other organisms can use to synthesize essential organic compounds such as proteins and nucleic acids. This process is crucial for the global nitrogen cycle and is primarily carried out by certain prokaryotic organisms, including bacteria and archaea. Our research uncovered a remarkable relationship between BNF and testosterone, the primary male sex hormone. In humans, testosterone is vital for the development of male reproductive tissues, such as the testicles and prostate, and it also promotes secondary sexual characteristics. We conducted molecular docking studies with testosterone and the nitrogenase enzyme, which comprises two components: the Fe protein (NifH) and the FeMo protein (NifDK). These components facilitate electron transfer and the conversion of N_2 to NH_3, respectively. Our results revealed that testosterone binds to NifH and NifDK with excellent binding affinities, demonstrating superb non-covalent bonding interactions. In parallel, we performed laboratory experiments by growing nitrogen-fixing bacteria in the presence and absence of testosterone. We measured their nitrogen fixation rates and confirmed that testosterone enhances BNF. It has been reported that certain Firmicutes can synthesize testosterone or promote its reabsorption through deconjugation, particularly in the human gut. Additionally, the human gut microbiota, which includes strains such as Klebsiella and Clostridiales, has the potential to fix nitrogen. Therefore, testosterone may enhance BNF in the human gut, providing essential nitrogen nutrition for malnourished individuals. This phenomenon might also apply to soil ecosystems, where testosterone is naturally present, potentially boosting nitrogen fixation in these environments as well.

Construction of cinnamaldehyde-loaded chitosan nanoparticles functionalized with DNase-I and their anti-biofilm activity against *Listeria monocytogenes*

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Abstract: *Listeria monocytogenes* tend to adhere to the surfaces of production equipment to form biofilms, leading to serious food safety concerns and economic losses. Cinnamaldehyde as a plant extract has broad-spectrum antibacterial activity and a good inhibitory effect on biofilms, however, it is responsive to environmental influences. In this study, DNase-I functionalized chitosan nanoparticles loaded with cinnamaldehyde (DNase-CS-CIN) were constructed to overcome bioavailability issues, and DNase-I functionalization could help the nanoparticles break through the natural barrier formed by extracellular matrix. The DNase-CS-CIN were subsequently characterized by ZetasizerNano, transmission electron microscopy (TEM), which results showed that the DNase-CS-CIN nanoparticles with smooth surface, nearly spherical shape and good stability. Specially, DNase-I was covalently grafted on the surface by FT-IR and XRD. The experimental results demonstrated that DNase-CS-CIN could reduce *L. monocytogenes* on stainless steel and polyurethane materials by 1.3 and 2 log colony-forming unit (CFU)/cm^2. Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) revealed that DNase-CS-CIN decompose extracellular DNA, destroy biofilm structure and damage the morphology of *L. monocytogenes* cells. Furthermore, DNase-CS-CIN treatment also resulted in DNA and protein leakage, decreased cell metabolic activity, further confirming the anti-biofilm activity of DNase-CS-CIN. In addition, following treatment with DNase-CS-CIN, the quorum sensing genes (*agrB*), virulence factors (*inlA*, *inlB*), and motility-related genes (*flaA*) of *L. monocytogenes* were significantly downregulated. The present results suggested the greater prospective of the application of DNase-CS-CIN as potential anti-biofilm agents as compared to native cinnamaldehyde and provides a new way for the development of anti-biofilm strategy in the future.

Keywords: nanoparticles, *Listeria monocytogenes*, biofilms, cinnamaldehyde, DNase I

Assessment of eggshell waste as a soil amendment in biosolarization

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Abstract: In recent decades, the demand for eggs in human diets has steadily risen, with global egg production projected to reach 90 million tons by 2030. This growth results in significant eggshell waste (ESW), with approximately 30% generated by industrial egg processing. Currently, most ESW is disposed of in landfills with minimal pre-treatment, contributing to environmental pollution through odor, microbial growth, and greenhouse gas (GHG) emissions. Developing safe and effective ESW disposal methods is essential for promoting sustainable food systems. Soil biosolarization (SBS) is an eco-friendly soil disinfestation technique combining organic waste addition into the soil with passive solar heating to control pests and enhance soil fertility. Previous studies have shown SBS can effectively incorporate agricultural byproducts, such as grape and tomato processing waste, to suppress weeds and improve soil health for subsequent crops. This study explores the potential of utilizing ESW as an amendment in SBS, offering a sustainable method for ESW management and soil enhancement. In our research, the feasibility of using ESW in SBS was evaluated through a laboratory simulation, with assessments conducted in both aerobic and anaerobic soil conditions. Key measurements included: (1) soil pH, volatile solids, electrical conductivity, and nutrient contents; (2) soil respiration and volatile emissions; (3) microbial community structure; and (4) phytotoxicity and weed inactivation. Results showed that ESW application increased soil pH and respiration, promoted the accumulation of pest-suppressing volatile compounds, and enhanced microbial diversity, including species richness and evenness, compared to control soils. Phytotoxicity tests indicated that while ESW-treated soils retained some volatile phytotoxicity after SBS, extended aeration mitigated this effect, allowing for successful biomass recovery. The SBS treatment also achieved complete weed inactivation, with high temperatures playing a critical role. These findings suggest that ESW can serve as a valuable amendment in SBS, offering a promising avenue for sustainable ESW disposal and soil health improvement.

Keywords: Eggshell waste (ESW), Soil biosolarization (SBS), Sustainable waste management, Microbial respiration, Soil microbiome

Pairwise encounters boost bacterial motion by transient velocity spikes

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Abstract: For swimming bacteria near surfaces, pairwise encounters inevitably occur and impact their social behavior. However, we know little about how the encounter events influence bacterial dynamics due to the limitations in tracking interplaying bacteria in 3D. Herein, we elucidated the motions of encountering *E. coli* by a combination of 3D holographic tracking experiments and hydrodynamic simulations. We find encounters with other cells induce transient yet remarkable fluctuations in swimming speed and angle of *E. coli*, concurrently diminishing their temporal correlations, in contrast to solitary cells. Notably, bacteria approaching each other in a face-to-face fashion both accelerate, whereas they both decelerate during pursuits. Generally, the motion of a pair of smooth-swimming *E. coli* is dictated by the relative angle, velocity, and intercellular distance, as validated by hydrodynamic simulations. The presence of the surface mitigates the velocity spikes during the encounter process. In contrast, the speed fluctuation of the cell is slightly amplified when a neighboring cell initiates a tumble. Additionally, the encounter process influences the timing of tumbles, i.e., tumble tends to occur before the two bacteria get in close proximity. Despite the impact of one encounter being transient, we reveal that *E. coli* gains propulsion advantage from encounter, thus providing insights into bacterial physiology and guidance for designing active microdevices.

Keywords: bacterial interactions, pairwise encounter, 3D tracking, digital holographic microscopy, tumbling motions

YtnP: one novel quorum quenching enzyme from *Bacillus amyloliquefaciens* W11 inhibits biofilms and spoilage of white radish by *Serratia marcescens*

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Abstract: The spoilage of fruits and vegetables caused by microorganisms poses significant challenges to food safety and food industry. *Serratia marcescens*, a microorganism commonly found in the environment and food production facilities, exhibits strong spoilage activity on fruits and vegetables. In this study, one novel lactonase (YtnP) derived from *Bacillus amyloliquefaciens* W11 was successfully expressed and utilized to combat the spoilage of white radish caused by *S. marcescens*. The recombinant YtnP demonstrated broad-spectrum activity against various AHL signaling molecules. Notably, YtnP exhibited remarkable thermal stability, retaining over 60% enzyme activity after exposure to 75°C for 1 h. Through mutagenesis of key amino acid residues, mutants H111A and H113A exhibited negligible enzyme activity against C6-HSL. H115A and H191A demonstrated reduced catalytic activity compared to the wild type. Conversely, mutant S154K exhibited enhanced catalytic activity against C6-HSL. Furthermore, infection models involving white radish and *Galleria mellonella* larvae confirmed that YtnP significantly reduced the toxicity of *S. marcescens* towards the host. Consequently, these findings highlight the potential of YtnP from *B. amyloliquefaciens* W11 as a food preservative against *S. marcescens*.

Keywords: Quorum quenching, *Raphanus sativus*, *Bacillus amyloliquefaciens*, *Serratia marcescens*, biofilm

Rapid amperometric determination of bacteria embedded in biocoatings

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Abstract: The dynamic and complex nature of biofilms poses a challenge in the characterization of biofilms, as established classical and molecular techniques require complex sample preparation and expensive imaging equipment. There is a need for a complementary technique that enables standardized and rapid characterization of biofilm composition and metabolic activity. Bioelectrochemical methods are a good option due to their sensitivity, fast response time, ease of use, and low cost. However, biofilm growth on the electrodes requires many hours or days, and the biofilm structure cannot be controlled, leading to poorly repeatable results. To overcome this challenge, in this study, *Pseudomonas aeruginosa* was immobilized in a non-conductive 2-hydroxyethyl cellulose (HEC) coating at an optimized concentration that allows the diffusion of nutrients and soluble species required for cell metabolic activity. When short-term amperometry at oxidative potential (400 mV vs. Ag) was carried out in the presence of 5 mM potassium ferricyanide as redox mediator and 20 mM glucose as carbon source, there was an increase in total charge (integration of current over time) from -259.5 µC ± 14.1 (10^5 CFU/mL) to -329.4 µC ± 20.7 (10^7 CFU/mL) compared to the blank electrode (-231.4 µC ± 16.9). Further increasing the cell concentration did not result in increased charge, indicating rate-limiting factors such as diffusion of the redox mediator and carbon source. In the absence of a redox mediator, the charge decreased from -5.2 µC ± 1.1 to -1.4 µC ± 0.3, confirming that a redox mediator is required to facilitate extracellular electron transfer via the EC' mechanism, as *P. aeruginosa* is a weak electricigen. The induction of viable but non-culturable state with UV-B (I = 36.5 W/m^2) showed a logarithmic decrease in culturability within 30 s of induction. The effect of induction on cells showed a 5.7-fold reduction in total charge compared to non-induced cells (10^7 CFU/mL), indicating a decrease in cellular respiration, which can be attributed to the toxicity of potassium ferricyanide as resazurin assay, a quick respiration experiment revealed similar time of maximum fluorescence for both induced and non-induced cells at lower induction time. With prolonged UV-B induction time, UV-B kills the cells rather than cause a switch to VBNC state as observed in the difference between the time to reach maximum fluorescence for the induced (induction time = 20 min) and non-induced cells, which indicates loss of respiration in the induced cells. The proposed method enables rapid detection of bacterial cells and their viability, which might find application in environmental and food monitoring.

Keywords: Chronoamperometry, viable but non-culturable, microbial bioelectrochemical systems, screen printed electrodes, bacteria immobilization

Preparation of chitosan/sodium carboxymethyl cellulose film loaded with halloysite nanotubes-zingerone and its impact on fish preservation effects.

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Abstract: Under refrigeration conditions, *Aeromonas sobria* bacteria thrive and can lead to food spoilage. Quorum sensing (QS) is a biological pathway present in a multitude of microorganisms, through which bacteria can regulate several of their physiological activities. Numerous substances have been identified as quorum sensing inhibitors (QSI); these interfere with the QS system and control the production of bacterial spoilage characteristics and virulence factors. In our previous research, zingerone exhibited effective anti-QS activity at sub-minimum inhibitory concentration (sub-MIC) levels. Therefore, in this study, chitosan and sodium carboxymethyl cellulose films loaded with halloysite nanotubes were fabricated and their physicochemical properties were characterized. These films were applied to sea bass fillets and their impact on the fillets during refrigeration was assessed. The results indicated that the films had a small diameter and smooth surface, without any beads or other defects. Their thermal stability, tensile strength, and other properties were suitable for refrigeration conditions. The electrospun fibers containing QSI significantly inhibited the total volatile basic nitrogen (TVB-N) without suppressing the growth of bacteria in the sea bass slices. Furthermore, the spoilage of muscle tissue in the sea bass fillets was significantly delayed during refrigeration. Quantitative analysis demonstrated that the films had a notable inhibitory effect on bacterial spoilage capabilities.

Valorization of bioactive compounds extracted or fermented from tea waste using lonic liquids

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Abstract: The valorization of bioactive compounds extracted or fermented from tea waste using ionic liquids presents a transformative opportunity to enhance the utility of this underutilized resource. This study investigates both extraction and fermentation processes to recover valuable phytochemicals, such as catechins and flavonoids, renowned for their numerous health benefits. Utilizing 1-butyl-3-methylimidazolium chloride (BMIMCL) and 1-ethyl-3-methylimidazolium chloride (EMIMCL) as green solvent alternatives, this research aims to optimize both methodologies for maximal yield and quality. Ionic liquids have demonstrated exceptional capabilities in preserving the stability of bioactive compounds, effectively minimizing degradation when compared to traditional solvents. However, the role of food microbiology in fermentation processes is equally crucial. By employing specific microbial strains, the fermentation of tea waste can transform complex phytochemicals into more bioavailable forms, thereby enhancing their potential applications. The interplay between microbial activity and the inherent compounds of tea waste can lead to the production of novel metabolites with added health benefits, making the fermentation process a vital component in the overall valorization strategy. This project emphasizes optimizing fermentation conditions, such as temperature, pH, and microbial selection, to maximize the extraction of valuable compounds through both methods. Monitoring specific volatile organic compounds (VOCs) during extraction and fermentation serves as real-time indicators of process progress, allowing for better control and timely adjustments, thus enhancing operational efficiency. Moreover, the economic viability of employing ionic liquids in conjunction with fermentation is reinforced by their capacity for recycling and reuse, significantly reducing operational costs. The extracted and fermented compounds possess substantial market potential across various sectors, driven by an increasing consumer demand for natural, functional ingredients. By extending the application of ionic liquid extraction and food microbiology in fermentation to diverse sources of traditional Chinese medicine (TCM), this research highlights the synergistic benefits of these approaches. The combination of ionic liquid extraction and fermentation not only improves the recovery of high-quality bioactive compounds but also promotes sustainability by utilizing green technologies. Ultimately, this study underscores the potential for integrated approaches that leverage ionic liquids and food microbiology to transform tea waste into economically viable and environmentally sustainable products, contributing to the advancement of the extraction industry and the promotion of functional food applications.

Keywords: Tea Waste, Ionic Liquid, Catechins, Caffeine, Volatile Organic Compound

Effect of obstacle size effect on the 3D motion behavior and biofilm formation

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Abstract: Motility determines the adaptability and ability of species to survive and spread in the environment, and is crucial for bacterial colonization, pathogenicity, and biofilm formation. The natural habitats of microorganisms are full of small particles which may adhere to surface as obstacles. Understanding the movement of bacteria in such complex environments is of enormous importance for deciphering key biological processes, from disease infection to fertility and reproduction, and ecosystem health. By using digital holographic microscopy (DHM), we demonstrate that the surface motion of *Pseudomonas aeruginosa (PAO1)* is influenced by silica obstacles in the range of 0.5~8 μm in size. The results indicate that the motility behavior and collision frequency of *PAO1* are significantly affected by the size of the obstacles, which is crucial for understanding the early events in bacterial adhesion and biofilm formation. The influence of obstacle size on motion behavior was non-monotonic. Near the obstacle of 5μm, the movement speed of bacteria increased by about 20%, and the collision frequency was lower. The findings of this study not only enhance our understanding of the motility mechanisms of *PAO1* in complex environments but also have significant implications for uncovering the molecular mechanisms of bacterial adhesion and biofilm formation, providing a scientific basis for the development of new antimicrobial strategies and antifouling materials.

Keywords: obstacle, bacterial motion, 3D tracking, digital holographic microscopy, adhesion

Physical, chemical and microbiological features of saline lakes in Europe

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Abstract: Saline lakes are defined as having salinity of at least 1 g/L. Inland saline lakes occur on every continent, and their total number and volume on Earth is comparable to that of all freshwater lakes. They are very diverse regarding their origin and physicochemical features, which determine their inhabiting microbial communities. In the presentation I will share the results of our research group conducted on soda lakes, which are special types of saline aquatic habitats having permanently alkaline pH. The Westernmost occurrence of these sites in Eurasia is within Europe (Hungary, Austria and Serbia). On the other hand, several other saline lakes could be found in Europe, which we have studied recently. Some of them are deep and have heliothermal character, which means that intensive solar water heating process below the slightly saline surface layer causes subsurface thermal maxima. A huge diversity of aerobic and anaerobic microbes thrive in these sites which were studied by the combination of classic microbiological techniques (cultivation and microscopic methods) with modern genomic tools (amplicon sequencing, metagenomics and prokaryotic genomics)

Keywords: Extremophiles, Alkaline habitat, Salt stress, Bacteria, Algae

Fungal Biofilms: Beneficial, Harmful, and Mysterious Frontiers

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Abstract: Most biofilm research has focused on bacterial biofilms and little is known about biofilms formed by fungi. Diverse fungi have demonstrated to be capable of colonizing surfaces and develop biofilms, either as single species or in association with other fungi or other microbes including algae and bacteria. Fungi have been detected within biofilms associated with rocks, acid mine drainage, pipe walls, buildings and historic monuments, medical implants, and water systems. This presentation will focus on describing the relevance of fungal biofilms, unveiling some of the aspects of fungal biofilm formation, and presenting the 'beneficial" and the 'harmful' aspects of fungal biofilms in the medical, environmental, and industrial fields. This presentation will also provide an overview of bioreactor systems and characterization tools used to study fungal biofilms. Finally, future research perspectives of fungal biofilms will also be discussed.

Keywords: biofilms, fungi, biotechnological applications, bioremediation

Quorum quenching driven biofouling control in membrane bioreactor for high-strength wastewater treatment

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Abstract: Membrane bioreactor (MBR) has been widely applied in high-strength wastewater treatment, with significant efforts focusing on sustainable and non-toxic strategies for biofouling mitigation. This study investigated the potential of the quorum quenching (QQ) bacterium *Brucella* sp. ZJ1 to control biofouling in a MBR system treating high-strength wastewater. Results demonstrate that under conditions of 1500 mg/L phenol, ZJ1 entered the VBNC state after 30 days, leading to a reduction in QQ activity of over 50%. The addition of the resuscitation-promoting factor (Rpf) facilitated the recovery of ZJ1 from the VBNC state, resulting in the strain exhibiting restored QQ activity. Compared to normal cells, VBNC cells showed significant alterations in morphology, extracellular polymeric substance production, functional groups, and the protein secondary structure. When applied in high-strength wastewater treatment, QQ-MBR extended the time to reach a transmembrane pressure of 35 kPa by 3∼10-fold compared with the control. After being operated in MBR for 40 days, QQ beads retained about 40% of their quorum sensing (QS) signals degradation activity. Biofouling reduction was driven by physical friction removal of microbial cells on the membrane surface, alongside a notable QQ effect, evidenced by 27-41% lower extracellular polymeric substance (EPS) concentrations and 60% lower QS signal levels. Metagenomic analysis revealed that QQ beads significantly reduced QS-related and EPS production genes while increasing QQ-related genes, effectively mitigating biofouling. This study underscores the role of QQ in reshaping the microbial community to sustainably reduce biofouling in high-strength wastewater MBR treatment.

Keywords: Quorum quenching, Membrane bioreactor, Biofouling, Microbial Community, High-strength Wastewater

After the biofilm: bacterial transfer, infections and hand hygiene in a healthcare environment

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Abstract: The spread of microbes, including multi-drug resistant microbes, occurs mostly via contaminated health care workers' (HCW) hands and surfaces (WHO 2017). Thus, HCWs are required to perform hand hygiene at several different moments when interacting with patients. HCWs are hand hygiene compliant when they perform hand hygiene at all of the required moments. Clearly, it is important for healthcare facilities to maintain high hand hygiene compliance rates. Unfortunately, in the absence of rigorous hand hygiene programs, HCW hand hygiene compliance can be low. A 5-year retrospective observational study that monitored 350 million hours at 10 hospitals to investigate whether an automated hand hygiene monitoring system (AHHMS) in conjunction with different interventions increased hand hygiene compliance (Arbogast et al 2022). Time series analysis showed that just implementation of the AHHMS did not yield a sustained improvement in hand hygiene, but AHHMS implementation with supplementary strategies resulted in a 46% increase ($P < .006$). Over the same period at one of the hospitals, the incidence of non–C. difficile infections decreased by 56% ($P = .0841$), while C. difficile infections increased by 60% ($P = .0533$) (Boyce et al. 2019). Interestingly, hand hygiene changed most drastically during hours in a day, with highest compliance at the beginning of HCWs' shifts (50%) that worsened (to 38%) as workload increased (Moore et al 2024). These findings suggest that interventions should be well timed during HCWs' shifts to maintain the hight levels of hand hygiene compliance and negatively affect hospital acquired infections.

Impacts of silver nanoparticles on freshwater biofilms

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Abstract: The ubiquity of silver nanoparticles (AgNPs) in freshwater ecosystems has long been a topic of apprehension due to their detrimental effects on macro-organisms in general. Nevertheless, it remains inadequately elucidated how freshwater biofilm microbial communities react to AgNP exposure. This investigation utilized modeled freshwater biofilms to examine their response and interaction with AgNPs through the application of metagenomic and metabolomic methodologies, as well as high-resolution microscopy. Metagenomic 16S rRNA sequencing analyses disclosed that biofilm microbial diversity did not undergo alteration subsequent to AgNP exposure. Further genotype examination underscored two pivotal taxa, namely Alphaproteobacteria and Betaproteobacteria, as the fundamental constituents of the multispecies biofilm upon exposure to AgNPs, which also elucidated the augmented production of extracellular polymeric substances (EPS). LS-MS/MS-based untargeted metabolomics demonstrated the significance of glycerophospholipid metabolism in sustaining biofilm integrity. Scanning electron microscopic assessment unveiled distinct biofilm structural phenotypes characterized by smoother biofilm attributes subjected to AgNPs. Collectively, the study offers a distinctive perspective on how multispecies biofilms react to a singular environmental stressor in the guise of silver nanoparticle exposure.

Keywords: Multispecies biofilm, Silver nanoparticles, Metagenomics, Metabolomics, Stress response

Selective succession and enrichment of pollutants in (Micro)plastic biofilms and environmental risks

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Abstract: Plastic pollution has emerged as a pressing global concern, with about 2 million tons of plastic currently afloat in the world's oceans. Microbes can rapidly colonize microplastics in the water to form a dynamic biofilm, utilizing extracellular polymeric substances as their shelter. Under environmental stress conditions, such as exposure to antibiotics, biofilms can promote gene transfer through mobile genetic elements and enable the rapid spread of antibiotic-resistant genes within the biofilm community. This study aims to understand the antibiotic-resistant genes in microplastic biofilms in their coastal weathering process. Our data showed that microplastic surface biofilm could enrich specific bacterial groups. Furthermore, the microplastic biofilm community enriched more antibiotic-resistant genes in the presence of environmental pollutants.

Advanced genomic sequencing-enhanced wastewater-based epidemiology for monitoring viruses and antibiotic-resistant pathogens

Xiaoqing Xu, Tong Zhang

The University of Hong Kong

Abstract: One Health emphasizes the intrinsic connections between human, animal, and environmental health, recognizing the environment as a vital indicator of population health and a reservoir reflecting the prevalence and trends of infectious diseases and antimicrobial resistance (AMR). Wastewater-based epidemiology (WBE) has emerged as a powerful, non-invasive tool for public health surveillance, capable of providing early warning signals of disease outbreaks and tracking the spread of antibiotic-resistant pathogens across communities. Our research aims to advance molecular detection technologies and leverage cutting-edge genomic sequencing, specifically using platforms like Nanopore, to significantly enhance WBE's capacity for comprehensive pathogen monitoring and detailed epidemiological assessment. During the COVID-19 pandemic, our sewage surveillance technologies proved highly effective. Hong Kong was the first case globally to implement WBE for statutory public health action. Our team established a city-wide sewage monitoring system that provided early warning signals, identified hidden cases, predicted outbreak trends, and quantified viral variants. Key advancements included the development of highly sensitive and precise detection techniques, real-time allelic assays, and high-resolution sequencing technologies. These innovations allowed for the rapid and accurate detection and differentiation of viruses, including evolving variants of concern. Our results underscored the system's capability to provide early signals ahead of clinical case detection for informing public health interventions, to conduct valuable epidemiological assessments, and to monitor viral evolution for capturing significant shifts in variant dynamics. These results highlight the critical role of wastewater surveillance in pandemic preparedness and response. Beyond viral surveillance, the World Health Organization has identified antibiotic resistance as one of the most pressing health threats in this century, necessitating robust monitoring strategies. To address this, we have collected and analyzed over 1,000 *E. coli* genomes from diverse sources using Nanopore genomic sequencing. Our goal is to decipher the connectivity between different habitats under the One Health framework and understand the dynamics of AMR spread. By elucidating these complex interconnections between human, animal, and environmental health sectors, our genomic surveillance initiatives provide multidimensional insights into the transmission of pathogens and resistance genes. Overall, the integration of advanced genomic sequencing into WBE represents a transformative advancement in public health surveillance, offering timely, population-level data for controlling disease spread and tackling AMR challenges. Our work lays the foundation for more resilient surveillance systems that can guide policy and strengthen global health management.

Keywords: One Health, Wastewater-based epidemiology, Sewage viral surveillance, Genomic sequencing, Antibiotic-resistant pathogens

Stopping the decay of *Geobacter* electroactive biofilm

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Abstract: Sustaining a metabolically active electroactive biofilm (EAB) is essential for the high efficiency and durable operation of microbial electrochemical systems. However, EABs usually decay during long-term operation, and the causes are equivocal. Here, we first report that lysogenic phages can cause *Geobacter sulfurreducens* EAB decay, and suggest that attack by phages is a primary cause of EAB decay. A cross-streak agar assay and bioinformatic analysis revealed the presence of prophages on the *G. sulfurreducens* genome, and a mitomycin C induction assay revealed the lysogenic to lytic transition of those prophages, resulting in a progressive decay in both current generation and the EAB. Furthermore, the addition of phages purified from decayed EAB resulted in accelerated decay of the EAB, thereafter contributing to a faster decline in current generation; otherwise, deleting prophage-related genes rescued the decay process. Next, we tried to seek approaches to rejuvenate decayed EABs. We report that introducing a competitive species of *Geobacter uraniireducens* is able to suspend prophage induction in *G. sulfurreducens* and thereby rejuvenate the decayed *G. sulfurreducens* EAB. The transcriptomic profile of *G. sulfurreducens* demonstrated that the addition of *G. uraniireducens* significantly affected the expression of metabolism- and stress response system-related genes and in particular suppressed the induction of phage-related genes. Mechanistic analyses revealed that interspecies ecological competition exerted by *G. uraniireducens* suppressed prophage induction. Our study provides the first evidence of an interaction between phages and electroactive bacteria, and provides a novel strategy to rejuvenate decayed EABs, having significant implications in bioelectrochemical systems

Keywords: *Geobacter* biofilm, Phage attack, Biofilm decay, Decay suppression, Ecological competition

Life style and bioprospecting of marine biofilm bacteria

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Abstract: Microorganisms are important components of the marine ecosystem. For a long time, microorganisms living in plankton have been studied extensively. However, a growing body of research shows that the species and functional diversity of attached-living microorganisms is severely underestimated and largely unknown. Our work in recent years has been directed on "Marine Biofilm Species-Functional Diversity and Resource Mining", including: 1) Marine biofilm species diversity and core functions; 2) Energy metabolism of typical biofilm bacteria; 3) Development of biofilm resources based on artificial intelligence. Through global sampling and metagenomic analysis, we constructed the world's first marine biofilm strain and core gene library, and systematically interpreted the species and functional diversity. We isolated rosebacters from marine biofilms and established a new model organism to study biofilm formation, bacterial energy metabolism, and carbon source utilization. It was found that Roseobacter can be oxidized under facultative anaerobic conditions through the sox gene cluster. The reduced sulfur element was used to obtain energy, and the regulatory mechanism of biofilms adapting to temperature changes was explored. On the basis of understanding the diversity of marine biofilms and the life characteristics of typical species, our recent work has established a biofilm culturable bacterial catalog and discovered more than 300 antibacterial peptide molecules with activity against pathogenic bacteria.

Keywords: marine biofilms, biodiversity, Roseobacter, antimicrobial peptide

Study on greenhouse gas emission characteristics of typical sewage treatment plants in the Beijing-Tianjin-Hebei region

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Abstract: Wastewater treatment is an important means of pollution prevention and an important area for promoting the reduction of greenhouse gas (GHG) emissions. In this study, GHG emissions of 4 typical wastewater treatment plants using AAO process in Beijing-Tianjin-Hebei region were calculated by emission factors, daily and seasonal variation characteristics of emissions. The study shows that the emission factors of typical sewage treatment plants in the Beijing-Tianjin-Hebei region obtained by field measurement are all within the emission factor range provided by the IPCC 2006 Guidelines for National Greenhouse Gas Inventories (2019 Revised Edition). However, it is quite different from the default value in the IPCC report. The average CH4 emission factor of typical wastewater treatment plants in the Beijing-Tianjin-Hebei region is 0.0030 kgCH4/kgCOD, which is only 40% of the IPCC default value. The average N2O emission factor is 0.0017 kgN2O/kgTN, one-tenth of the default value. It is found that greenhouse gas emissions are affected by environmental factors such as reaction stage, structure of biosphere, dissolved oxygen and water temperature. The emissions of aerobic ponds are higher than those of anaerobic and anoxic ponds, while the greenhouse gas emissions of underground sewage treatment plants are more stable and have little daily change. Dissolved oxygen has a significant positive correlation with carbon dioxide and nitrous oxide emissions. Seasonal monitoring results shows that direct greenhouse gas emissions generally reach their maximum value in summer, while indirect greenhouse gas emissions mostly occur in winter. In general, the proportion of greenhouse gas emissions from sewage treatment plants was electricity emissions > direct emissions > pharmaceutical emissions. Electric power emissions accounted for 41%-89.1%, direct emissions accounted for 5.6%-54.7%, and pharmaceutical emissions accounted for 0.5%-28.1%. Adopt energy-saving combination technology, multi-channel water reuse, resource recycling, energy structure optimization and carbon capture technology to reduce the carbon emission intensity of typical sewage treatment plants in the Beijing-Tianjin-Hebei region, so that the sewage treatment industry can realize the transformation from energy consumption factories to resource recycling factories.

Keywords: Wastewater treatment plant, greenhouse gas, emission characteristics, emission factor correction, seasonality

Effect of microplastics and antibiotics on the microbiome and resistomes on activated sludge in wastewater treatment process

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Abstract: Antibiotics and microplastics (MPs) are ubiquitous in the environment, exerting adverse effects on ecological systems. Wastewater treatment plants (WWTPs) are regarded as hotspots for various pollutants, often detecting the coexistence of MPs and antibiotics. Plastisphere (the biofilm on microplastics) may enrich pathogens and antibiotic resistance genes (ARGs) which can cause risks to the ecological environment by discharging into receiving waters. Metagenome was applied to investigate the microbial composition, functions and ARGs of the polyvinyl chloride (PVC) plastisphere in lab-scale reactors, and revealed the effects of tetracycline (TC) and/or Cu (II) pressures on them. The results indicated that the plastisphere provided a new niche for microbiota showing unique functions distinct from the activated sludge (AS). Particularly, various potentially pathogenic bacteria tended to enrich in PVC plastisphere which had more potential ARG hosts and a stronger correlation with ARGs. The ARGs abundances increased after exposure to TC and/or Cu (II) pressures, especially tetracycline resistance genes (TRGs), and the results further showed that TRGs with different resistance mechanisms were separately enriched in plastisphere and AS. The exogenous pressures from Cu (II) or/and TC also enhanced the association of potential pathogens with TRGs in PVC plastisphere. Moreover, when using polyethylene (PE) and sulfamerazine (SM1) as selective pressures to investigate their effects on extracellular antibiotic resistance genes (eARGs) in AS, the results showed that exposure to PE MPs and antibiotics increased the relative abundance of eARGs, with a notable increase in the abundance of proteins encoding the type IV secretion system (T4SS), leading to active release of eARGs. The enhancement of eARGs may further accelerate the spread of ARGs in the environment. The study outlines the mechanisms of eARGs release in AS, which is of significant importance for the risk assessment of antibiotic resistance.

Keywords: Microplastics, Antibiotics, Microbiome, Resistome, Activated sludge

Microalgal-bacterial granular sludge: a novel low-carbon wastewater treatment process sustained by natural light

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Abstract: Urban wastewater treatment predominantly relies on biological processes, particularly the activated sludge method, which efficiently removes organic matter and nutrients but is heavily dependent on aeration. However, growing concerns regarding the environmental unsustainability of conventional wastewater treatment methods, such as high energy consumption and substantial greenhouse gas emissions, etc, have prompted a shift toward more sustainable alternatives. In this context, the light-motivated non-aerated microalgal-bacterial granular sludge (MBGS) process has emerged as a promising solution. This review synthesizes recent advancements and outlines future directions for the non-aerated MBGS process in wastewater treatment and reclamation. The MBGS technology utilizes oxygen generated by microalgae during photosynthesis as an eco-friendly alternative to mechanical aeration. This innovative approach captures solar energy while exploiting the synergistic relationships between microalgae and bacteria, enhancing carbon fixation and facilitating efficient pollutant removal. Key environmental factors that influence the performance of the non-aerated MBGS process, including light intensity, temperature, stirring conditions, and influent composition, are critically analyzed. Recent research elucidates the formation mechanism of MBGS from activated sludge, highlighting the importance of mobility under environmental stresses such as shear forces and nutrient deficiencies. Furthermore, this process can achieve a target of carbon-nertrality with inorganic carbon addition to the MBGS system. Notably, this study reveals that the carbon-oxygen metabolism between microalgae and bacteria within MBGS can be fully coupled under illuminated conditions, enabling effective pollutant degradation in outdoor environments without artificial aeration. Furthermore, the non-aerated MBGS process fosters a balanced ecosystem, converting wastewater pollutants into valuable biomass that can be repurposed as biofertilizer and feed for agricultural applications. By aligning with national objectives for carbon neutrality and enhancing environmental sustainability, the microalgal-bacterial granular sludge technology stands as a transformative advancement in wastewater management. This review aims to deepen the understanding of the non-aerated MBGS process and its potential to address critical challenges in urban wastewater treatment while promoting resource recovery.

Keywords: Microalgal-Bacterial Granular Sludge, Wastewater Treatment, Carbon Neutrality, Pollutant Removal, Resource Recovery

Rapid recognition of potential microbial resources for bioremediation of organochlorine pesticides and flame retardants

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Abstract: Microbe-mediated remediation becomes a desire method for removal of persistent organic pollutants (POPs) due to its eco-friendly and sustainable nature. The improvement of practical feasibility requires constructing comprehensive species pool, while it is still limited by the rapid recognition of potential microbial resources from environment. Here, we established indexes in assessing microbial tolerance to various organochlorine pesticides (OCPs) and flame retardants (FRs) and explored large-scale multi-POPs tolerance pattern of soil bacteria in forests across China. We demonstrated that diversity, community composition, and relative abundances of POP-tolerant microbes were significantly related to POPs' distribution and concentration. By recognizing over a hundred of genera composed of POP-tolerant species, we found that OCP-tolerant taxa were already wide-distributed, while bacterial communities were more responsive to the contamination level of FRs and FR-tolerant taxa were accumulated along the increase of FRs pollution. Our developed indexes successfully recognized well-known POPs-degrading genera, including *Rhodococcus*, *Bacillus*, *Arthrobacter*, *Stenotrophomonas*, and *Pseudomonas*, as well as a series of versatile taxa affiliated with families Ktedonobacteraceae, Acetobacteraceae, Solirubrobacteraceae, and Nocardiaceae, which were extremely rare and likely ignored in laboratory-scale experiments. Together, our findings provide valuable clues to expand the library of POPs-degrading candidates that is helpful in screening microbial resources for bioremediation.

Keywords: Organochlorine pesticides (OCPs), Flame retardants (FRs), Forest soil, Microbial tolerance, Bioremediation

The development of membrane bio-contactors for improving nitrogen removal

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Abstract: In conventional membrane processes, such as MBRs, we normally attempt to inhibit the biofilm growth on membranes. Over two decades of research, I sometimes think that what will happen if we let the bacteria grow on the membranes freely? Differing to previous studies, we recently incubated functional bacteria on hollow fiber membranes (aged membranes) that are widely used for wastewater treatment. The findings are: i) the hollow fiber membranes can act as satisfactory biocarriers for biofilm development; ii) the counter diffusion of carbon source and nitrate from the two sides of membrane can improve denitrification largely, with much lower COD usage and lower effluent SS; iii) a pilot-scale study further corroborated these results. In addition, the membrane isolation of fermentative bacteria and denitrifiers in our process can contribute to the improved performance. Overall, the roles of membrane were extended from solid/water separation to bacteria incubation or isolation.

Keywords: Membrane bioreactor, Membrane fouling, Biofilms, Wastewater treatment

Engineering ‘trap then release’ biofilms for microplastics removal

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Abstract: The Micro/nanoplastics (MNPs) are prevalent pollutants to the environment globally, where they pose health risks to humans and wildlife. Moreover, they serve as vectors for microbial pathogens and adsorbing pollutants like heavy metals and antibiotics. Hence, this raises the need to detect and remove MNP pollution. Firstly, to mitigate MNP pollution in aquatic environments, we engineered microplastic aggregating microbes to trap MNPs into larger clusters, facilitating their removal by simple filtration or sedimentation. This was achieved by enhancing biofilm formation via mutations that upregulate c-di-GMP synthesis. We further incorporated an enzymatic dispersal step using protease to release captured MNPs for convenient recovery, creating a sustainable "capture-and-release" system. Additionally, we improved microplastic detection with a refined Raman spectroscopy protocol, achieving high digestion efficiency and recovery rates for various MNPs in seafood, ensuring accurate monitoring with minimal interference. This integrated approach underscores the dual need for innovative biological solutions and improved analytical methods to manage MNP pollution, safeguard ecosystems, and protect public health effectively.

Hybrid of benthic bioturbation and membrane aerated biofilm ecologically *in-situ* eliminates overloaded nitrogen in sediments of freshwater system

Le Han

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Abstract: managing the overloaded endogenous N in sediments is crucial toward a healthy aquatic ecosystem, considering its long-term continuous release to trigger eutrophication and even cause malodorous black phenomenon in rivers and lakes. We proposed that the endogenous nitrogen in freshwater system would be in-situ eliminated by a hybrid ecological process of benthic bioturbation via macroinvertebrate and biofilm uptake via aerated gas permeable membrane (MABR). Such a hypothesis was verified after a 42-day experiment, with following conclusion drawn: (1) Tubificids as nutrient pump facilitated release of nitrogen in sediment. (2) Biofilm on MABR guaranteed water quality by microbial uptake of released nutrients. (3) Denitrification and anammox rate were improved by 1 and 3 folds, respectively. Overall, the hybrid of invertebrate and MABR improved microbial community.

Keywords: nitrogen, MABR, bioturbation, Denitrification, anammox

Enhanced nitrogen removal in anammox coupled with heterotrophic denitrification processes via directly doing waste activated sludge

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Abstract: Inducing heterotrophic denitrification into anammox systems can reduce nitrate (NO_3^-) accumulation. However, achieving a precise balance between anaerobic ammonium-oxidizing bacteria (AnAOB) and heterotrophic denitrifying bacteria remains challenging yet critical for efficient nitrogen removal in a single reactor. This study investigated the feasibility of using fresh waste activated sludge (WAS) as both a carbon source and a functional microbial community to enhance the performance of an anammox process coupled with heterotrophic denitrification. During long-term reactor operation, dosing fresh WAS immediately increased total nitrogen removal efficiency to 94.1%, significantly greater than that in the bioreactor without WAS addition (86.6%). The initial microbial products (MP) released from fresh WAS were rich in CHON and CHONS compounds, characterized by unsaturation and reduction, making them ideal electron donors for denitrification. Removing the initial MP from WAS facilitated the release of biopolymers and low-molecular-weight neutrals, ensuring a continuous supply of electron donors. The presence of electron transfer mediators (e.g., humus-like substances) in MP also promoted anammox activity. Additionally, the abundance of partial denitrifying bacteria (e.g., *Limniobacter*) increased from 15.9% to 22.2% with WAS supplementation, which in turn supported the growth of AnAOB (e.g., *Candidatus Kuenenia*). Functional gene analysis confirmed an increase in genes associated with the degradation of polymeric compounds, amino acids, and aromatic compounds, potentially supporting denitrification. A notable increase in nitrate reductase genes (e.g., *napAB*, *narGHI*) was observed, likely promoting partial denitrification from NO_3^- to nitrite (NO_2^-), thereby helping to maintain a balance between AnAOB and heterotrophic denitrifying bacteria. This study demonstrates a novel strategy of using fresh WAS to mediate an anammox coupled denitrification process, significantly enhancing nitrogen removal efficiency.

Keywords: Waste activated sludge, Microbial products, Anammox, Partial denitrification, Functional microbial community

Intensifying wastewater treatment with sulfur bacterial biofilms

Di Wu

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Abstract: Climate change poses escalating challenges to the water sector, including extreme weather, deteriorating water quality, and outdated infrastructure. To address these issues, a shift from conventional water treatment and supply to an integrated management approach is essential. This approach promotes a circular, sustainable water economy with minimal carbon output. Modern advancements in wastewater treatment technologies are pivotal for utilities aiming to minimize resource consumption. These technologies encompass innovations in microbiology, intensified processes, energy conservation, decreased chemical dependence, and effective carbon capture and utilization. The speaker has actively contributed to the development of innovative, eco-friendly wastewater treatment solutions, with a particular focus on utilizing the sulfur cycle biological technologies. These efforts are targeted at bridging the gaps in knowledge regarding environmental process innovation, intensification, and integration. By equipping the water sector with necessary insights and techniques, this work facilitates the industry’s shift towards decarbonization, paving the way for a transition to a net-zero water sector. This strategic move not only aligns with global sustainability goals but also ensures long-term resilience and efficiency in water management practices.

Keywords: Wastewater Treatment, Sulfur Cycle, Sulfur Bacterial Biofilms, Process Integration, Process Intensification

Molecular biology–opportunities and challenges

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Abstract: Biofilms are an important component of many complex systems including septic systems which may include anaerobic digesters and, in tertiary systems, aerobic treatment processes. My lab has conducted DNA community studies of each component of tertiary treatment processes. Since biofilms can reduce treatment efficacy and lead to high maintenance costs, there is a pressing need to identify bacterial contaminants and develop remedial strategies. I will discuss our strategies to help a local industrial partner deal with ongoing biofouling issues and suggest possible remedies.

Keywords: Biofilms, Treatment efficacy, Bacterial contaminants, Remedial strategies, Biofouling issues

Soil biofilm induction to increase crop production and bioremediation: a novel approach

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Abstract: Soil biofilms, complex microbial communities encased in a self-produced matrix, are pivotal to soil health, nutrient cycling, and plant growth. The novel approach of inducing soil biofilm formation is through the application of biofilm biofertilizers (BFBFs). They are specialized formulations containing beneficial biofilms which secrete specific biochemicals that break dormancy of soil microbial seed bank. This enhances microbial diversity, abundance, and hence cell density-dependent quorum sensing and biofilm formation in the soil. These soil biofilms with diazotrophs, nutrient solubilizers, growth hormone producers, biocontrolling agents etc. increase biological nitrogen fixation, mineral nutrient availability, plant growth, and pest and pathogen suppression. In addition, an ultrafast transfer of the biofilm-associated electrons takes place in the soil biofilms, triggering quantum effects, and hence it provides electrical energy which can be converted to biological energy via ATP production. The biofilm electron pool and the produced ATP catalyze biochemical reactions in the soil-plant system. Thus, this acts as a natural microbial fuel cell linking the soil biofilm, mycorrhizal network and the plant, facilitating soil bioremediation processes too. That results in enhanced bioremediation of environmental pollutants, including heavy metals and organic contaminants, thereby contributing to soil detoxification. This dual functionality not only supports sustainable agricultural production but also addresses pressing environmental concerns related to soil contamination. In this manner, the BFBF application uplifts the plant growth in a holistic approach by enhancing ecosystems through the quantum effects for sustainability. The induction of soil biofilms also leads to improved soil aeration and water retention. Furthermore, the biofilm matrix serves as a protective habitat for beneficial microbes, enabling them to thrive in the soil environment, thus bolstering the overall health of the agroecosystem. It can be concluded that by integrating the BFBF into agricultural practices, we can create resilient, productive soils that support sustainable food production and ecological restoration, ultimately contributing to global food security and environmental sustainability.

Keywords: Soil biofilms, Plant growth, Bioremediation, Biofilm biofertilizer, Agroecosystems

Influence of shear stress, flow fluctuations, and cell motility on biofilm formation

Judy Yang

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Abstract: Biofilms play a critical role in pathogenic contamination of drinking water, contribute to biofilm-related diseases, alter sediment erosion rates, and are used in wastewater treatment. In this talk, I will discuss how physical factors, including hydrodynamic conditions, flow fluctuations, and bacterial motility, influence biofilm development, focusing on *Pseudomonas putida* and *Pseudomonas aeruginosa*. First, I will discuss how hydrodynamic conditions and microscale surface roughness control early-stage *Pseudomonas putida* biofilm growth. Through microfluidic experiments and numerical simulations, we found that high flow conditions suppress biofilm growth, with a critical local velocity for development identified at approximately 50 μm/s, correlating with P. putida's swimming speed. Microscale surface roughness enhances early-stage biofilm formation by increasing low-flow areas. The critical shear stress for biofilms to develop on rough surfaces is 0.9 Pa, significantly higher than the 0.3 Pa on smooth surfaces. Our findings offer essential insights for predicting and managing early-stage biofilm development in drinking water pipelines and bioreactors. Second, I will discuss how flow fluctuations impact the development of *Pseudomonas putida* biofilms. While most research emphasizes steady flows, fluctuations are prevalent in natural and engineered systems. Our microfluidic experiments revealed that biofilm growth under fluctuating conditions follows a three-phase process (lag, exponential, and fluctuation phases) instead of the four-phase model under steady flow. Furthermore, we demonstrated that low-frequency fluctuations promote biofilm growth, while high-frequency fluctuations inhibit it. This phenomenon occurs because an adjustment time is required for biofilm to regrow when the flow switches from high to low flow conditions. We proposed a theoretical model that explain these growth patterns and provides suggestions on the application of flow fluctuations on biofilm management. Lastly, I will discuss how bacterial cell motility impacts the development of *Pseudomonas aeruginosa* biofilms. Our microfluidic experiments demonstrated that motility enhances biofilm formation by orienting cells towards surfaces, thereby increasing attachment and density. We propose a theoretical model predicting cell orientation based on local flow velocity and swimming speed, which reveals that motility can enhance biofilm density by up to tenfold. Our results shed light on the role of cell motility in biofilm formation and offer potential strategies for managing biofilms in environmental ecosystems and medical applications.

Keywords: Biofilm, shear stress, flow fluctuations, cell motility, microfluidic experiments

Screening and Inhibition mechanism of natural active ingredients on biofilm

Zaixiang Lou

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Abstract: Separation and enrichment of phenolics from peony flowers were performed to improve the anti-biofilm and antibacterial activities for the first time. Through several times of separation, the purity of phenolics components increased significantly, and the anti-biofilm and antibacterial activities of phenolics components against *E. coli* and *S. aureus* were also significantly improved. Finally, the phenolics of peony flowers in the eluent of silica gel column chromatography (PPF-ESGCC) was found to exhibit the highest anti-biofilm and antibacterial activities. The inhibition rates of PPF-ESGCC on biofilms of *E. coli* and *S. aureus* were 77.93%, and 87.03% respectively, at a very low concentration (1/2 MIC, 0.235 mg/mL). It was found that the biofilm inhibition was achieved by inhibiting their swimming, swarming, twitching motilities, exopolysaccharide (EPS) production and quorum sensing (QS). Moreover, there was a positive dose-dependent relationship (r = 0.75 to 1) between the inhibition rates and concentrations of PPF-ESGCC during the critical biofilm-formation stage (1-3 d). Chemical composition analysis showed the PPF-ESGCC comprised of gallic acid, kaempferol-7-O-glucoside, and apigenin-7-O-glucoside. In conclusion, PPF- ESGCC exhibited strong inhibitory effect on biofilm formation and gallic acid, kaempferol-7-O-glucoside, apigenin-7-O-glucoside might play a crucial role in inhibiting biofilm formation. Meanwhile, this study indicated that PPF-ESGCC, a new natural QS inhibitor and biofilm inhibitor, could be used as a novel intervention strategy to enhance the safety and quality of food.

Diverse functions of the type VI secretion system in complex communities

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Abstract: Many significant Gram-negative pathogens employ the type VI secretion system (T6SS) to deliver a diverse array of toxins into both prokaryotic and eukaryotic cells within biofilm communities. The T6SS functions as a contractile macromolecular structure consisting of a membrane-anchored complex and a unique double tubular needle that extends across the cell. Acting as a formidable weapon for bacteria, the T6SS can breach various cell types, including those found in complex biofilm environments. The full capabilities of the T6SS have yet to be recognized, requiring comparative studies of various organisms. I will discuss how effectors and pivotal initiators regulate the assembly process and how effectors influence diverse functions in different host-microbe interaction models.

Keywords: secretion system, interspecies interaction, effector, contractile sheath, T6SS

Decoding the microbiome volatilome: insights from food waste prevention and valorization

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Abstract: In a world facing increasing resource pressures due to rapid population growth, balancing food security with the environmental impact of the food industry has become critical. A key aspect of this balance is the challenge of reducing food waste and loss. Human society is not alone in its quest for food sources, as there is constant competition with microorganisms for these resources. To gain an edge in this ongoing battle, it is essential to deepen our understanding of the interactions between microbial communities and the food they colonize. Our lab explores these microbial interactions by examining changes in microbial communities in tandem with the volatile organic compounds (VOCs) they emit as they metabolize various food substrates. In this presentation, we will discuss three case studies: 1) extending the shelf-life of strawberries through surface treatments and analyzing the impact on surface microbes, 2) studying the fermentation and spoilage of *Citri Reticulatae Pericarpium* (CRP) under different environmental conditions, and 3) investigating solid-state fermentation of tea waste for caffeine production. In all of these studies, VOCs were directly measured using proton-transfer-reaction mass spectrometry (PTR-MS) to capture volatile fingerprints of microbial metabolism. Additionally, respiration measurements provided further insights into how different substrates, environmental conditions, and treatments influenced microbial activity. In the strawberry shelf-life study, we observed consistent patterns between key VOCs and microbial changes. At low temperatures, VOCs were primarily alcohols and esters, which were positively correlated with decomposer microorganisms such as *Penicillium sp.* and *Alternaria tenuissima*. At room temperature, amines became more prevalent, showing negative correlations with biocontrol microorganisms such as *Meyerozyma guilliermondii* and *Meyerozyma caribbica*.In the CRP fermentation study, we identified significant interactions between microbial communities and VOCs during storage. Bacteria and fungi played key roles in modulating VOC production, influencing the aroma and quality of CRP. Correlation analyses revealed strong associations between microbial phyla such as *Ascomycota*, *Bacteroidota*, *Fusobacteriota*, and *Proteobacteria*, and key VOC markers that discriminated aging periods of CRP. Notably, *Ascomycota* and *Campylobacterota* were positively correlated with VOCs such as 2-methylbutanal and elemol, providing insights into microbial contributions to CRP's chemical profiles during aging. In the tea waste fermentation study, we observed increased abundance of *Bacillus* and *Panibacillus* species, which were positively correlated with caffeine extraction. The fungal community was dominated by *Aspergillus*, which also showed a positive correlation with caffeine extraction. Hydrogen sulfide, methanol, and acetaldehyde were identified as the major VOCs produced during fermentation, and their gradual increase suggests they may be key microbial metabolites involved in the process. This research underscores the critical role of microbial communities in influencing food quality and safety, offering new perspectives on how microbial interactions can be leveraged to reduce food waste and enhance food preservation.

Keywords: Microbiome, Volatile Organic Compounds, Food Waste, Food Storage, Solid State Fermentation

Biofilm Electrochemistry: from characterization to electrofermentation

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Abstract: Biofilms comprise of microorganisms encased in self-produced extracellular polymeric matrix, which provide mechanical stability, resistance to antimicrobials, and favors adhesion to nearly any surfaces. When biofilms grow onto electrodes, they are termed electroactive biofilm (EABs). EABs are capable of extracellular electron transfer (EET) to and from solid acceptor, through direct or mediated mechanism. EABs are beneficial to wastewater treatment and could find applications in advanced bioprocesses. A thorough comprehension of the mechanism underlying EET is needed for biofilm management and to develop productive EABs for bioprocesses, biomedical, and biosensing applications. The EET mechanisms are investigated through a combination of electrochemical techniques, molecular biology and microscopy techniques. Following early studies on strong electricigens like *Geobacter* sp. and *Shewanella* sp., recent research has shown that most prokaryotes and even few eukaryotes can be classified as EAB under specific conditions, thus extending the validity of electrochemical methods for biofilm analysis. Further, EET in weak electricigens is advantageous to design novel bioprocess like electrofermentation, in which biopolymers in biofilms are produced at higher yield and with different properties than conventional fermentation processes. In this presentation, I will show recent results on EABs research in our group, with particular regard to the methodology for EAB characterization and its Bioprocess applications.

Keywords: Electroactive biofilms, electrofermentation, biofilm characterization, weak electricigens

Characterization of 3D bacterial adhesion and detachment dynamics

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Abstract: Micro-scale three-dimensional dynamic characterization offers a novel perspective to understand surface adhesion behavior of microorganism and the mechanisms of biofouling formation. However, achieving high-precision, non-invasive three-dimensional observations of microbial dynamic behaviors remains challenging. Recently, we developed a three-dimensional imaging technique based on optical interference—Digital Holographic Microscopy (DHM)—and advanced high-throughput, high-precision three-dimensional tracking algorithms. In DHM measurements, an interference pattern is formed around the target sample, allowing us to capture real-time holographic image sequences using a high-speed camera. Combined with optical diffraction reconstruction and multi-particle three-dimensional tracking algorithms, we successfully obtained the 3D trajectories and morphological changes of microorganisms. This method enables observations ranging from the interface to hundreds of micrometers away without the need for labeling, allowing simultaneous tracking of multiple targets with a three-dimensional localization resolution of less than100 nm. Utilizing DHM, we examined the three-dimensional dynamic behaviors of various bacteria on diverse polymer surfaces (including hydrophobic polymer brushes and biodegradable, low-surface-energy coatings) and under different electrical environments. Our findings reveal that bacteria actively respond to the physicochemical properties of interfaces, which is a significant aspect of their near-interface behavior, comparable to or even exceeding physical interactions. This provides new insights for designing the next generation of marine antifouling materials.

Keywords: bacteria adhesion, surface detachment, antibiofouling, 3D imaging

Constructions and applications of biofilm living materials

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Abstract: Engineered living materials (ELMs) are functional materials with biological characteristics created by using genetic engineering technology and engineering ideas, which is a hot topic in the cross research of synthetic biology and materials science. In recent years, Dr Huang has innovatively built a highly flexible and functionally adjustable microbial engineering living material platform based on microbial biofilm, and explored their applications in bioremediation and disease treatment. The main research work is summarized in the following aspects:

(1) To overcome the problems such as the difficulty of regeneration and programming of traditional materials, a new platform of programmable and printable living material for bacterial biofilm was successfully built by using synthetic biology technology. The living material of *Bacillus subtilis* biofilm has the viscoelasticity of hydrogel, which can be used in biofabration such as 3D printing; By means of gene knockout and nuclear magnetism, the correlations between biofilm components and bacteria 'environmental viability (antibiotic tolerance), structure and physical and chemical properties of materials (hydrophobicity and viscoelasticity) were revealed.

(2) The engineered bacterial biofilm can efficiently display functional domains and enzymes. Due to its strong environmental tolerance and self-replication, it can realize the functions of biocatalysis and bioremediation, with green and sustainable development characteristics. I will give some examples of smart ELMs constructed by genetic engineering in our group, which have potential application in the heavy metal ion detection, the toluene degradation and virus defensing materials.

Keywords: Bacterial biofilm, Engineered living materials, biocatalysis, bioremediation

Emerging nanotechnologies for targeting pathogenic bacterial biofilms

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Abstract: Biofilms are prevalent in chronic wounds and once formed are very hard to remove, which is associated with poor outcomes and high mortality rates. Biofilms are comprised of surface-attached bacteria embedded in an extracellular polymeric substance (EPS) matrix, which confers increased antibiotic resistance and host immune evasion. Here, we report several novel nanotechnologies to do this, based on protease-functionalized nanogel carriers of antibiotics. Such active antibiotic nanocarriers, surface coated with the EPS degrading enzymes, “digest” their way through the biofilm EPS matrix, reach the buried bacteria and deliver a high dose of antibiotic directly on their cell walls, which overwhelms their defenses. We demonstrated their effectiveness against six wound biofilm-forming bacteria, S. aureus, P. aeruginosa, S. epidermidis, K. pneumoniae, *E. coli* and E. faecalis. We confirmed a 6-fold decrease in the biofilm mass and a substantial reduction in bacterial cell density. Encapsulating an equivalent concentration of ciprofloxacin into the Alcalase-coated nanogel particles boosted their antibacterial effect much further, reducing the bacterial cell viability to below detectable amounts. The Alcalase-coated nanogel particles showed very low cytotoxicity to human adult keratinocyte cells, inducing a very low apoptotic response in these cells. Overall, we demonstrated that the Alcalase-coated nanogels loaded with a cationic antibiotic elicit very strong biofilm-clearing effects against wound-associated biofilm-forming pathogenic bacteria. This approach may breathe new life into a wide variety of existing antibiotics, helping to overcome antibiotic resistance. It has the potential to become a very powerful treatment of chronically infected wounds with biofilm forming bacteria.

Keywords: antibiotic nanoparticles, active delivery, protease functionality, antimicrobial resistance

Microbial strategies for enhancing plant stress tolerance in future farming systems

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Abstract: Plant growth and nutrition are adversely affected by various factors such as water stress, high temperature, and plant pathogens. Soil and rhizospheres are complex environments with high carbon concentrations, oxygen, nutrients, and microorganisms. Rhizosphere-inhabiting microbes such as beneficial bacteria and pathogenic fungi compete for nutrients and niches Plant-associated microbes play a vital role in the growth and development of their hosts under biotic and abiotic stresses. Plant-beneficial microorganisms belong to several genera and are able to modulate plant physiological process, helping them to survive in their environment. The use of a rhizosphere microbiome for plant growth stimulation and the biological control of fungal disease can lead to improved crop productivity. Mechanisms used by plant-growth-promoting rhizobacteria (PGPR) to protect plants from soilborne pathogens include antibiosis, the production of lytic enzymes, indole-3 acetic acid production, decreasing ethylene levels by secreting 1-aminocyclopropane-1-carboxylate deaminase, competition for nutrients and niches, parasitism and induced systemic resistance. The use of secondary metabolites produced by endophytic microorganisms for biological control and induced resistance to plant pathogens shows great promise for sustainable agriculture, as it offers an environmentally friendly alternative to synthetic fungicides. However, the performance of biocontrol microbes depends on their environment and interactions among plants and pathogens as well. Thus, the physiological properties of biological control microbes, their interactions with other microorganisms, including pathogens, and the mechanisms involved in the plant-beneficial effect under hostile climatic conditions still need to be researched. The future prospects of the biological control of plant disease and plant stress tolerance by microbiome are promising.

Keywords: plant microbiome, abiotic, biotic, stresses, plant benefits, biocontrol

Multi-modal imaging unveils complex biofilm dynamics of probiotic lactobacillus strains from traditional Kazakh dairy

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Abstract: Bacterial biofilms play crucial roles in microbial ecology, host-microbe interactions, and probiotic functionality. However, the biofilm-forming capabilities of traditional food-derived probiotic strains remain largely unexplored. Here, we present a groundbreaking multi-modal approach to characterize biofilm formation in Lactobacillus strains isolated from artisanal Kazakh dairy products, integrating MALDI-TOF mass spectrometry with cutting-edge microscopy techniques. We identified 26 distinct Lactobacillus isolates from 63 traditional dairy samples using rapid MALDI-TOF MS profiling. Employing a novel combination of laser capture microdissection and atomic force microscopy, we visualized biofilm architecture with unprecedented spatiotemporal resolution. Strikingly, Lactobacillus helveticus strain Lh-13 demonstrated remarkable biofilm complexity, featuring intricate three-dimensional structures and a dense extracellular matrix. Time-resolved imaging revealed a highly dynamic biofilm development process, while quantitative atomic force microscopy analysis provided new insights into surface topography and intercellular adhesion forces within the biofilm. Notably, we discovered a strong positive correlation between robust biofilm formation and other key probiotic traits, including enhanced acid/bile tolerance and antimicrobial activity. Furthermore, we introduce a new metric for quantifying biofilm structural complexity, the "biofilm architecture index," which shows potential as a predictor of probiotic efficacy. Our findings not only shed light on the sophisticated biofilm-forming capabilities of dairy-derived Lactobacillus strains but also establish a powerful analytical framework for investigating bacterial community formation across diverse microbial ecosystems. This study opens new avenues for rational design of next-generation probiotics and provides a deeper understanding of the role of biofilms in microbe-host interactions, with far-reaching implications for human health, food science, and microbial ecology.

Keywords: Lactobacillus, biofilms, multi-modal microscopy, MALDI-TOF MS, probiotic, microbe-host interactions

Biofilm mediated bioremediation of pesticides contaminated sites

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Abstract: Overuse of pesticides in agricultural soil and industrial wastewater containing dyes contaminate the environment severely and are toxic to animals and humans as well, so their removal from the environment is essential. The present study was focused on the bioremediation of pesticides (Cypermethrin (CYP) and Imidacloprid (IMI)) and dyes (Malachite Green (MG) and Congo Red (CR)) using biofilm of bacteria isolated from pesticides polluted agriculture soil and effluents from the textile industry. From pesticides polluted soil, four bacteria, namely, Bacillus thuringiensis (OP554568), Enterobacter hormaechei (OP723332), Bacillus sp. (OP586601), Bacillus cereus (OP586602) and from dyes polluted soil, three bacteria i.e., L. sphaericus (OP589134), Bacillus sp. (OP589135) and Bacillus sp. (OP589136), were identified based on 16S rDNA analysis. Biofilm of individual and mixed cultures of indigenous bacterial isolates was developed and tested for their ability to degrade pesticides (CYP and IMI) and dyes (MG and CR). UV-visible and FTIR spectroscopy was used for the confirmation of CYP, IMI, MG and CR degradation. From all, the mixed culture of B. thuringiensis + Bacillus sp. (5A) (g7) showed the highest degradation (46.2%) against CYP (100µL) and the mixed culture of B. thuringiensis + E. hormaechei + Bacillus sp. (5A) + B. cereus (g11) highly degraded (70.0%) IMI (100µL) within 10 days of incubation at 37 °C. Mixed culture of Bacillus sp. (CF3) + Bacillus sp. (DF4) (g6) showed the highest degradation (86.76%) against MG (100µL) and mixed culture of L. sphaericus + Bacillus sp. (CF3) highly degraded (30.78%) CR (100µL). UV–Vis spectral analysis revealed the major peak at 224 nm of CYP, 263nm of IMI, 581nm of MG and 436nm of CR, which completely disappeared after biofilm treatment. FTIR analysis showed several major peaks which are completely or partly disappeared and the appearance of many new peaks after biofilm treatment. As a result of this study, it was concluded that the biofilm of these bacteria could be suitable agents for the bioremediation of pesticides and dyes. This study expresses an ecofriendly approach for the bioremediation of harmful contaminants from the environment, like pesticides and dyes.

Keywords: Biofilm, Bioremediation, Cypermethrin, Imidacloprid, Congo red, Malachite green

Cyanobacterial biofilms as a strategy to revitalize and innovate the inoculant technology in agriculture

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Abstract: Cyanobacteria have been explored as a biofertilizer since almost 1930s, and mostly in rice cultivation. In the last five decades, their roles across crops for their multi-and diverse attributes including nitrogen fixation, photosynthesis, production of growth stimulating compounds, chemically diverse metabolites, polysaccharides, biofuels etc. towards enriching soil and produce is being documented. Their use as biofertilizers has often been hampered by their sensitivity to grazers or chemicals in the soil environment or competitiveness, with other resident microflora. As naturally occurring biofilms which proliferate in a diverse range of environments, cyanobacteria possess the ability to house diverse microbes in the matrix of polymeric substances, which aids in attachment to biotic or abiotic surfaces; therefore, our investigations were aimed towards mimicking the natural milieu by optimization of *in vitro* development of biofilms using an agriculturally important filamentous cyanobacterium (*Anabaena torulosa*) as matrices, with partners as beneficial bacteria/fungi endowed with nutrient mobilizing potential, in terms of growth and PGP traits. Metabolite profiling using untargeted GC-MS illustrated interesting insights. Evaluation of biofilm formulations as inoculants in diverse crops-cereals, vegetables, legumes and flowers, under field and protected cultivation environments, revealed synergism in terms of the PGP traits and the capacity to maintain the metabolic activity, upto harvest stage with increased survival in the rhizosphere, enhancement in growth, yields, and nutrient availability and fortification of produce. Our in-depth investigations, both in lab and field demonstrated that these biofilms as novel organic options for the eco-sustainable management of C-N, thereby, the biotic component of Earth.

Bio solubilization of Eppawala Rock Phosphate (ERP) by fungal-bacterial biofilms and its impact on crop enhancement of potatoes (*Solanum tuberosum* L)

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Abstract: Eppawala rock phosphate (ERP) in Sri Lanka shows promise as an alternative to Triple Super Phosphate (TSP), though its low solubility hinders its use. Recent researches indicate that fungal-bacterial biofilms (FBBs) could enhance the effectiveness of ERP by enhancing its solubility. This study evaluated the effectiveness of ERP solubilized by FBBs and their impact on soil properties and potato crop performance. Fungi and bacteria were isolated, screened for phosphate-solubilization, and developed into FBBs. These FBBs were tested with ERP (< 20 µm), and the most effective FBBs were evaluated under field experiments for their influence on soil properties and potato crop performance. The FBB-treated and untreated ERP were analyzed using scanning electron microscopy (SEM) and spectrophotometry. The FBB composed of *Bacillus subtilis*, *Brevibacillus brevis*, and *Penicillium polonicum* demonstrated the highest significant ($P < 0.05$), phosphate solubilization and the lowest medium pH. Molecular analysis confirmed the involvement of microorganisms in biofilm formation. Spectrophotometric analysis showed changes in phosphate bands, and SEM images revealed surface deformation in biofilm-treated ERP, indicating successful phosphate solubilization. Using FBBs with ERP, particularly when pretreated, enhanced leaf chlorophyll content, fresh weight, and tuber number in potatoes, though not significantly different from TSP treatment. Additionally, biofilm-enriched ERP resulted in the highest soil available phosphorus and the lowest soil pH. Thus, biofilm-enriched ERP outperforms the recommended TSP dosage in potato cultivation under field conditions.

Keywords: Eppawala rock phosphate, Solubilization, Biofilms

Enhancing industry / academic partnerships

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Abstract: Funding agencies look favorably upon grants that include industrial partnerships under the pretense that industry provides input that makes benchtop research more applicable to real world solutions. The Center for Biofilm Engineering at Montana State University has a 35year history of engaging industrial partners into their research programs. This presentation will explore the benefits and challenges of industrial/academic partnerships in terms of education, research and impact. These ideas will be explored through two case studies focusing on the development of new antibiofilm biocides and clean-in-place strategies. Finally, the presentation will explore the importance of these partnerships in context of regulatory decision making.

Keywords: regulatory science, industrial partnerships, product development

Metabolic engineering of non-model microorganisms

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Abstract: Non-model microorganisms possess unique and versatile metabolic characteristics, offering great opportunities as cell factories for biosynthesis of target products. However, lack of efficient tools for pathway engineering represents a big challenge to unlock the full production potential of these microbes. For function of heterologous DNA, a system of microfluidics-based large volume electroporation was developed to increase the probability of finding rare transformants. Next, a CRISPR-mediated genetic modification system was implemented to pathway engineer for degrading polyethylene glycol terephthalate into succinate acid, transforming white pollution into raw materials for green degradable plastics. These systems transform non-model strains into microbial cell factories, opening new avenues for engineering non-model microorganisms for industrial application.

Keywords: metabolic engineering, non-model microorganisms, transformation, natural products

Heterogeneity of metabolites excreted by fungal, bacterial and fungal-bacterial biofilms

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Abstract: Environmental conditions and physical interactions among microbial cells may alter the metabolites released by them. The present study examined the functional differences of metabolites excreted by three biofilms viz., a fungal biofilm of *Aspergillus* sp. (FB), a bacterial biofilm of *Enterobacter* sp. (BB), and their mixed-culture biofilm (FBB). All three biofilms were formed under *in vitro* conditions and their cell-free exudates were analyzed for functional properties using Fourier Transform Infrared (FTIR) spectroscopy. During the data analysis, FTIR spectrogram was divided into five windows (W1-W5) such as W1, 2800 - 3000 cm-1; W2, 1500 - 1800 cm-1; W3, 1200 - 1500 cm-1; W4, 900 - 1200 cm-1; W5, 500 - 900 cm-1. The data were extracted from each window and analyzed by cluster analysis to see the similarities and differences among data. Functional molecules produced by the FBB were clustered separately, showing the distinctiveness of molecules produced by FBB. All three biofilms showed a higher accumulation of functional molecules during their mature stages rather than in the early stages of their development. The study emphasizes the importance of developing specific biofilms for improved metabolic activities over microbes at the species level.

Keywords: Biofilms, Metabolites, FTIR

Biofilm formation and production of extracellular polymeric substances by perchlorate reducing microorganisms isolated from serpentine soils in Sri Lanka

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Abstract: In this study, pure cultures of a perchlorate-reducing bacteria (PRB, n=1) and perchlorate-tolerant fungi (n=3) isolated from serpentine soils of Ussangoda National Park (UNP), Sri Lanka, (6°05'55"N 80°59'12"E), were screened for biofilm formation and their bioremediation potential for perchlorate ions. Given the elemental similarity between UNP's soils and Martian regolith, overarching aim of this study is to evaluate the perchlorate reduction capabilities of microbial isolates under Martian environmental conditions. The bacterial (A) and three fungal species (W, Y, P), were first prepared as monocultures in perchlorate reducing bacteria selective media (0.02 mol dm^{-3} $NaClO_4$). Then mixed fungi-bacteria cultures (AP, AW, AY) were prepared and screened for their ability for perchlorate reduction efficiency by FTIR analysis. AW demonstrated the highest perchlorate reduction efficiency (60.9%) followed by AY (48.8%) while AW indicated low efficiency (1.6%) after three weeks of incubation. Building on these findings, biofilm formation in AP, AY and AW were investigated qualitatively using Congo Red agar method which indicates the production of extracellular polymeric substances (EPS). Further, a UV spectrophotometry-based Congo Red (0.1 and 0.2 mg/L) broths were used as a quantitative approach for assessment of the biofilms formed. The biofilm structures were confirmed by microscopic observations. Results showed that microbial combinations AP and AW formed biofilms after 72 hrs, while AY has taken 14 days for positive indication of the biofilm. At 0.1 mg/l concentration of the stain, compared to the average absorbance of the control, (1.516) AP biofilm reported a significantly higher average absorbance (2.587) and a similar pattern was found at the higher concentration of the stain. These findings underscore the potential of the biofilms formed using PRBs and perchlorate-tolerant fungi for efficient perchlorate reduction under extreme environmental conditions, offering promising candidates for bioremediation applications on Mars and perchlorate contaminated sites on Earth. Future work will focus on refining the quantification of biofilm biomass and efficiency of Perchlorate reduction in contaminated soils and mass culturing of selected biofilms.

Keywords: Perchlorate-reducing bacteria, Biofilm formation, Martian soil, Serpentine soil, Extracellular polymeric substances

The potential of fungal biofilms in desert soil rehabilitation

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Abstract: Xinjiang Province, located in the northwest of China, holds immense importance both geographically and strategically. It borders eight countries, making it a vital bridge for international trade, economic cooperation, and cultural exchanges. Th Rikke Meyer its harsh conditions, the desert is dotted with oases that support agriculture and sustain life. Furthermore, the northern part of Xinjiang is known for its fertile grasslands and forests, which are essential for the region's livestock and agricultural industries. The plains and deserts of Xinjiang are dominated by soil types such as brown calcareous soil, light brown calcareous soil, grey desert soil, and aeolian sandy soil. However, due to the arid climate, high evaporation rates, high groundwater levels, and inefficient irrigation practices, soil salinization and alkalization can be seen there. Some areas of Xinjiang suffer from soil fertility problems, particularly low organic matter content and deficiencies in nutrients like nitrogen and phosphorus. Therefore, it is necessary to develop the soil conditions to overcome these issues. Microbial biofilms play a crucial role in agriculture, in the challenge of harsh environments and climatic change. Especially, fungal-fungal biofilms and fungal-bacterial biofilms can improve the soil fertility and plant growth promotion, when used as Biofilm biofertilizers (BFBFs). In this study, we focus on understanding biofilm formation and utilization of fungal biofilms to improve soil fertility in desert environments. Macrofungi which are taxonomically known as basidiomycetes are associated with all living plants as Arbuscular Mycorrhizal (AM) symbionts. In recent years, AM fungi (AMF) have shown significant effects in improving saline-alkali soil environments and promoting grass growth. To date, from Xinjiang, over 1000 species of fungi have been reported. Among these *Russula* and *Lactarius* are common examples of AMF with the potential bioprotectants promoting plant growth. In addition to this, *Trichoderma* is a common soil fungus genus, which consists of over 100 species. These species have been utilized to develop crop yield in saline-alkali land. Studies have shown that *Azotobacter chroococcum* and *Trichoderma viride* can develop biofilm which can induce plant defense enzyme activities in cotton and wheat seedlings. Cotton wilt disease caused by *Fusarium* has led to a significant reduction in cotton production in Xinjiang. Therefore, further studies are required to develop BFBFs to enhance crop production in Xinjiang. Even though the environmental factors are harsh in Xinjiang region, bacteria, mosses, algae, lichens, and other microorganisms are abundant in this soil, as well as lower plants and their secreted substances interact with the soil to form a tightly layered shell on the surface. In the future, these microbial resources can be utilized to develop fungal biofilms to address these fertility issues.

Keywords: Fungal biofilms, Phosphorus deficiency, Saline alkali soil, Desert, *Trichoderma,* Xinjiang Province

Kanamycin promotes biofilm viability of MRSA strains showing extremely high resistance to kanamycin

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Abstract: *Staphylococcus aureus* is widely distributed in environment and can cause various human infection and food poisoning cases. Also, this pathogen is a typical biofilm former, which further complicates its pathogenicity. Antibiotics have been widely used to eliminate pathogenic bacteria, but their indiscriminate use has also led to the widespread emergence of drug-resistant bacteria, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA). In this study, the effect of antibiotics on biofilm formation of MRSA strains 875 and 184 was explored. Firstly, MRSA 875 belongs to SCC*mec* type IV, ST239, carrying the *atl*, *icaA*, *icaD*, *icaBC*, and *aap* genes, and MRSA 184 belongs to SCCmec type II, ST5, carrying the *atl*, *icaD*, *icaBC*, *aap*, and *agr* genes. Then, a total of 8 antibiotics have been selected, including kanamycin, gentamycin, cipprofloxacin, erythromycin, meropenem, penicillin G, tetracycline, vancomycin. Minimum inhibitory concentrations (MICs) of each antibiotic were determined, and MIC of MRSA 875 and 184 to kanamycin/gentamicin are 2048/64 μg/mL and 2048/4 μg/mL, respectively. A total of 10 concentrations, ranging from 1/128 to 4 MIC with 2-fold, were used to study biofilm formation. Biofilm biomass and viability were determined during different phases, including initial adhesion (8 h), proliferation (16 h), accumulation (24 h) and maturation (48 h). Importantly, kanamycin at specific concentrations showed significant promotion of biofilm biomass and biofilm viability, with none of such observation acquired from other antibiotics. This study provides scientific basis and new research ideas for the quality control technology of microorganisms and safety prevention of MRSA.

Keywords: *S. aureus*, biofilm, SCC*mec*, MLST, antibiotics stress

Development and verification of Crossing Priming Amplification on rapid detection of virulent *L. monocytogenes*: in-depth analysis on the target and further application on food screening

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Abstract: Virulent *Listeria monocytogenes* is responsible for listeriosis, a highly fatal foodborne illness that impairs human health. One of the major causes of its pathogenicity is the carriage of virulence factor, listeriolysin O, encoded by *hylA* gene. Herein, the specificity and conservativity of *hylA* gene sequences within 6305 *L. monocytogenes* genomes and non-*L. monocytogenes* bacteria were determined, with most conservative region selected. Then, a crossing priming amplification (CPA) assay targeting *hylA* conservative region and producing a detectable color reaction based on calcein was established to apply an isothermal condition (63°C) to identify virulent *L. monocytogenes* within 60 min. Forty-four reference strains including two standard *L. monocytogenes* strains and forty-two strains of other species were selected to assess sensitivity and specificity of this CPA assay. As shown, the proposed CPA assay was not easily prone to false positive with strong specificity and presented sensitivity in genomic DNA with 5.66 pg/μL. The practicality of the CPA assay had been further confirmed in four artificially contaminated rice-flour products, including mantou, rice noodle, crystal pastry, radish pastry. According to the results, the limit of detection in all 3 artificially contaminated rice-flour products was 10^5 CFU/mL, and comparing with regular polymerase chain reaction (PCR), it was 100 times higher. In conclusion, the developed CPA method has advantages of high sensitivity, specificity and quick measurement in the detection of virulent *L. monocytogenes*, showing considerably feasible promise for future food safety.

Keywords: In-depth target analysis, Crossing Priming Amplification, Rapid Detection, *L. monocytogenes*, Rice-flour products

Isolation and identification of quorum quenching bacteria in membrane bioreactor activated sludge and its inhibition of biofilm formation

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Abstract: Membrane Bioreactor (MBR) is a wastewater treatment process that has received much attention in recent years, but the biofouling problem on the surface of MBR membrane has restricted the further promotion of this technology and needs to be solved. The activated sludge in the reactor is the core of degrading pollutants and consists of a large number of microorganisms. Among them, Quorum Sensing (QS) bacteria regulate the formation of biofilm, leading to a series of membrane biofouling problems such as decreased membrane flux and reduced MBR water purification efficiency. The use of Quorum Quenching (QQ) technology can reduce the formation of biofilm in MBR and inhibit membrane fouling. In recent years, research on the application of QQ technology in membrane fouling prevention has received much attention. However, only a limited number of efficient QQ bacteria have been successfully isolated and purified, and more efficient QQ bacteria resources need to be explored. In this study, QS strains were isolated and screened from the activated sludge of actual MBR using the reporter strain CV026 indicator plate method. The QQ strains were isolated and screened by the unique carbon source culture method, and the QQ function of the strains were verified using the reporter strain A136 indicator plate method. The strains were classified and identified by Gram staining, physiological and biochemical and 16S rRNA gene sequencing methods, and phylogenetic tree construction. The ability of the isolated QQ bacteria to inhibit the biofilm formation of typical QS model bacteria was initially verified by co-culture method. In this study, 13 strains of QS bacteria and 12 strains of QQ bacteria were successfully isolated, among which *Acinetobacter* sp. MFY16 was able to degrade the N-Octanoyl-DL-Homoserine Lactone (C8-HSL) signal molecule basically completely within 6 h and effectively inhibit the biofilm formation of *Pseudomonas aeruginosa* PAO1.

Keywords: Quorum sensing, Quorum quenching, Membrane bioreactor, N-Acyl homoserine lactone, Biofilm

Fabrication of antibacterial nanomedicine by phenolic network

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Abstract: *Pseudomonas aeruginosa* (*P. aeruginosa*) corneal ulcer with a characteristic of circumferential abscess is a very serious acute suppurative corneal ulcer commonly found in ocular surface diseases, and often causes devastating damage to entire cornea cause in a very short period of time[1,2]. Here, we innovatively use 3,4,5-trihydroxybenzaldehyde (THBA), ε-poly-L-lysine (EPL), copper ions (Cu^{2+}), and tobramycin (TOB) to prepared the positively charged modular nanoparticles (NPs) with uniform size and pH response with one-step co-assembly method through such functions as polyphenol-metal coordination, hydrogen bonding, π-π stacking, hydrophobicity, and electrostatic adsorption. Dialysis method indicated that the THBA-Cu-TOB NPs released TOB rapidly at pH 6.5 and Cu^{2+} continuously at pH 5.5. Importantly, the THBA-Cu-TOB NPs (40 μg/mL, TOB content: 2.6 μg/mL) showed superior ability of biofilm penetration and eradication in comparison with free TOB (160 μg/mL). In addition, it is proved that the THBA-Cu-TOB NPs not only play a role of anti-inflammation by affecting the TLR4/NF-κB signaling pathway to reduce the secretion of inflammatory cytokines (i.e., TNF-α, IL-6, and IL-1β), but also are able to eliminate reactive oxygen species (ROS) and promote the migration of HUVECs. It is suggested that fluorescently labeled THBA-Cu-TOB NPs have an outstanding performance in ocular surface retention time and corneal permeability. In animal models, the THBA-Cu-TOB NPs can effectively eliminate bacterial infection, reduce inflammation, and promote corneal wound healing in accordance with ophthalmic clinical score, histopathology, and immunofluorescence evaluation. In general, based on the characteristics of modular NPs multi-component synergies, we build the THBA-Cu-TOB NPs with biological adhesion and permeability, high load capacity of drugs, and pH response for efficient drug delivery and intelligent release for expectation of overcoming such difficulties as short residence time in ocular surface and low bioavailability, and improving the effective treatment to *P. aeruginosa* corneal ulcer at the same time.

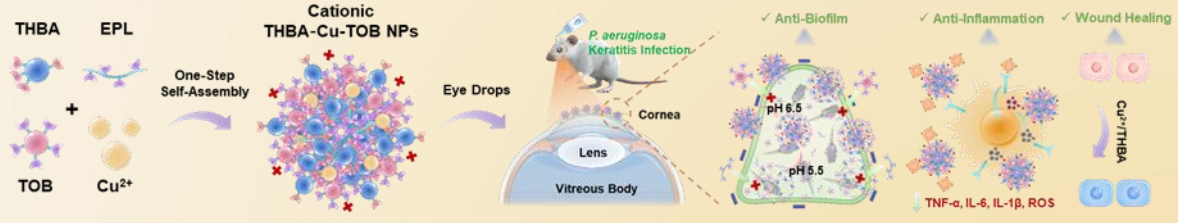

Fig. 1 Schematic diagram of the application in the synergistic treatment of infectious corneal ulcer.

Keywords: Polyphenols, Biofilm, Antibacterial, Inflammation

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