SCIENTIFIC PROGRAM

3rd International Conference on Biofilms

Asia-Pacific Biofilms 2021

May 11-16, 2021 | Guangzhou, China

Asia-Pacific Biofilms 2021

On behalf of the Organizing Committee, you are cordially invited to attend the virtual conference of the 3rd International Conference on Biofilms (Asia-Pacific Biofilms 2021), held on May 11-16 of 2021, in Guangzhou, China.

Asia-Pacific Biofilms 2021 will cover subjects including characteristics of biofilms, quorum sensing in biofilms, industrially- and clinically- relevant biofilms and emerging technologies on biofilms. In the relevant fields, distinguished scholars are invited for keynote or invited presentations, and young scientists with latest research findings from various disciplines are also invited for oral presentations. This is undoubtedly the best opportunity for participants to present the recent progress and foster new collaboration. Asia-Pacific Biofilms 2021 also builds a bridge between Chinese and international universities or enterprises.

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
Zhenbo Xu
Organizing Committee

Organization

Organizers

South China University of Technology

Southern University of Science and Technology

AEIC Academic Exchange Information Center

Supporting parties

China Society for Microbiology General Microbiology Professional Committee

Overseas Chinese Society for Microbiology (SinoMicro)

ESCMID Study Group for Biofilms

ELSEVIER

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Mark Shirtliff

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Founder and Honorable President

Mark Shirtliff
(1969-2018)

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

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

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

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

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

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

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

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

We hope that you will participate in making Asia-Pacific Biofilms 2021 a successful follow-up to the ChinaBiofilms 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.

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.

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.

AEIC Academic Exchange Information Center

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

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

Agenda

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

May 11th Registration	
16:00-18:00	**Registration and Meeting platform test**
May 12th Workshop	
9:00-10:30	**Animal models for biofilm infections** **Modeling biofilm-associated wound infections. Kendra Rumbaugh** **Orthopedic models of biofilm infection. Janette Harro**
10:30-11:00	**Meet the speakers**
11:00-12:30	**Standard methods for biofilms** **Standardized laboratory bench top flow through and batch reactors for growing a reproducible biofilm. Darla Goeres** **Laboratory reactors for real time imaging of biofilm bacteria. Paul Stoodley**
12:30-13:00	**Meet the speakers**
14:30-16:00	**Getting your article published in Biofilm** **Tom Coenye, Birthe Kjellerup**
16:00-16:30	**Meet the speakers**
16:30-17:30	**Standardized development and detection of bacterial biofilms** **Yulong Tan, Su Ma, Zhenbo Xu**
17:30-18:00	**Meet the speakers**

Biofilm

Getting your article published in Biofilm

ASIA-PACIFIC BIOFILMS 2021

Dr. Tom Coenye & Dr. Birthe Kjellerup
Senior Editors
@BiofilmJournal
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ELSEVIER

Researcher Academy
On Campus

Writing your paper

Ethics and you

Choosing a journal

Biofilm

Writing your paper

Are you ready to write a paper?

- You should consider publishing if you have information that advances understanding in a certain scientific field
- This could be in the form of
  - Presenting new, original results or methods
  - Rationalizing, refining, or reinterpreting published results
  - Reviewing or summarizing a particular subject or field

Next Step: a strong manuscript

A good manuscript…

- Contains a scientific message that is novel, clear, useful, and exciting
- Conveys the authors' thoughts in a logical manner such that the reader arrives at the same conclusions as the author
- Make editor feel like he has learnt something useful!
- Is well-organized and focused, and as SHORT as possible

Building an article from the ground up

Top Tip!

Pick a "model" article from the journal and copy its style.
There is a reason why it was published there!

Your paper – Your Way

Benefits for the author:

- ✓ You submit **one** file to Editorial Manager for review
- ✓ After **15 clicks:** Paper has been submitted
- ✘ No uploading multiple files, lots of information etc.

Make it easy for the reviewers – Recommendations:

- Font (easy to read) & Font size (11 or 12)
- Include line & page numbers
- Line spacing (1.5 or 2)
- Margins (1 inch)
- Use headings
- Figures and Tables – easy to read

When the paper has been accepted:

- Submit an **editable** format (i.e. word) to make publication faster

Pay attention to…

- Novelty of data/interpretation
- Length – SHORT is good!
- Scientific language
  - Write with clarity, objectivity, accuracy, and brevity.
  - Sentence construction – use active voice
  - Tenses
  - Grammar
- Use English
- The style and requirements of the journal you have chosen to submit to
- Read Guide for Authors *before* you start writing!

Top tip!

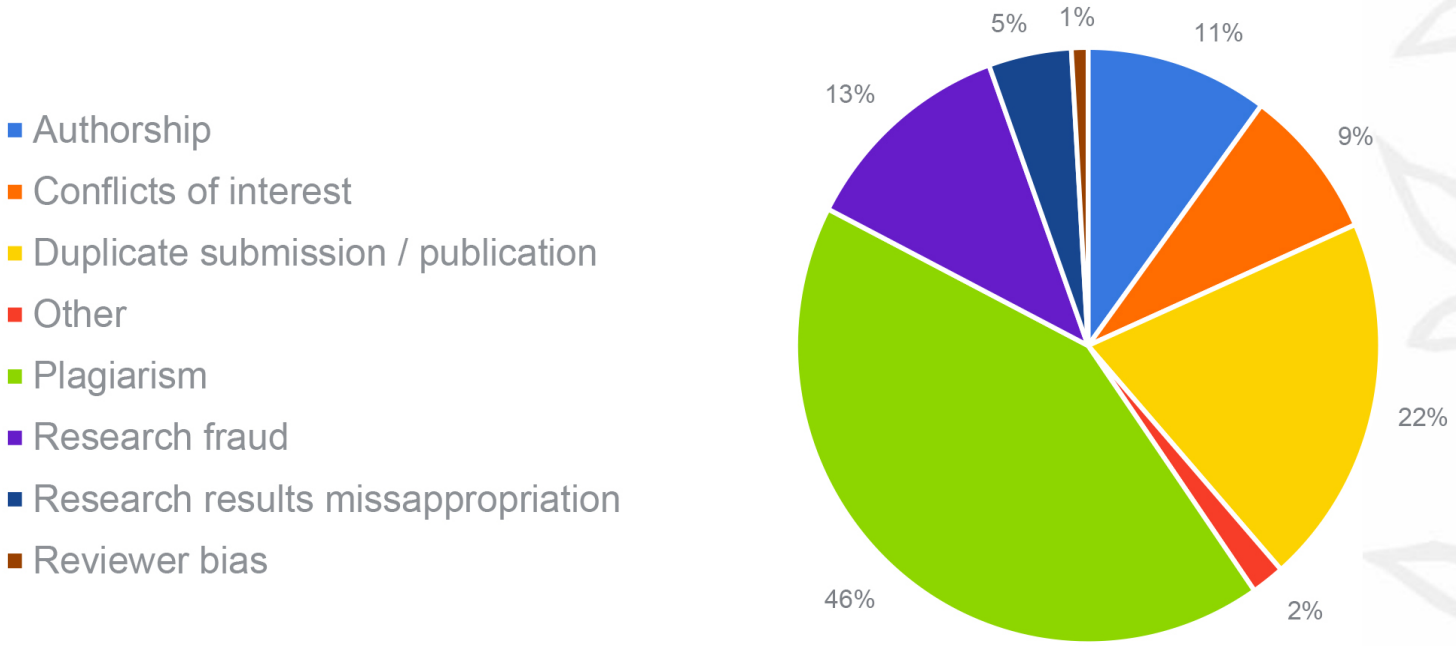
- Make a decision about which journal you would like to submit to first, even before beginning to write.
- In that way, you can follow the style of the journal, and shape the focus of the paper.
- Repeat: Read Guide for Authors *before* you start writing!

Ethics and you

Ethics issues in publishing

- Plagiarism – different forms / severities
- Duplicate submission / duplicate publication
- Inappropriate acknowledgement (prior research/researchers)
- Inappropriate identification of all co-authors
- Conflict of interest
- Scientific misconduct

Sample of cases reported to Elsevier Journals publishing staff in 2012



Plagiarism

"Plagiarism is the appropriation of another person's ideas, processes, results, or words without giving appropriate credit, including those obtained through confidential review of others' research proposals and manuscripts."

M. Errami & H. Garner, A tale of two citations
Nature 451 (2008): 397-399

Federal Office of Science and Technology Policy, 1999

Work that can be plagiarized includes –

- Words (Language)
- Ideas
- Findings
- Writings
- Graphic Representations
- Computer Programs
- Diagrams

- Graphs Illustrations
- Information
- Lectures
- Printed Material
- Electronic Material
- Any Other Original Work

Paraphrasing

Paraphrasing is restating someone else's ideas while not copying their actual words verbatim.

It is unacceptable to:

- Use exact phrases from the original source without enclosing them in quotation marks
- Emulate sentence structure even when using different words
- Emulate paragraph organization even when using different wording or sentence structure

Statement on Plagiarism
Department of Biology, Davidson College.
www.bio.davidson.edu/dept/plagiarism.html

Detection of plagiarism

- CrossCheck – Huge database of 30+ million articles, from 50,000+ journals, from 400+ publishers
- Software alerts Editors to any similarities between the article and this huge database of published articles
- Many Elsevier journals check every submitted article using CrossCheck

Correct citation is key

Crediting the work of others (including your advisor's or your own previous work) by citation is important for at least three reasons:

- To place your own work in context
- To acknowledge the findings of others on which you have built your research
- To maintain the credibility and accuracy of the scientific literature

Authorship: do's and don'ts

- **Ghost authors:** leaving out authors who should be included
- **Gift authors:** including authors when they did not contribute significantly
- **Check criteria for each journal**
- **Do not submit a manuscript if not all authors have seen the paper and approved its submission**

Author disputes

- Must be resolved by authors
- Editors cannot adjudicate or act as judge
- If the article has not been published, the editor has to get agreement from all authors about any changes
- If the article is already published, changes can be published as a correction, but needs agreement from all authors with justification

Conflicts of interest

- Direct financial – employment, stock ownership, grants, patents
  - A University Researcher, who owns stock in a large oil company, conducts an experiment on the environmental effects of oil drilling.
- Indirect financial – honoraria, consultancies, mutual fund ownership, expert testimony
  - A University Researcher, who is developing and testing a new technology, is also a consultant for a financial services firm that weighs investments in new technologies.
- Career and intellectual – promotion, direct rival
- Institutional
  - A Researcher submits an article to a journal for which the Editor-in-Chief is a Professor in the Researcher's department.
- Personal belief
  - A Doctor who abides by traditional healing procedures writes a paper on emerging current medical technologies.

The proper way to handle potential conflicts of interest is through transparency and disclosure. This means disclosure of the potential conflict in your cover letter to the Journal Editor and/or in the online submission form.

Scientific misconduct can end badly…

Retraction Watch

Tracking retractions as a window into the scientific process

Former Cardiff researcher found guilty of misconduct "very disappointed," calls process "unprofessional"

with 2 comments

Yesterday, we reported on the second retraction in a case at Cardiff University, which had found misconduct by a former scientist. *Cancer Research*, which published the retraction, said that scientist, Rossen Donev, could not be reached.

Donev responded to our request for comment this morning: Read the rest of this entry »

Written by ivanoransky
November 12, 2013 at 7:00 am

Posted in uk retractions

Pages
About Adam Marcus
About Ivan Oransky
The Retraction Watch FAQ, including comments policy
The Retraction Watch Store
The Retraction Watch Transparency Index
Upcoming Retraction

Third retraction appears for orthopedic su… investigation, lawsuits

leave a comment »

In July, we reported on the unfortunate math of Harish Hosalkar, a San … orthopedic surgeon who was at the center of an institutional investigat… integrity of his data, two lawsuits and three retractions.

At the time, we were waiting on the third retraction, in the journal *Orth… Reviews*. It has now arrived.

The article was titled "Open reduction and internal fixation of displaced … fractures in adolescents," and Hosalkar wrote it with Gaurav Parikh, Jam… and Bernd Bittersohl. Read the rest of this entry »

Written by amarcus41
November 11, 2013 at 11:50 am

Posted in erroneous data, freely availab… press, united states, unreliable findings

Second retraction stemming from Cardiff i…

with 5 comments

A second retraction of a paper by a Cardiff University researcher found … misconduct has appeared.

In April, a Cardiff investigation found that Rossen Donev, a former rese… university, had manipulated images in four different papers. Donev, wh…

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comments on this story

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Stories by keywords
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Published online 1 November 2011 | *Nature* **479**, 15 (2011) | doi:10.1038/479015a
Updated online: 1 November 2011
Updated online: 8 December 2011

News

Report finds massive fraud at Dutch universities

Investigation claims dozens of social-psychology papers contain faked data.

Ewen Callaway

When colleagues called the work of Dutch psychologist Diederik Stapel too good to be true, they meant it as a compliment. But a preliminary investigative report (go.nature.com/tqmp5c) released on 31 October gives literal meaning to the phrase, detailing years of data manipulation and blatant fabrication by the prominent Tilburg University researcher.

"We have some 30 papers in peer-reviewed journals where we are actually sure that they are fake, and there are more to come," says Pim…

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24 February 2011 Last updated at 11:38 GMT

German minister loses doctorate after plagiarism row

Germany's defence minister has been stripped of his university doctorate after he was found to have copied large parts of his work from others.

Mr Guttenberg failed to name sources for pa… PhD thesis

Karl-Theodor zu Guttenberg, an aristocrat who lives in a Bavarian castle, admitted breaching standards but denied deliberately cheating.

Analysis revealed that more than half of his thesis had long sections lifted word-for-word from the work of others.

So far the German Chancellor, Angela Merkel, has stood by the minister.

The University of Bayreuth decided that Mr Guttenberg had "violated scientific duties to a considerable extent".

It deplored the fact that he had lifted sections of text without attribution.

Last week Mr Guttenberg said he would temporarily give up his PhD title while the university investigated the charges of plagiarism. He admitted that he had made "serious mistakes".

His thesis - Constitution and Constitutional Treaty: Constitutional Developments in the US and EU - was completed in 2006 and published in 2009.

Chancellor Merkel insisted on Monday that she was standing by her defence minister, who was seen as something of a rising star in her conservative coalition.

Related Sto…
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Plagiarism ro… minister drops
German minis… denies plagiar…

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Disgraced South Korean scientist guilty of faked stem cell research

A South Korean scientist who falsely claimed to have ach… breakthroughs in stem cell research has been found guilt…

Prof Hwang Woo-suk

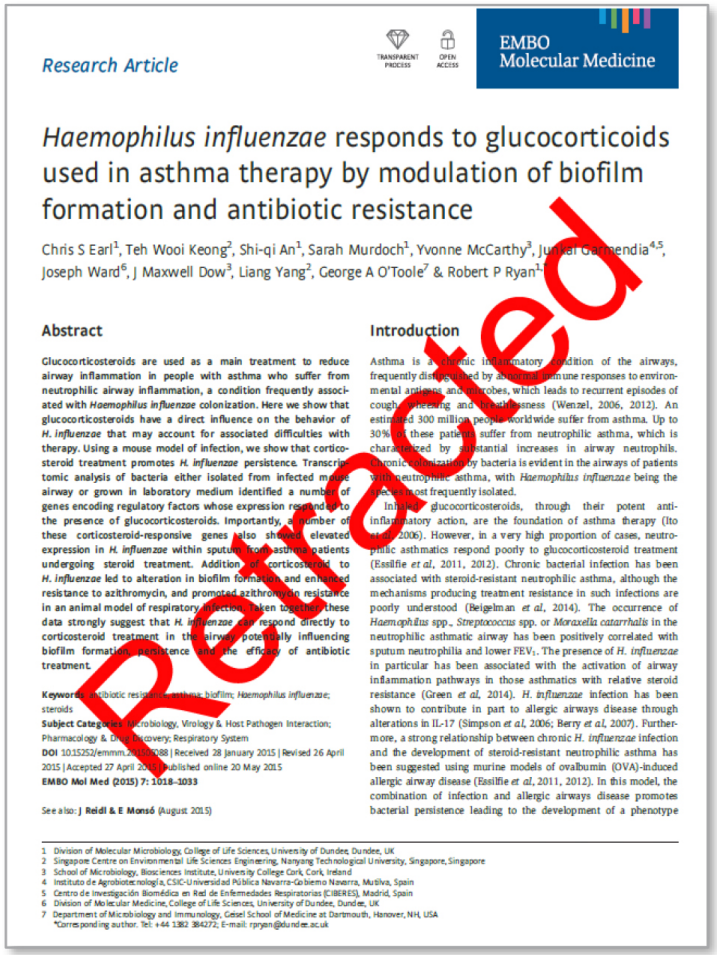
6:27AM GMT 26 Oct 2009

Hwang Woo-suk, 56, once a scientist with rock-star like status for bringin… South Korea to the forefront of stem cell studies, had also been on trial o… charges of misusing state funds and violating bioethics laws.

"He was guilty of fabrication," the Seoul court said in a verdict in the trial that stretched more than three years and included painstaking details about the scientific work Hwang and his team had performed at Seoul National University.

The court also said that Hwang illegally diverted a portion of the money h… received for research for his personal use.

"But he has shown he has truly repented for his crime," the court said in …



Research Article

EMBO Molecular Medicine

Haemophilus influenzae responds to glucocorticoids used in asthma therapy by modulation of biofilm formation and antibiotic resistance

Chris S Earl[1], Teh Wooi Keong[2], Shi-qi An[1], Sarah Murdoch[1], Yvonne McCarthy[3], Junkal Garmendia[4,5], Joseph Ward[6], J Maxwell Dow[3], Liang Yang[2], George A O'Toole[7] & Robert P Ryan[1,*]

Retracted

Abstract

Glucocorticosteroids are used as a main treatment to reduce airway inflammation in people with asthma who suffer from neutrophilic airway inflammation, a condition frequently associated with *Haemophilus influenzae* colonization. Here we show that glucocorticosteroids have a direct influence on the behavior of *H. influenzae* that may account for associated difficulties with therapy. Using a mouse model of infection, we show that corticosteroid treatment promotes *H. influenzae* persistence. Transcriptomic analysis of bacteria either isolated from infected mouse airway or grown in laboratory medium identified a number of genes encoding regulatory factors whose expression responded to the presence of glucocorticosteroids. Importantly, a number of these corticosteroid-responsive genes also showed elevated expression in *H. influenzae* within sputum from asthmatic patients undergoing steroid treatment. Addition of corticosteroid to *H. influenzae* led to alteration in biofilm formation and enhanced resistance to azithromycin, and promoted azithromycin resistance in an animal model of respiratory infection. Taken together these data strongly suggest that *H. influenzae* can respond directly to corticosteroid treatment in the airways potentially influencing biofilm formation, persistence and the efficacy of antibiotic treatment.

Keywords antibiotic resistance; asthma; biofilm; *Haemophilus influenzae*; steroids
Subject Categories Microbiology, Virology & Host Pathogen Interaction; Pharmacology & Drug Discovery; Respiratory System
DOI 10.15252/emmm.201505088 | Received 28 January 2015 | Revised 26 April 2015 | Accepted 27 April 2015 | Published online 20 May 2015
EMBO Mol Med (2015) 7: 1018-1033

See also: **J Reidl & E Mensó** (August 2015)

Introduction

Asthma is a chronic inflammatory condition of the airways, frequently distinguished by abnormal immune responses to environmental antigens and microbes, which leads to recurrent episodes of cough, wheezing and breathlessness (Wenzel, 2006, 2012). An estimated 300 million people worldwide suffer from asthma. Up to 30% of these patients suffer from neutrophilic asthma, which is characterized by substantial increases in airway neutrophils. Chronic colonization by bacteria is evident in the airways of patients with neutrophilic asthma, with *Haemophilus influenzae* being the species most frequently isolated.

Inhaled glucocorticosteroids, through their potent anti-inflammatory action, are the foundation of asthma therapy (Ito *et al*, 2006). However, in a very high proportion of cases, neutrophilic asthmatics respond poorly to glucocorticosteroid treatment (Essilfie *et al*, 2011, 2012). Chronic bacterial infection has been associated with steroid-resistant neutrophilic asthma, although the mechanisms producing treatment resistance in such infections are poorly understood (Beigelman *et al*, 2014). The occurrence of *Haemophilus* spp., *Streptococcus* spp. or *Moraxella catarrhalis* in the neutrophilic asthmatic airway has been positively correlated with sputum neutrophilia and lower FEV_1. The presence of *H. influenzae* in particular has been associated with the activation of airway inflammation pathways in those asthmatics with relative steroid resistance (Green *et al*, 2014). *H. influenzae* infection has been shown to contribute in part to allergic airways disease through alterations in IL-17 (Simpson *et al*, 2006; Berry *et al*, 2007). Furthermore, a strong relationship between chronic *H. influenzae* infection and the development of steroid-resistant neutrophilic asthma has been suggested using murine models of ovalbumin (OVA)-induced allergic airway disease (Essilfie *et al*, 2011, 2012). In this model, the combination of infection and allergic airways disease promotes bacterial persistence leading to the development of a phenotype

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6 Division of Molecular Medicine, College of Life Sciences, University of Dundee, Dundee, UK
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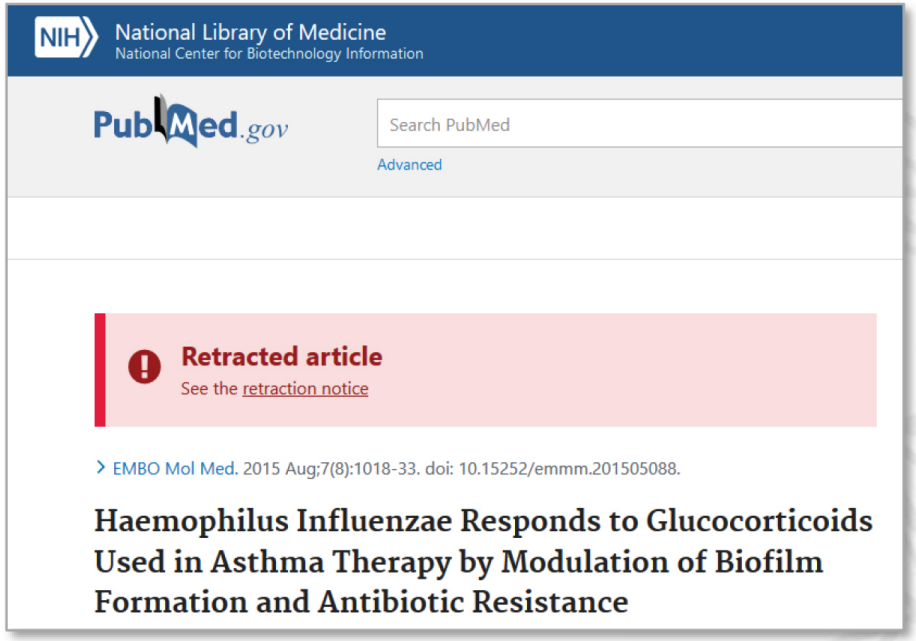

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Retracted article
See the retraction notice

EMBO Mol Med. 2015 Aug;7(8):1018-33. doi: 10.15252/emmm.201505088.

Haemophilus Influenzae Responds to Glucocorticoids Used in Asthma Therapy by Modulation of Biofilm Formation and Antibiotic Resistance

A retracted article will *not* be removed from the databases

What are your responsibilities as an author?

- Report data that is real, unfabricated and original
- Shared responsibility – when in doubt ask for the raw data!
- Declare any conflicts of interest
- Ensure proper authorship
- Submit to one journal at a time
- Make sure to cite others' work carefully and properly

Choosing a journal

The Measure of Impact: Impact factor

The impact factor of a journal for a particular year is the average number of citations for that year, for articles published in the preceding two years

$$\text{IF for year } x = \frac{\text{total citations in year } x \text{ for articles published in years } x-1 \text{ and } x-2}{\text{total number of articles published in years } x-1 \text{ and } x-2}$$

Used in an attempt to describe the quality of a journal

- The higher the impact factor, the better the journal
- It is influenced by editorial policies of journals and turnover of research
  - Example – in a "fast" area, there will be more recent citations
  - Reviews are better cited, so journals with only reviews will have a high IF

The measure of impact: other metrics

- **Journal level**
  - **CiteScore**
    https://www.elsevier.com/authors-update/story/impact-metrics/citescore-a-new-metric-to-help-you-choose-the-right-journal
  - **Eigen Factor**
    eigenfactor.org
  - **SJR**
    scimagojr.com
  - **SNIP**
    Scopus.com

- **Personal**
  - **H-index**
    Scopus.com
    a scholar with an index of *h* has published *h* papers each of which has been cited in other papers at least *h* times. Thus, the *h*- index reflects both the number of publications and the number of citations per publication.

- **Article level**
  - **PlumX**
    https://plumanalytics.com/
  - **Altmetrics**
    http://altmetrics.org/

Where to publish?

Do **not** just descend the Impact Factor stairs.

Top journals
(Nature, Science, Lancet, Cell…)

↓

Field-specific top journals

↓

Other field-specific journals

↓

National journals

What should I look for in a journal?

- A journal that is read by colleagues that work in the same field
- A journal that has the highest impact in your field
  (not necessarily the highest IF!)
- A journal that is fast in publishing
- A journal where the manuscripts are easy to prepare
- A journal that is easy to find on the web

What should I look for in a journal?

- Investigate all candidate journals to find out
  - Aims and scope
  - Types of articles that are published
  - Readership
  - Current hot topics
- Ask help from your supervisor or colleagues
  - The supervisor (who is sometimes the corresponding author) has at least co-responsibility for your work.
    You are encouraged to chase your supervisor if necessary.
- Articles in your references will likely lead you to the right journal

Top tip! Narrow down to 3 potential journals

- 1st choice = the ‘reach’ (*Biofilm*, of course)
- 2nd choice = the ‘backup’
- 3rd choice = the ‘fall back’

All done?

Biofilm

Biofilm focuses on hypothesis- or discovery-driven studies on microbial cells that grow in multicellular communities and demonstrate different gene expression, growth rate, behavior and appearance to those that are in planktonic (free-living) state.

journals.elsevier.com/biofilm

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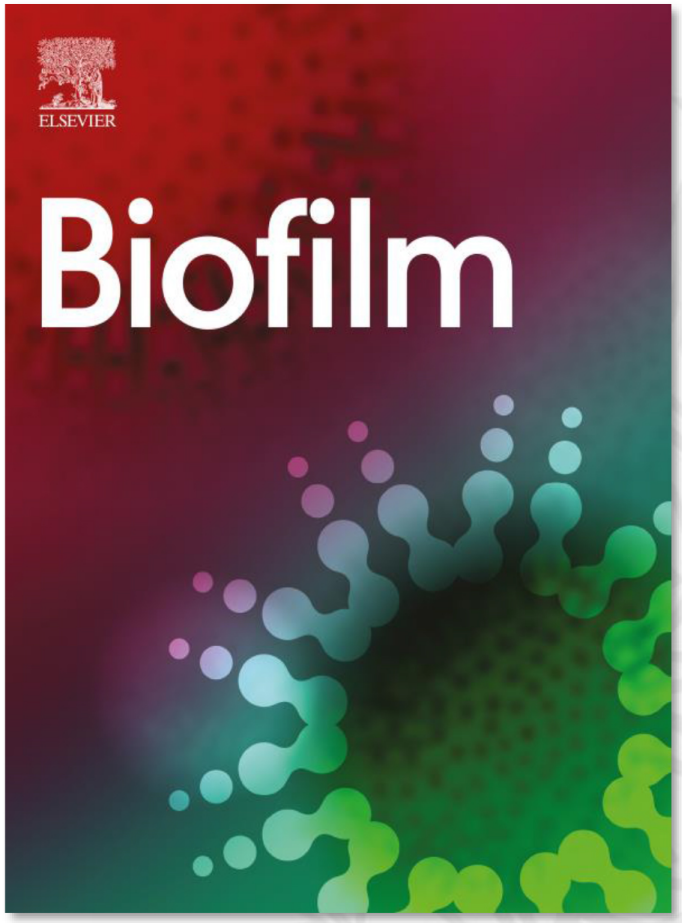
Tom Coenye
Ghent University, Gent, Belgium

Birthe Veno Kjellerup
University of Maryland, College Park, Maryland, USA

Ákos T. Kovács
Technical University of Denmark (DTU), Kongens Lyngby, Denmark

Darla Goeres
Montana State University, Bozeman, Montana, USA



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41 specialists from all over the world

Editorial board

41 specialists from all over the world

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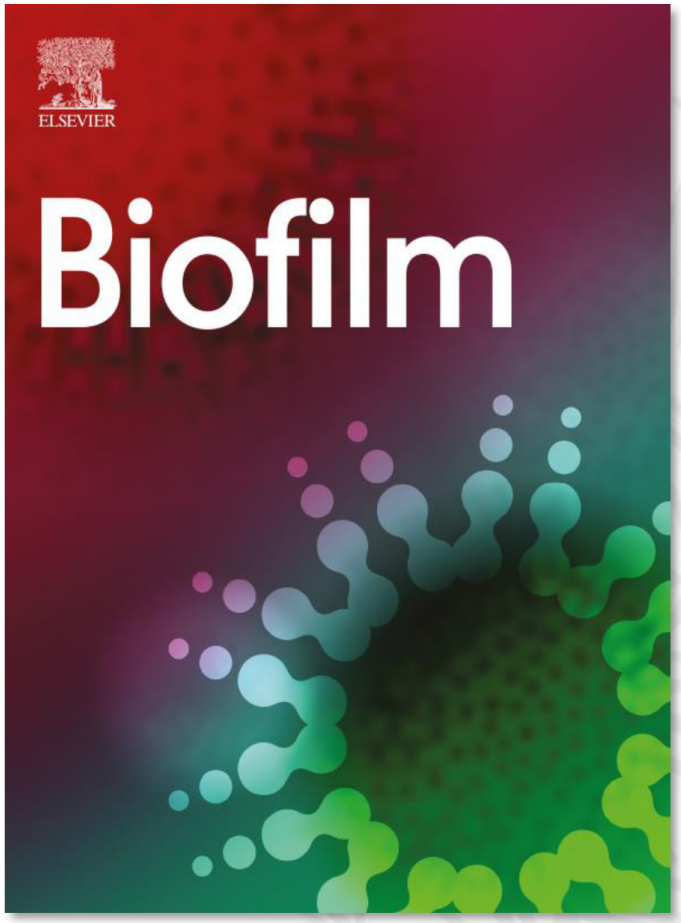
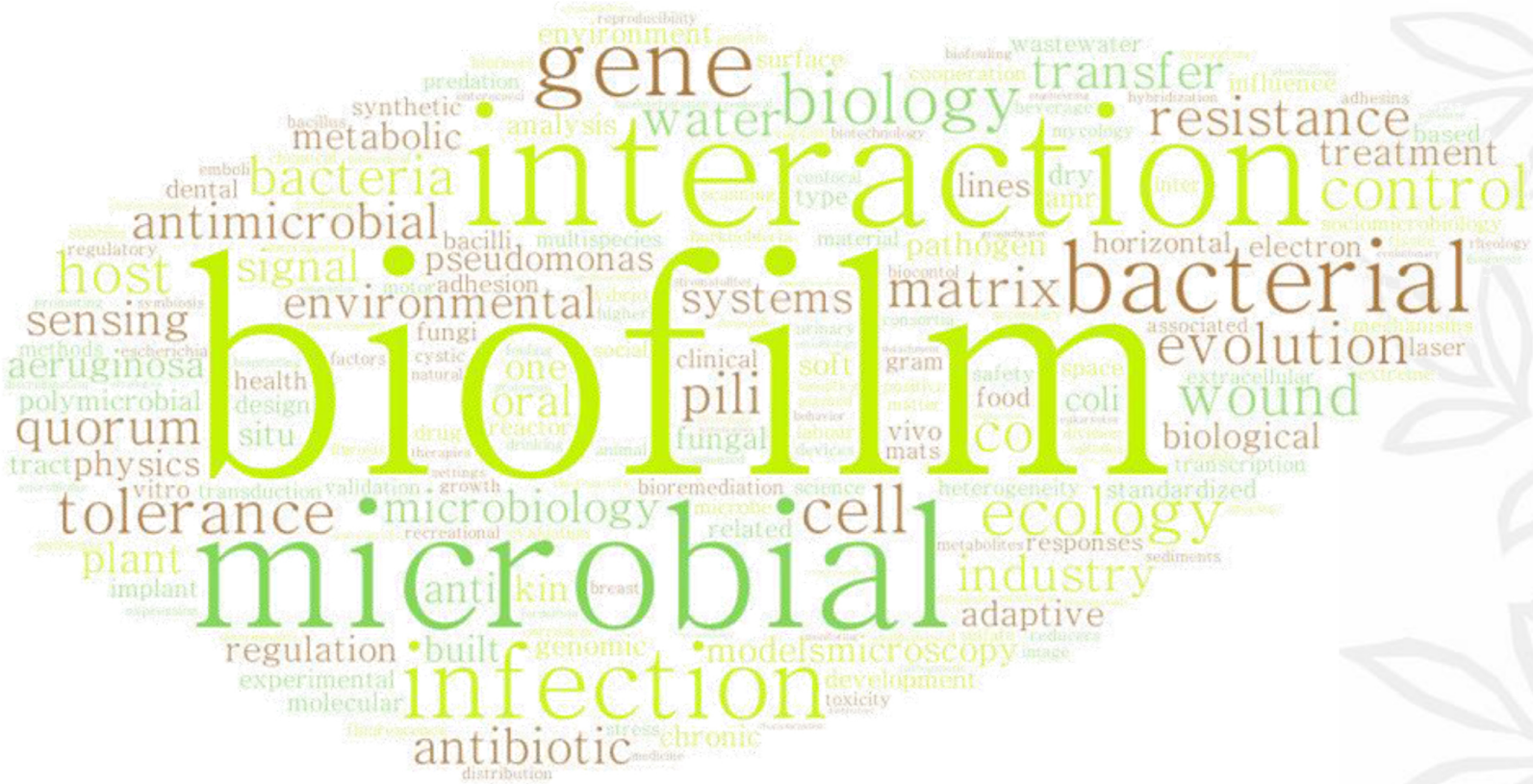
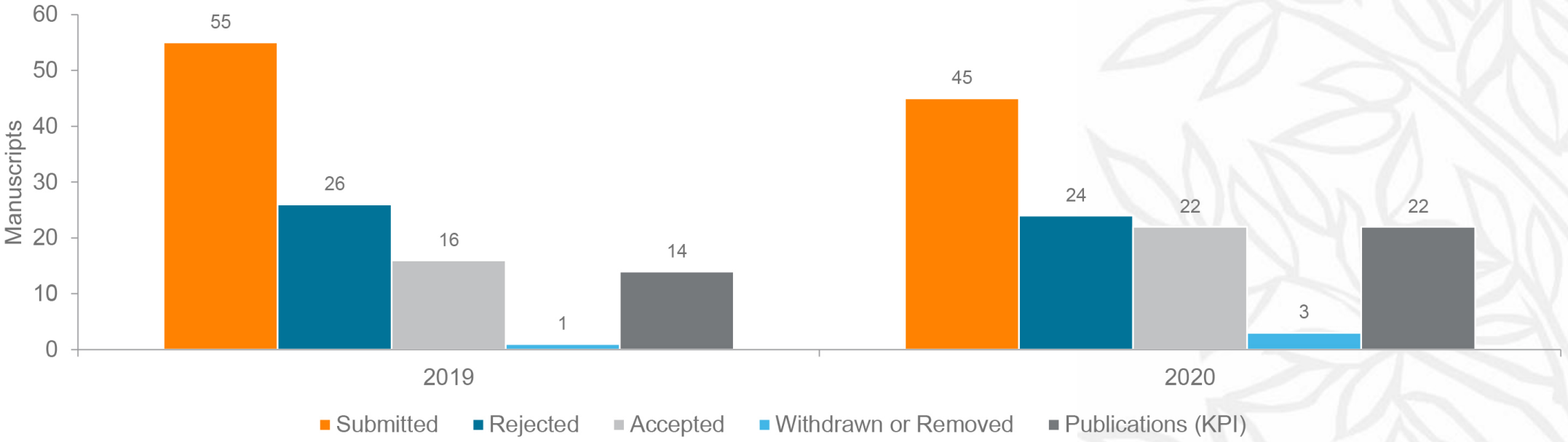


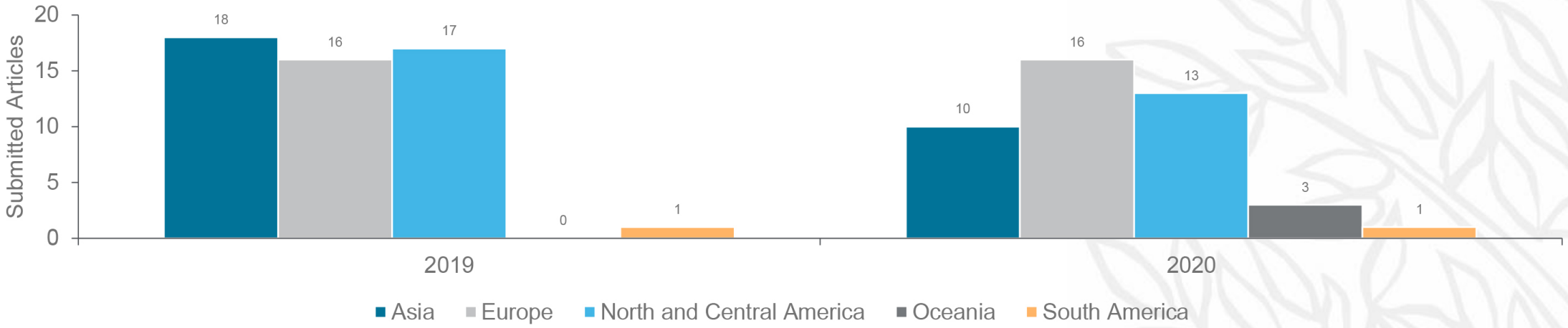
Topics covered by the Editorial Board

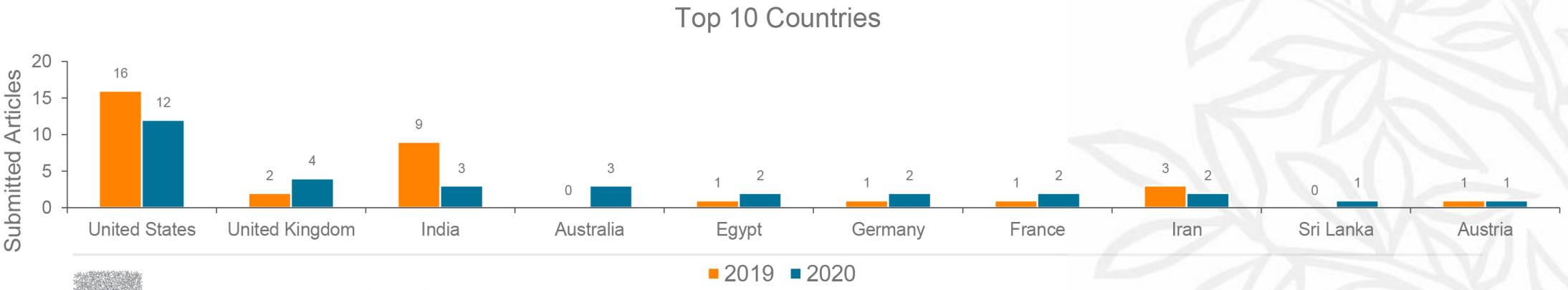


Some key numbers



Submitted Articles by Region and Country





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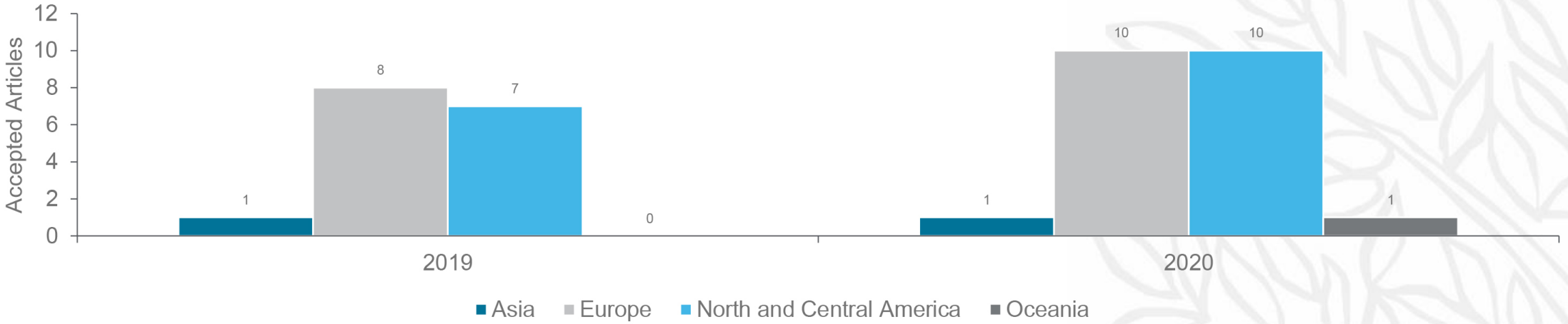
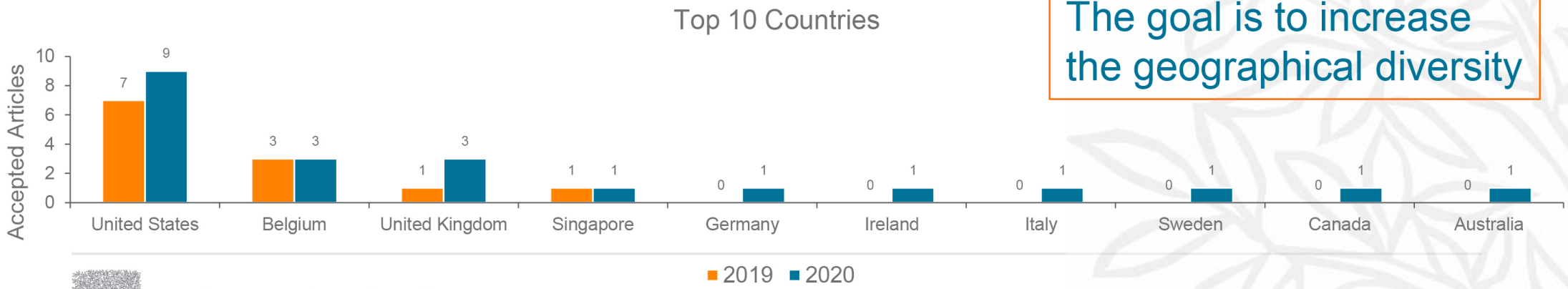
Accepted Articles by Region and Country





The goal is to increase the geographical diversity

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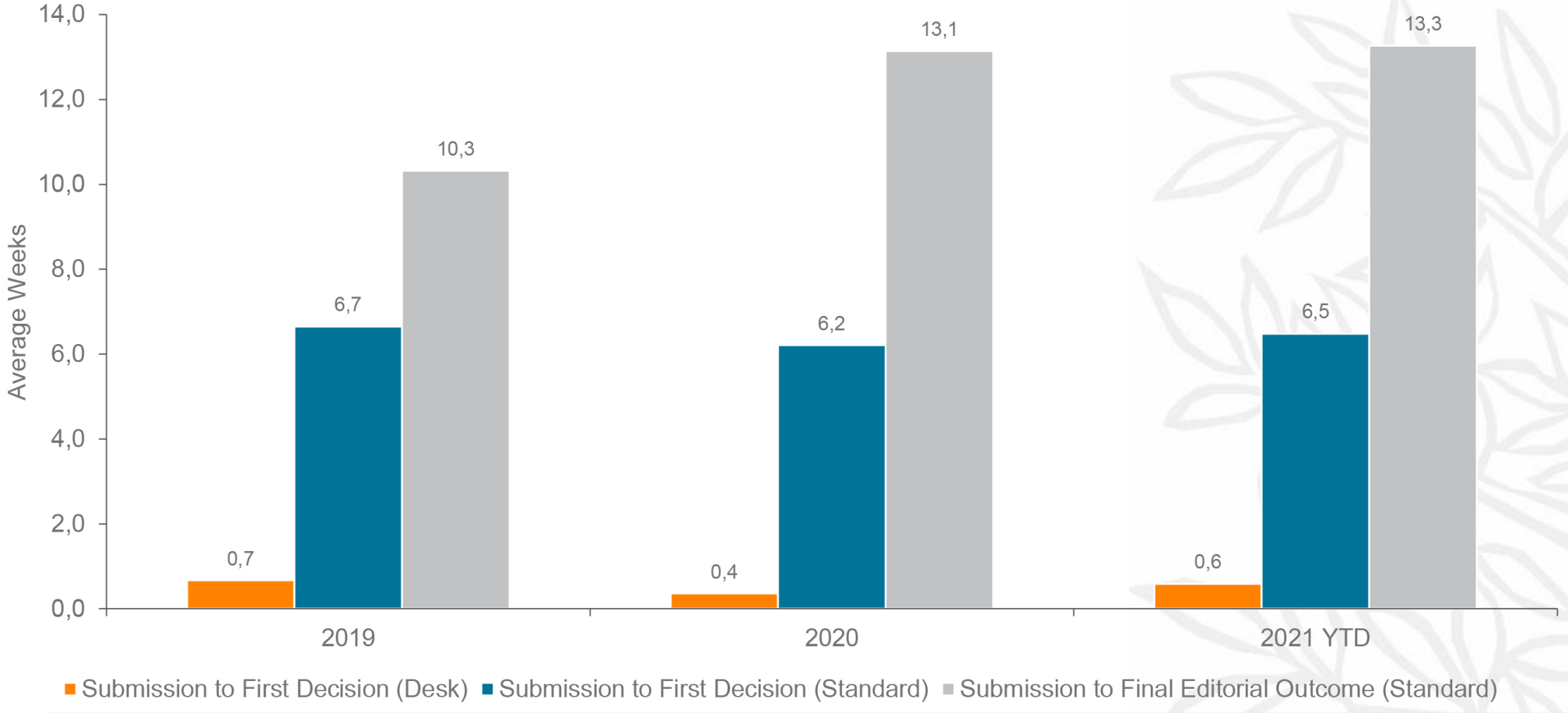
Average Editorial Speed (overall average: 4.8 weeks)



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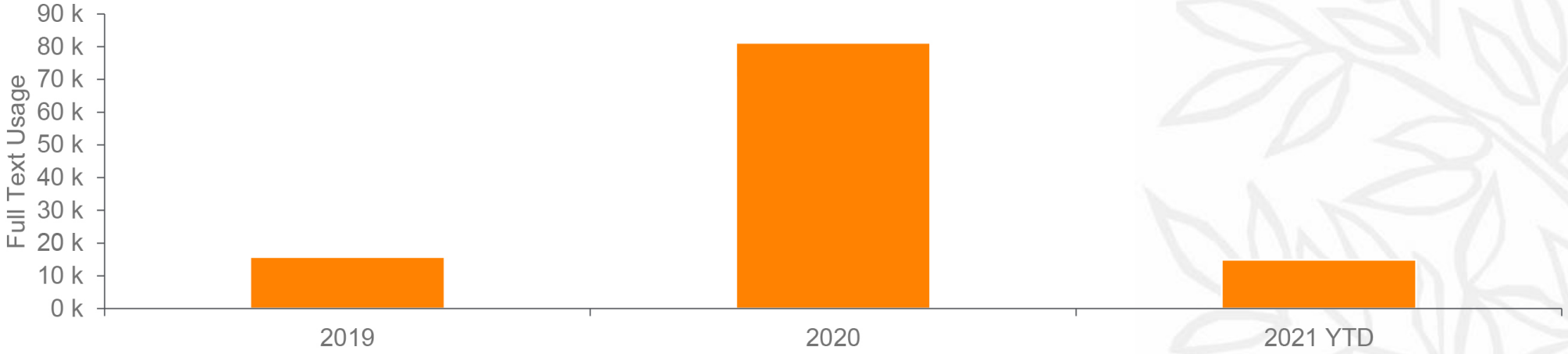

Type	2019	2020	2021 YTD
Total	15,783	81,234	14,948

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ScienceDirect

Most Downloaded Articles From ScienceDirect, 2020 (Published All Time)

Downloads	Downloads (lifetime)	Article Title	Authors	Publication Year	Document Type
10,464	12,503	The future of biofilm research – Report on the '2019 Biofilm Bash'	Tom Coenye, Birthe Kjellerup, Paul Stoodley, Thomas Bjarnsholt	2020	Full-length article
6,288	7,524	Microsystems for biofilm characterization and sensing – A review	Sowmya Subramanian, Ryan C. Huiszoon, Sangwook Chu, William E. Bentley, Reza Ghodssi	2020	Review article
5,965	7,087	Weak acids as an alternative anti-microbial therapy	Binu Kundukad, Gayathri Udayakumar, Erin Grela, Dhamanpreet Kaur, Scott A. Rice, Staffan Kjelleberg, Patrick S. Doyle	2020	Full-length article
5,280	6,458	Biofilm mechanics: Implications in infection and survival	Erin S. Gloag, Stefania Fabbri, Daniel J. Wozniak, Paul Stoodley	2020	Review article
4,436	6,342	Minimum information guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates	Jontana Allkja, Thomas Bjarnsholt, Tom Coenye, Paul Cos, Adyary Fallarero, Joe J. Harrison, Susana P. Lopes, Antonio Oliver, Maria Olivia Pereira, Gordon Ramage, Mark E. Shirtliff, Paul Stoodley, Jeremy S. Webb, Sebastian A.J. Zaat, Darla M. Goeres, Nuno Filipe Azevedo	2020	Full-length article
3,410	3,840	Forming and waking dormant cells: The ppGpp ribosome dimerization persister model	Thomas K. Wood, Sooyeon Song	2020	Full-length article
3,348	4,176	Widespread cryptic viral infections in lotic biofilms	Alexandra T. Payne, Abigail J. Davidson, Jinjun Kan, Marc Peipoch, Raven Bier, Kurt Williamson	2020	Full-length article
3,105	3,771	Cellular chaining influences biofilm formation and structure in group A Streptococcus	Artur Matysik, Foo Kiong Ho, Alicia Qian Ler Tan, Anuradha Vajjala, Kimberly A. Kline	2020	Full-length article
2,829	3,630	Impact of metal ions on structural EPS hydrogels from aerobic granular sludge	Simon Felz, Hugo Kleikamp, Jure Zlopasa, Mark C.M. van Loosdrecht, Yuemei Lin	2020	Full-length article
2,511	4,873	Into the well—A close look at the complex structures of a microtiter biofilm and the crystal violet assay	Kasper Nørskov Kragh, Maria Alhede, Lasse Kvich, Thomas Bjarnsholt	2019	Full-length article

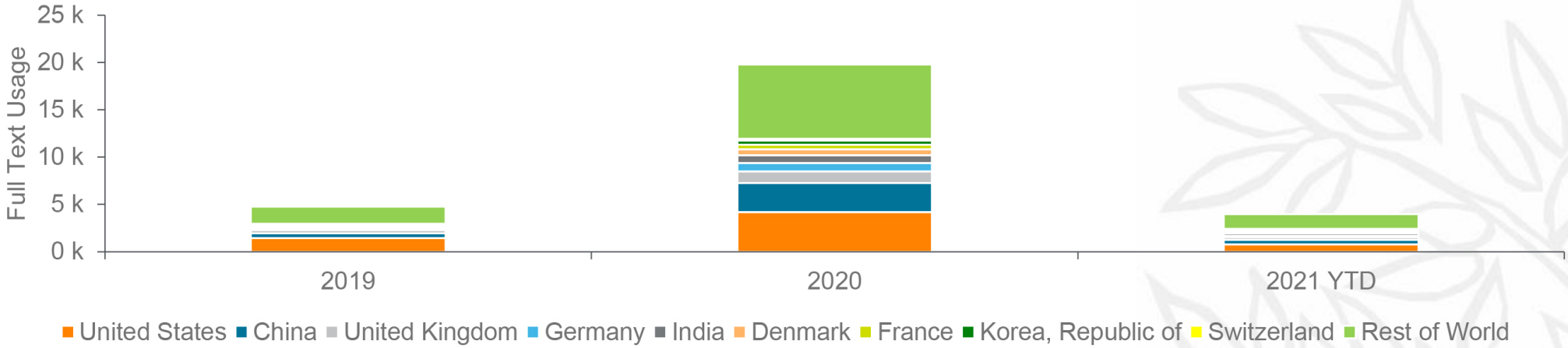
Biofilm

ScienceDirect

Most Downloaded Articles From ScienceDirect, 2021 YTD (Published All Time)

Downloads	Downloads (lifetime)	Article Title	Authors	Publication Year	Document Type
1,655	12,503	The future of biofilm research – Report on the '2019 Biofilm Bash'	Tom Coenye, Birthe Kjellerup, Paul Stoodley, Thomas Bjarnsholt	2020	Full-length article
1,122	7,087	Weak acids as an alternative anti-microbial therapy	Binu Kundukad, Gayathri Udayakumar, Erin Grela, Dhamanpreet Kaur, Scott A. Rice, Staffan Kjelleberg, Patrick S. Doyle	2020	Full-length article
974	6,342	Minimum information guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates	Jontana Allkja, Thomas Bjarnsholt, Tom Coenye, Paul Cos, Adyary Fallarero, Joe J. Harrison, Susana P. Lopes, Antonio Oliver, Maria Olivia Pereira, Gordon Ramage, Mark E. Shirtliff, Paul Stoodley, Jeremy S. Webb, Sebastian A.J. Zaat, Darla M. Goeres, Nuno Filipe Azevedo	2020	Full-length article
888	7,524	Microsystems for biofilm characterization and sensing – A review	Sowmya Subramanian, Ryan C. Huiszoon, Sangwook Chu, William E. Bentley, Reza Ghodssi	2020	Review article
718	2,796	Biofilm dispersion: The key to biofilm eradication or opening Pandora's box?	Jasper Wille, Tom Coenye	2020	Review article
668	6,458	Biofilm mechanics: Implications in infection and survival	Erin S. Gloag, Stefania Fabbri, Daniel J. Wozniak, Paul Stoodley	2020	Review article
595	2,996	Potential biofilm control strategies for extended spaceflight missions	Luis Zea, Robert J.C. McLean, Tony A. Rook, Geoffrey Angle, D. Layne Carter, Angela Delegard, Adrian Denvir, Robin Gerlach, Sridhar Gorti, Doug McIlwaine, Mononita Nur, Brent M. Peyton, Philip S. Stewart, Paul Sturman, Yo Ann Velez Justiniano	2020	Review article
500	4,873	Into the well—A close look at the complex structures of a microtiter biofilm and the crystal violet assay	Kasper Nørskov Kragh, Maria Alhede, Lasse Kvich, Thomas Bjarnsholt	2019	Full-length article
461	461	Do results obtained with RNA-sequencing require independent verification?	Coenye T.	2021	Editorial
430	3,840	Forming and waking dormant cells: The ppGpp ribosome dimerization persister model	Thomas K. Wood, Sooyeon Song	2020	Full-length article

ScienceDirect Usage: Top 10 Countries & Regions by Last Year



Country or Region	2019	2020	2021 YTD
United States	1,436	4,176	788
China	505	3,086	475
United Kingdom	358	1,222	263
Germany	161	873	178
India	128	829	228
Denmark	165	646	112
France	105	475	154
Korea, Republic of	42	423	111
Switzerland	52	206	111
Unknown	11,038	61,472	10,988
Rest of World	1,793	7,826	1,540
Total	**15,783**	**81,234**	**14,948**

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1 Cite Share

Milieu matters: An *in vitro* wound milieu to recapitulate key features of, and probe new insights into, mixed-species bacterial biofilms.

Kadam S, Madhusoodhanan V, Dhekane R, Bhide D, Ugale R, Tikhole U, Kaushik KS.

Biofilm. 2021 Apr 3;3:100047. doi: 10.1016/j.bioflm.2021.100047. eCollection 2021 Dec.

PMID: 33912828 Free PMC article.

Most in vitro studies employ refined laboratory media to study biofilms, representing conditions that are not relevant to the infection state. To mimic the wound milieu, in vitro **biofilm** studies often incorporate serum or plasma in growth conditions, or employ clot or matr ...

2 Cite Share

Regulation of Cunninghamella spp. **biofilm** growth by tryptophol and tyrosol.

Khan MF, Saleem D, Murphy CD.

Biofilm. 2021 Mar 26;3:100046. doi: 10.1016/j.bioflm.2021.100046. eCollection 2021 Dec.

PMID: 33898970 Free PMC article.

The molecules had a comparatively minor impact on the **biofilm** growth of C. elegans and C. echinulata and on the growth of these fungi on agar plates. Finally, when exogenous tyrosol or tryptophol was added to previously grown C. blakesleeana **biofilm**, detachment was ...

3 Cite Share

Transcriptional profiling of biofilms formed on chilled beef by psychrotrophic meat spoilage bacterium, *Pseudomonas fragi* 1793.

Wickramasinghe NN, Ravensdale J, Coorey R, Dykes GA, Chandry PS.

Biofilm. 2021 Feb 17;3:100045. doi: 10.1016/j.bioflm.2021.100045. eCollection 2021 Dec.

PMID: 33718862 Free PMC article.

At the same time, the **biofilm** growth was assessed by fluorescent staining and imaging using confocal laser scanning microscope. ...The results show that protein synthesis and cellular multiplication cease after the **biofilm** population maximum has reached....

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Increasing your chances of having your paper accepted in *Biofilm*

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Are you ready to write a paper for *Biofilm*?

- You should consider publishing if you have information that advances understanding in the field
- This could be in the form of presenting new, original results; reinterpreting published results; or reviewing a particular subfield
- Check our aims and scope: https://www.journals.elsevier.com/biofilm

Are you ready to write a paper for *Biofilm*?

- Purely descriptive studies will only be considered for publication in *Biofilm* if they contain a substantial body of research data and report on an important advancement in the field

- We like to see *more* than just observations; novel insights and mechanisms are strongly preferred

- Think about the models that you use!

Are you ready to write a paper for *Biofilm*?

- *Biofilm* will typically not publish papers that are preliminary, i.e. lack sufficient amounts of novel data, papers that merely extend known observations/mechanisms, or case reports

- Examples of topics unlikely to be high on the priority list:
  - Repeating work already done with another organism
  - Work strictly focused on a local context, without broader implications
  - Work with natural products/plant extracts without identification of active compound and/or mechanism of action

Are you ready to write a paper for *Biofilm*?

- Feel free to discuss potential submission with one of the senior editors prior to submission
- While this does not guarantee anything, it can help in making a decision on whether you are ready to submit to *Biofilm*
- Also: feel free to reach out after a rejection – we are there to provide more explanation if needed

Volunteer for Peer Review with Biofilm

Introducing Volunpeers

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Introducing *Volunpeers*

Volunteer to perform peer review for *Biofilm*

- Aim is to offer quick, constructive and fair peer-review process, to engage reviewers actively into the publishing process and to provide mentorship to researchers (especially early career) to provide meaningful and constructive reviews to their peers
- Sign-up to the initiative now as a “volunpeer” reviewer for Biofilm, by creating an account on our editorial system: https://www.editorialmanager.com/bioflm/default.aspx
- Ensure to:
  - provide your Scopus Author/Researcher/PubMed Author/ORCID ID
  - provide your Keywords/areas of expertise
  - Answer “yes” to: “Are you willing to review for this journal”
- You will then be discoverable by editors in the peer review system as “volunpeer”

Biofilm

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ASIA-PACIFIC BIOFILMS 2021

Dr. Tom Coenye & Dr. Birthe Kjellerup
Senior Editors
@BiofilmJournal
Tom.Coenye@UGent.be & bvk@umd.edu

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May 13th Medical Microbiology

Session 1

Chairs: Birthe Kjellerup & Zhenbo Xu

9:00-9:25	Meeting platform test
9:25-9:35	Opening ceremony Birthe Kjellerup, Zhenbo Xu
9:35-10:00	Experimental evolution as a tool to study biofilm biology Tom Coenye, Ghent University, Ghent
10:00-10:25	Medical devices and infection control - current challenges and opportunities Helmut Thissen, Monash University, Melbourne
10:25-10:40	Bacterial chemical signal regulation of biofilm development Lianhui Zhang, South China Agricultural University, Guangzhou
10:40-10:55	Meet the speakers

Session 2

Chairs:Matthew Parsek & Yue Qu

10:55-11:20	*Pseudomonas aeruginosa* uses a versatile repertoire of exopolysaccharides and proteins to build biofilms Matthew Parsek, University of Washington, Seattle
11:20-11:45	Mechanical circulatory support - a major advance but with the price of serious infection risk David McGiffin, Monash University, Melbourne
11:45-12:00	A nuclear-dbf2 related (NDR) kinase exerts isoform-specific control over *Aspergillus fumigatus* pathogenic fitness Jarrod Fortwendel, University of Tennessee Health Science Center, Memphis
12:00-12:15	Intracellular glycosyl hydrolase shapes bacterial cell fate, signaling, and the biofilm development of *Pseudomonas aeruginosa* Luyan Ma, Institute of Microbiology of the Chinese Academy of Sciences, Beijing
12:15-12:30	Mouse and human cell models of *Bordetella pertussis* biofilm development Rajendar Deora, The Ohio State University, Columbus
12:30-14:00	Meet the speakers 12:30-12:45 / Networking 12:45-14:00

Session 3 **Chair: Kimberly Kline**	
14:00-14:25	**Identification of small molecules that interfere with c-di-GMP signaling and induce dispersal of *Pseudomonas aeruginosa* biofilms** Tim Tolker-Nielsen, University of Copenhagen, Copenhagen
14:25-14:40	**Dissecting the mechanisms of *Entercocccus faecalis* biofilm-associated infection** Kimberly Kline, Nanyang Technological University, Singapore
14:40-14:55	**Application of nucleic acid-based methods to study and modulate multispecies biofilms** Nuno Azevedo, University of Porto, Porto
14:55-15:10	**War and Peace: Polymicrobial interactions during cystic fibrosis airway infection** Dominique Limoli, University of Iowa, Iowa City
15:10-15:25	**Proteogenomic determinants of biofilm-associated antimicrobial resistance in *Pseudomonas aeruginosa*** Jeremy Webb, University of Southampton, Southampton
15:25-15:40	**Memory and cooperativity during reversible and irreversible attachment in young biofilms** Calvin Lee, University of California, Los Angeles
15:40-15:55	Meet the speakers
Session 4 Student Presentations **Chair: Zhao Cai**	
15:55-17:05	**Application of transposon inserted mutant sequencing analysis in identifying genetic determinants of bacterial biofilms** Jing Wang, Southern University of Science and Technology, Shenzhen **Low concentrate of antibiotics enhance biofilm formation in *Staphylococcus aureus*** Ziqi Liu, South China University of Technology, Guangzhou **Molecular epidemiology analysis of *Pseudomonas aeruginosa* infections carrying *qnrVC* genes from Guangzhou, China** Jinqiong Lin, First Affiliated Hospital of Guangzhou Medical Univ., Guangzhou **Antibacterial self-assembled nanodrugs composed of berberine derivatives and rhamnolipids against *Helicobacter pylori* biofilms** Xiaonan Chen, Sun Yat-Sen University, Guangzhou **Mucus penetration enhanced lipid polymer nanoparticles improve the eradication rate of *Helicobacter pylori* biofilm** Pengyu Li, Sun Yat-Sen University, Guangzhou **Polymicrobial interaction between *Lactobacillus* and *Saccharomyces cerevisiae*** Xin Lin, South China University of Technology, Guangzhou ***C. albicans* augments *S. aureus* quorum sensing during polymicrobial infections** Olivia Todd, University of Tennessee Health Science Center, Memphis
17:05-18:00	Networking

May 14th Medical Microbiology

Session 5

Chair: Paul Stoodley

9:00-9:25	Synovial fluid induced *Staphylococcus aureus* aggregation and biofilm formation in periprosthetic joint infection (PJI) Paul Stoodley, The Ohio State University, Columbus
9:25-9:50	The role of *in vivo* biofilm migration in ventricular assist device (VAD) infections Anton Peleg, Monash University, Melbourne
9:50-10:05	To be determined Janette Harro, University of Maryland, Baltimore
10:05-10:20	The role of TNF in host immunity to *Staphylococcus aureus* Nathan Archer, Johns Hopkins University, Baltimore
10:20-10:35	Co-expression of *ECE1* and *ALS3* in *C. albicans* independent of hyphal formation is capable of damaging vaginal epithelial cells Zhenbo Xu, South China University of Technology, Guangzhou
10:35-10:50	Meet the speakers

Session 6

Chair: Liang Yang

10:50-11:15	Biofilm degradation in wound infections Kendra Rumbaugh, Texas Tech University Health Sciences Center, Lubbock
11:15-11:40	Bacterial interspecies interactions and evolution in multispecies biofilms Mette Burmolle, University of Copenhagen, Copenhagen
11:40-11:55	Impact of clinical lipid formulations on Candida biofilm formation and incidence of candidiasis Brian Peters, University of Tennessee Health Science Center, Memphis
11:55-12:10	Nosocomial *P. aeruginosa* regulates alginate biosynthesis and T6SS during adaptive and convergent evolution for coinfection in critically ill COVID-19 patients Liang Yang, Southern University of Science and Technology, Shenzhen
12:10-12:25	Cyclic di-GMP diverges to control antibiotic synthesis in *Lysobacter* Guoliang Qian, Nanjing Agricultural University, Nanjing
12:25-14:00	Meet the speakers 12:25-12:40 / Networking 12:40-14:00

Session 7 **Chair: Yue Qu**	
14:00-14:25	**To know and not just to believe regarding biofilms and the infectious microenvironment** **Thomas Bjarnsholt, University of Copenhagen, Copenhagen**
14:25-14:40	**A critical role of biomaterial surface chemistry and environmental cues on biofilm formation of *Staphylococcus capitis*** **Yue Qu, Monash University, Melbourne**
14:40-14:55	**Non-attached biofilm aggregates in chronic infections - and how to model them *in vitro*** **Kasper Kragh, University of Copenhagen, Copenhagen**
14:55-15:10	**Sticking it to bacterial resistance using honey-inspired antimicrobial materials** **Lewis Blackman, CSIRO, Canberra**
15:10-15:25	**Prevention of oral diseases by anti-biofilm dental materials** **Lei Cheng, Sichuan University, Chengdu**
15:25-15:40	**Pleural empyema-related pathogens and biofilms** **Ke Wang, The First Affiliated Hospital of Guangxi Medical University, Nanning**
15:40-15:55	**Meet the speakers**
Session 8 **Chair: Qingbin Guo**	
15:55-16:10	**Interactions between bacteria and their dead siblings** **Xiangjun Gong, South China University of Technology, Guangzhou**
16:10-16:25	**Mixed-species biofilm formation of *L. monocytogenes* in food processing plants and its inactivation by low-energy X-ray irradiation.** **Xinyi Pang, National University of Singapore, Singapore**
16:25-16:40	**Strategies for structural characterization of EPS from biofilm** **Qingbin Guo, Tianjin University of Science and Technology, Tianjin**
16:40-16:55	**Novel drug delivery system against biofilms infections** **Haiyan Hu, Sun Yat-Sen University, Guangzhou**
16:55-17:10	**Application of photodynamic inactivation for eradicating planktonic and sessile bacteria** **Jingjing Wang, Shanghai Ocean University, Shanghai**
17:10-18:00	**Meet the speakers 17:10-17:25 / Networking 17:25-18:00**

May 15th Basic and Foodborne Microbiology

Session 9

Chair: Chuanwu Xi

9:00-9:25	Environmental surveliene of SARS-CoV-2 to inform exposure risks Chuanwu Xi, University of Michigan, Ann Arbor
9:25-9:50	Biofilm Standard Methods: Enabling for innovation in the marketplace Darla Goeres, Montana State University, Bozeman
9:50-10:05	Bacterial sensing by histidine kinases: chemical and gas Wei Qian, Institute of Microbiology of the Chinese Academy of Sciences, Beijing
10:05-10:20	Study on the regulation of multi-factor interactions during the *Vibrio parahaemolyticus* biofilm formation Yong Zhao, Shanghai Ocean University, Shanghai
10:20-10:35	Naftifine derivatives inhibit biofilm formation of multidrug-resistant *Staphylococcus aureus* and potentiate antimicrobials Chunlei Shi, Shanghai Jiaotong University, Shanghai
10:35-10:50	Meet the speakers

Session 10

Chair: Liang Yang

10:50-11:15	YdiV--- An ongoing story begins with the biofilm Lichuan Gu, Shandong University, Jinan
11:15-11:40	Opportunities and challenges of surface plasmon resonance (SPR) on biofilms Chii-Wann Lin, National Taiwan University, Taipei
11:40-11:55	The removal mechanism of *Listeria monocytogenes* biofilm by combined effect of acidic electrolyzed water and alkaline electrolyzed water Jianxiong Hao, Hebei University of Science and Technology, Shijiazhuang
11:55-12:10	Biofilm: What can we learn from *Bacillus cereus* Yu Ding, Jinan University, Guangzhou
12:10-12:25	Mechanism of natural clays against single biofilm formation of *Staphylococcus aureus* on stainless steel surface Nor Ainy Mahyudin, Universiti Putra Malaysia, Serdang
12:25-14:00	Meet the speakers 12:25-12:40 / Networking 12:40-14:00

Session 11 **Chair: Lei Yuan**	
14:00-14:25	**Biofilm formation and control in the dairy industry** **Steve Flint, Massey University, Palmerston North**
14:25-14:40	**Molecular mechanism of membrane disintegration and dysfunction in foodborne pathogens with ultrasonic treatment** **Mingming Guo, Zhejiang University, Hangzhou**
14:40-14:55	***Cronobacter* biofilm formation and control strategies in the food industry** **Yingwang Ye, Hefei University of Technology, Hefei**
14:55-15:10	**Influence of quorum sensing on formation of viable but nonculturable (VBNC) cells of beer-spoilage lactic acid bacteria growing in biofilms** **Yang Deng, Qingdao Agricultural University, Qingdao**
15:10-15:25	**Discovery of anti-biofilm terpenoid compounds: (+)-nootkatone as an example** **Renyou Gan, Institute of Urban Agriculture, Chengdu**
15:25-15:40	**Inhibitory effect of *Lactobacillus plantarum* metabolites against biofilm formation by *Bacillus licheniformis* isolated from milk powder products** **Lei Yuan, Yangzhou University, Yangzhou**
15:40-15:55	**Research on the role of quorum sensing in biofilm of *Leuconostoc citreum* and its application in dairy fermentation** **Rihua Xu, Inner Mongolia University, Hohhot**
15:55-16:10	**Meet the speakers**
Session 12 Student Presentation **Chair: Yingdan Zhang**	
16:10-17:15	**Monitoring the 3D morphology of growing biofilms** **Weixiong Zhang, South China University of Technology, Guangzhou** **Molecular epidemiology characteristics of 146 CRE infections in Guangzhou, China** **Chen Peng, First Affiliated Hospital of Guangzhou Medical University, Guangzhou** **Molecular epidemiology and antibiotics resistance analysis of non-typing *H. influenzae* after the introduction of the Hib vaccine in Guangzhou, China** **Shuxian Wen, First Affiliated Hospital of Guangzhou Medical Univ., Guangzhou** **SPR on biofilms: with biofilm associated genes screening in *S. aureus* as an example** **Yuting Luo, South China University of Technology, Guangzhou** **Delineating the Upc2A regulon in *Candida glabrata*** **Yu Li, St. Jude Children's Research Hospital, Memphis** **Control and impact of glycogen utilization & synthesis in *C. albicans* mediated VVC** **Jian Miao, University of Tennessee Health Science Center, Memphis** **In vitro evaluation of biofilm formation by LAB into different stress conditions** **Fedrick Mgomi, Yangzhou University, Yangzhou**
17:15-18:00	**Networking**

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Session 13

Chair: Junyan Liu

9:00-9:25	Biofilms in intermittent and continuous flow drinking water distribution systems Stefan Wuertz, Nanyang Technological University, Singapore
9:25-9:50	Biofilm-mineral interactions, insights from engineered biomineralization applications and the urinary tract Robin Gerlach, Montana State University, Bozeman
9:50-10:05	Synergistic metabolism and community structure in algal-bacterial counter-diffusion biofilms for treating biogas slurry Rongchang Wang, Tongji University, Shanghai
10:05-10:20	Biofilms lifestyle of *Comamonas* in the environmental context Bin Cao, Nanyang Technological University, Singapore
10:20-10:35	Electroanalysis of *Candida albicans* biofilms: a suitable real-time tool for antifungal testing Enrico Marsili, Nazarbayev University, Astana
10:35-10:50	Meet the speakers

Session 14

Chair: Birthe Kjellerup

10:50-11:15	The Biofilm way of thinking in bioremediation Birthe Kjellerup, University of Maryland, College Park
11:15-11:40	Industrial characterization system for biofilms on materials Hideyuki Kanematsu, National Institute of Technology, Tokyo
11:40-11:55	Motility selection contributes to collective antibiotic tolerance in bacterial swarms Yilin Wu, Chinese University of Hong Kong, Hong Kong
11:55-12:10	Conditional Privatization Stabilize Bacterial Cooperation Fan Jin, Shenzhen Institute of Advanced Technology, Shenzhen
12:10-12:25	A variant *ECE1* allele contributes to reduced pathogenicity of *Candida albicans* during vulvovaginal candidiasis Junyan Liu, University of Maryland, College Park
12:25-14:00	Meet the speakers 12:25-12:40 / Networking 12:40-14:00

Session 15 Antimicrobial and Anti-Biofilms **Chair: Yulong Tan**	
14:00-14:25	Antimicrobial resistance and antibiotic therapy in biofilm-related infection Po-Ren Hsueh, National Taiwan University Hospital, Taipei
14:25-14:40	New antibiofilm strategy against fungal/bacterial polymicrobial biofilms Yulong Tan, Qingdao Agricultural University, Qingdao
14:40-14:55	Study on construction of bacterially anti-adhesive surface, its mechanism and application Jing Lin, Guangzhou University, Guangzhou
14:55-15:10	New uses for old drugs-Diclazuril inhibits biofilm formation of *Staphylococcus aureus* Jinxin Zheng, Shenzhen Nanshan Hospital, Shenzhen
15:10-15:25	pH and light-responsive polycaprolactone/curcumin@ZIF-8 composite films with enhanced antibacterial activity Jianyu Su, South China University of Technology, Guangzhou
15:25-15:40	QSIs from Some TCMs and Some Non-natural QSIs Aiqun Jia, Hainan University, Haikou
15:40-15:55	Meet the speakers
Session 16 Biofilm for application **Chair: Gamini Seneviratne**	
15:55-16:10	Biofilm vs microbial inoculation in biofertilization Gamini Seneviratne, National Institute of Fundamental Studies, Sri Lanka
16:10-16:25	Agriculturally beneficial biofilms as inoculants for sustainable and integrated nutrient and disease management: from lab to land Radha Prasanna, ICAR-Indian Agricultural Research Institute, New Delhi
16:25-16:40	The application of biofilm biofertilizer to increase crop yield and soil fertility status Sudadi Sudadi, Universitas Sebelas Maret, Surakarta
16:40-16:55	Enhanced recovery of biophotosensitizer from microalgal biofilm by photosyn thetic electrons extraction towards photolytic removal of antibiotic in wastewater Jian Sun, Guangdong University of Technology, Guangzhou
16:55-17:10	Application of the kombucha biofilm for the development of functional beverages Viduranga Waisundara, Australian College of Business & Technology, Kandy
17:10-17:25	Fouling microorganisms in the reservoirs of the groundwater treatment system Litvinenko Zoia, Institute of the Water and Ecology Problems, Far Eastern Branch, Russian Academy of Sciences, Khabarovsk
17:10-18:00	Closing ceremony / Award announcement

1.Speeches

Experimental evolution as a tool to study biofilm biology

Tom Coenye

Ghent University, Ghent

Abstract: An important factor contributing to failure of antimicrobial therapy is that *in vivo* bacteria form biofilms, i.e. aggregated and structured communities of cells belonging to one or more species, embedded in a self-produced polymeric matrix. Biofilm cells are phenotypically very different from planktonic cells and the microenvironment in these biofilm aggregates (e.g. reduced O_2 levels due to aerobic respiration, increased pH due to accumulation of waste products, lower levels of nutrients, …) leads to an altered metabolism linked to reduced susceptibility. In addition, bacteria adapt to changing conditions during chronic (biofilm-related) infections and rapidly evolve new phenotypes.

To predict what will happen when biofilms are exposed to conventional and/or experimental therapies, researchers have started to turn to experimental evolution. In these studies, bacteria are repeatedly exposed to an antibiotic and/or other compounds under controlled conditions, and their adaptation is closely monitored. This approach allows to study adaptation of microbial biofilms in real time, allows to predict the outcome of treatments and allows to predict the likeliness of resistance development.

In my presentation I will discuss the potential of experimental evolution for biofilm biology, using recent results obtained in my research group at Ghent University with *Burkholderia cenocepacia* and *Pseudomonas aeruginosa* biofilms.

Medical devices and infection control – current challenges and opportunities

Helmut Thissen

Monash University, Melbourne

Abstract: The infection risk associated with medical devices has become an exceptionally important research topic over the last few years due mainly to two reasons: Firstly, all medical devices have an associated infection rate over the lifetime of the device, with some remaining at an unacceptably high level. Secondly, with medical devices being the leading source of e.g. nosocomial infections, they also play an important part in the fight against antimicrobial resistance (AMR). Here we have developed highly effective coating platforms that reduce non-specific biointerfacial interactions while also allowing the display or release of antimicrobial agents in controlled densities and ratios. Specifically, we have employed crosslinked poly(ethylene glycol) and poly(2-hydroxypropyl acrylamide) polymer coatings that can be deposited in a single step to achieve effective control over non-specific biointerfacial interactions, including protein fouling, pathogen colonisation and biofilm formation. Moreover, we have incorporated antimicrobial compounds in these coatings ranging from peptides to quorum sensing inhibitors. Finally we have also used nanotopography features to create bactericidal surfaces. Our surface analytical results combined with our detailed analysis of the biological response in vitro and in vivo provide evidence that the infection risk associated with medical devices can be reduced significantly. It is expected that our concept of robust, one-step coatings offering multiple layers of defence against infections will find applications in a broad range of medical devices.

Pseudomonas aeruginosa uses a versatile repertoire of exopolysaccharides and proteins to build biofilms

Matthew Parsek

University of Washington, Seattle

Abstract: The *P. aeruginosa* biofilm matrix is a complex, multi-component mesh encasing cells in a variety of exopolysaccharides (EPS; Psl, Pel, and alginate) in addition to matrix proteins (CdrA and ecotin), and extracellular DNA (eDNA). Psl is a neutral, mannose-rich, branched polysaccharide important for the initial attachment of cells to a surface. In a mature biofilms, Psl localizes primarily to the periphery of aggregates where it interacts with matrix adhesin protein CdrA. CdrA is present in the matrix as both call-associated and released versions, with both forms playing roles in the stabilization and integrity of the biofilm through its interactions with Psl and Pel. Pel is a positively charged polysaccharide comprised of *N*-acetylglucosamine and *N*-acetylgalactosamine which localizes to the biofilm stalk and surface interface between biofilm and surface, where it also interacts with eDNA. While biofilm communities of *P. aeruginosa* are linked with a number of chronic human infections, mucoid *P. aeruginosa* infections exclusively arise as a result of colonizing the unique niche of the CF lung. We found that mucoid *P. aeruginosa* can form biofilms without the use of Psl and CdrA, if grown in a high calcium environment. This represents a novel finding, as previous reports have indicated that the majority of non-mucoid and mucoid *P. aeruginosa* strains rely upon Psl for attachment to surfaces, and CdrA for structural robustness. It has previously been reported that *P. aeruginosa* EPS rely on shared sugar precursors, with the enzyme AlgC serving as a critical check point for the synthesis of alginate, Psl, and LPS. While CdrA has been shown to interact with Psl, Pel, and itself within the biofilm matrix, our study demonstrates that CdrA does not interact with alginate, nor is CdrA required for the formation of a crosslinked calcium-alginate biofilm. Instead, the calcium-gelled alginate matrix appears to rely solely on calcium-alginate crosslinking for support, though it may still be possible that different structural matrix proteins contribute to the maintenance of the mucoid biofilm. Indeed, due to the disperse arrangement of cells within the alginate hydrogel matrix, we believe that unidentified matrix-associated proteins are very likely interacting with and being retained by the alginate polymer strands.

Mechanical circulatory support–a major advance but with the price of serious infection risk

Professor David McGiffin,

Department of cardiothoracic surgery and transplantation

The Alfred Hospital, Melbourne, Australia

Abstract: The development of mechanical circulatory support was a huge advance in the management of patients with end-stage heart disease. Third-generation continuous flow pumps (Heartware and Heartmate 3 ventricular assist devices) provide support for patients as a bridge to cardiac transplantation, for destination therapy (for patients who are ineligible for cardiac transplantation), a bridge to decision (where eligibility for cardiac transplantation is uncertain) and rarely, as a bridge to myocardial recovery. However, ventricular assist devices are at risk for infection which can substantially contribute to morbidity and even mortality. The classification of infections in patients with ventricular assist devices (VAD) is

- VAD-specific infection – driveline infection, pump pocket infection, pump or cannula infection
- VAD-related infection – endocarditis, mediastinitis, bloodstream infection
- Non-VAD infection – (unrelated to the presence of a VAD) such as urinary tract infection, respiratory tract infection

The VAD driveline is the major site of VAD-specific infection. 10 to 20% of driveline is develop an infection. Bacteria are the dominant cause of both early and late infection and the most common pathogen is Gram-positive bacteria, Staphylococcus aureus and epidermidis (accounting for 50% of VAD infections) and Gram-negative bacteria such as Pseudomonas and Klebsiella. Fungal infection is uncommon. The microbial basis of infection is biofilm formation because of microbial adherence to the external driveline surface with migration along the driveline in its tissue tunnel.

Prevention of driveline infection is important with strategies that reduce infection such as peri-implant antimicrobial prophylaxis, specific surgical techniques, anchoring of the driveline to prevent traction injury to the tissue tunnel and thorough driveline exit site care by the patient.

Systemic manifestations such as fever, elevated white cell count and systemic inflammation occur in only half the patients with VAD infection. Imaging such as ultrasound of the driveline tunnel, PET imaging and white cell SPECT-CT may be required to confirm the diagnosis.

Driveline and pump infections can be hard to eradicate and may require surgical procedures such as debridement of infected tissue around the driveline, repositioning of the driveline and antibiotic impregnated beads. Explantation of the VAD and cardiac transplantation may be the only way of eradicating the infection.

Elimination of VAD drivelines and replacement with transcutaneous energy transfer systems will be a major step forward in preventing VAD infection.

Intracellular glycosyl hydrolase shapes bacterial cell fate, signaling, and the biofilm development of *Pseudomonas aeruginosa*

Jingchao Zhang[a], Huijun Wu[b,c], Di Wang[b], Chenxi Zhang[a], Kun Zhao[a]#, Luyan Z. Ma[b,c]#

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Abstract: Biofilm formation is one of most important factors that leading to persistent infections. Exopolysaccharide Psl (ePsl) synthesized from *psl* operon, is a critical biofilm matrix polysaccharide in *Pseudomonas aeruginosa*, an opportunistic pathogen that can cause life-threatening chronic infections in cystic fibrosis patients and immunocompromised individuals. PslG, a glycosyl hydrolase encoded by *pslG* (a gene within *psl* operon), can degrade ePsl to disrupt *P. aeruginosa* biofilms when supplied exogenously or released to extracellular. However, it remains elusive about the functions of intracellular PslG and why a polysaccharide synthesis genes cluster requires a glycosyl hydrolase. Here, we systematically studied the *pslG* knock-out mutants (Δ*pslG*) at both a single cell and community level. Even though Δ*pslG* reduces ePsl production, swimming motility, and bacterial attachment on surface, unexpectedly, loss of *pslG* does not significantly affect the total biofilm biomass or the formation of ePsl fiber and trails, which has been shown to guide bacterial exploration and microcolony formation. Strikingly, Δ*pslG* shapes the localization of ePsl on bacterial periphery, resulting in long chains of bacterial cells. Moreover, lacking of PslG alters the signaling function and structure of ePsl and changes the relative level of cyclic-di-GMP molecule within mother cell and daughter cell during cell division. Consequently, Δ*pslG* shows faster microcolony formation in a flow-cell and more uneven bacterial distribution in an air-liquid interface biofilm than that of PAO1. Our results revealed the important role of an intracellular glycosyl hydrolase on cell fate and the biofilm development of *P. aeruginosa*.

Identification of small molecules that interfere with c-di-GMP signaling and induce dispersal of *Pseudomonas aeruginosa* biofilms

Jens Bo Andersen[1], Louise Dahl Hultqvist[1], Charlotte Uldahl Jansen[2], Tim Holm Jakobsen[1], Martin Nilsson[1], Morten Rybtke[1], Jesper Uhd[2], Blaine Gabriel Fritz[1], Roland Seifert[3], Jens Berthelsen[1], Thomas Eiland Nielsen[1,4], Katrine Qvortrup[2], Michael Givskov[1,4], Tim Tolker-Nielsen[1]*

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Abstract: Microbial biofilms are involved in a number of infections that cannot be resolved, as microbes in biofilms resist host immune defenses and current antibiotic therapies. With no strict biofilm-antibiotic in the current pipelines, there is an unmet need for drug candidates that enable the current antibiotics to efficiently deal with biofilm bacteria. We employed a high throughput screening approach to identify chemical compounds that reduce the intracellular c-di-GMP content in *Pseudomonas aeruginosa*. This led to the identification of a small molecule that efficiently depletes *P. aeruginosa* for c-di-GMP, inhibits biofilm formation and disperses established biofilm. A combination of the anti-biofilm compound with standard of care antibiotics resulted in improved eradication of biofilms *in vitro*, as well as in a murine biofilm infection model. Genetic analyses provided evidence that the anti-biofilm compound specifically stimulates the activity of the c-di-GMP phosphodiesterase BifA in *P. aeruginosa*. Our work constitutes a proof of concept for c-di-GMP phosphodiesterase-activating drugs administered in combination with antibiotics as a viable treatment strategy for otherwise recalcitrant infections.

Application of nucleic acid-based methods to study and modulate multispecies biofilms

Nuno Filipe Azevedo, Assistant Professor

Laboratory of Process Engineering, Environment, Biotechnology and Energy (LEPABE), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal

Abstract: It is now widely-accepted that most naturally-occurring biofilms are constituted by more than one species of microorganisms. These multispecies biofilms might exhibit a different behavior from the single-species biofilms that are studied in the lab, and hence affect the conclusions being drawn from a study. For instance, it is well-known that clinical multispecies biofilms may exhibit increased tolerance to antimicrobial agents. One of the most important parameters to evaluate in multispecies biofilms is the prevalence of the different species. Traditionally, this is accomplished by standard plate counts on nutrient-rich or selective agars. However, in a recent study, it has been shown that 3 different methods (plate counting, q-PCR and FISH) provide very different values for the prevalence of each biofilm population, even when these methods have been previously optimized and provide similar results on planktonic populations [1]. Another important aspect is the modulation of the population structure of a biofilm. Employing nucleic acid mimics (NAMs) coupled with delivery vectors, we are working on strategies to achieve selective depletion of an undesirable species in a multispecies biofilms [2]. This talk will address these two works and also briefly discuss an initiative (MIABiE) that aims to promote the reproducibility between biofilm experiments [3].

References:

[1] Lopes, S. et al.: Scientific Reports. 2018; [2] Santos, R. et al.: Adv. Drug Del. Rev. 2018; [3] Allkja J. et al.: Biofilm. 2020.

War and Peace: Polymicrobial interactions during cystic fibrosis airway infection

Dominique Limoli

University of Iowa, Iowa City

Abstract: Microbes often live in communities composed of multiple species; where interactions among community members impact both the individual constituents and the surrounding environment. Identifying strategies to harness these interactions will open avenues for new antimicrobial strategies. Here we developed a system to visualize interspecies behaviors at initial encounters, on a single cell level. By live imaging two prevalent pathogens known to be coisolated from chronic illnesses, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, we observed *P. aeruginosa* can sense *S. aureus* secreted factors from a distance and move towards *S. aureus* colonies by type IV pili (TFP) mediated motility. The ability of *P. aeruginosa* to sense other bacterial species requires the coordination of multiple *P. aeruginosa* regulatory systems, including the Pil-Chp chemosensory system and second messenger signaling by both cAMP and c-di-GMP. These studies lend insight into how *P. aeruginosa* senses and responds to other bacterial species and how *P. aeruginosa* controls the direction of TFP motility on surfaces.

Proteogenomic determinants of biofilm-associated antimicrobial resistance in *Pseudomonas aeruginosa*

Jeremy Webb

University of Southampton, Southampton

Abstract: Here, we validate a model system to identify genes involved in biofilm growth and biofilm-associated antibiotic resistance. We firstly use a genomics-driven workflow to fully assemble and complete the *Pseudomonas aeruginosa* strain MPAO1 genome (the parental strain of the widely utilized *P. aeruginosa* transposon mutant collection). Unique and conserved MPAO1 genes were identified by comparative genomics with the PAO1 reference strain and genes missed within existing assemblies by proteogenomics. Among over 200 unique MPAO1 genes, we identified six general essential genes that were overlooked when mapping public Tn-seq data sets against PAO1. Genomic data were integrated with phenotypic data from an experimental workflow using a user-friendly, soft lithography-based microfluidic flow chamber for biofilm growth and a screen with the Tn-mutant library in microtiter plates. The screen identified hitherto unknown genes involved in biofilm growth and antibiotic resistance and provides new mechanistic insights into biofilm-associated antimicrobial resistance.

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Memory and cooperativity during reversible and irreversible attachment in young biofilms

Calvin Lee

University of California, Los Angeles

Abstract: What are bacteria doing during "reversible attachment," the period of transient surface attachment when they initially engage a surface, besides attaching themselves to the surface? Can an attaching cell help any other cell attach? If so, does it help all cells or employ a more selective strategy to help either nearby cells (spatial neighbors) or its progeny (temporal neighbors)? Using community tracking methods at the single-cell resolution, we suggest answers to these questions based on how reversible attachment progresses during surface sensing for *Pseudomonas aeruginosa* strains PAO1 and PA14. Although PAO1 and PA14 exhibit similar trends of surface cell population increase, they show unanticipated differences when cells are considered at the lineage level and interpreted using the quantitative framework of an exactly solvable stochastic model. Reversible attachment comprises two regimes of behavior, processive and nonprocessive, corresponding to whether cells of the lineage stay on the surface long enough to divide, or not, before detaching. Stark differences between PAO1 and PA14 in the processive regime of reversible attachment suggest the existence of two surface colonization strategies. PAO1 lineages commit quickly to a surface compared to PA14 lineages, with early c-di-GMP-mediated exopolysaccharide (EPS) production that can facilitate the attachment of neighbors. PA14 lineages modulate their motility via cyclic AMP (cAMP) and retain memory of the surface so that their progeny are primed for improved subsequent surface attachment. Based on the findings of previous studies, we propose that the differences between PAO1 and PA14 are potentially rooted in downstream differences between Wsp-based and Pil-Chp-based surface-sensing systems, respectively.

Synovial fluid induced *Staphylococcus aureus* aggregation and biofilm formation in periprosthetic joint infection (PJI)

Paul Stoodley

The Ohio State University, Columbus

Abstract: Bacterial biofilm formation is an important virulence factor in chronic periprosthetic joint infection (PJI) and other foreign body involved surgical site infections. Biofilm formation protects bacteria from antibiotics and host immunity. Most *in vitro* and *in vivo* experiments studying PJI initiate biofilm formation on implant material and other surfaces by inoculating with homogeneously mixed planktonic cultures. However, it seems unlikely that such high concentration of bacteria would enter the surgical site during the primary surgery, and thus the question arises how can cells survive IV antibiotics, antimicrobial irrigants and host-defenses before they develop into mature biofilms? A number of recent studies have shown that *S. aureus* forms aggregates in synovial fluid and these aggregates confer protection against antibiotics - leading to the hypothesis that synovial fluid induced aggregation (SFIA) may provide initial protection prior to attachment and the subsequent development of biofilms. Using time lapse imaging and flow cytometry we characterize the kinetics, the degree and the role of host components of SFIA in *S. aureus* strains and other PJI Enterobacteriaceae. We find that SFIA occurs within seconds and is a common among species and strains, but to highly variable degrees. These findings and the consequences for PJI will be discussed.

The role of TNF in host immunity to *Staphylococcus aureus*

Nathan Archer

Johns Hopkins University, Baltimore

Abstract: *Staphylococcus aureus* is the leading cause of skin and soft tissue infections and has become a major health burden due to the emergence of antibiotic-resistant strains. To develop alternative therapies to antibiotics, we sought to understand the protective immune mechanisms against *S. aureus* skin infections mediated by tumor necrosis factor (TNF). TNF is a proinflammatory cytokine that is rapidly induced upon *S. aureus* exposure and whose inhibition is associated with increased risk of *S. aureus* infections in humans. However, the contribution of TNF or the cognate receptors, TNFR1 and TNFR2, to host defense against *S. aureus* skin infections is unclear. Therefore, to determine the host defense role of TNF, we used an *in vivo* mouse model of *S. aureus* skin infection whereby TNF, TNFR1, or TNFR2 deficient mice and wildtype (wt) mice were intradermally injected with bioluminescent *S. aureus* and monitored for 14 days. TNF, TNFR1, and TNFR2 deficient mice exhibited increased lesion sizes and bacterial burden compared to wt mice. Interestingly, TNFR1 deficient mice had a significant defect in neutrophil recruitment and abscess formation but not NETosis, whereas the TNFR2 deficient mice had normal neutrophil recruitment and abscess formation but a marked defect in NETosis. Furthermore, we discovered that the promotion of TNF expression via pan-caspase inhibition induced significant reductions in bacterial burden and lesion sizes compared to control mice. Taken together, these findings indicated that TNF signaling via TNFR1 and TNFR2 directs differential neutrophil responses for protection against *S. aureus* skin infections, which has implications in the development of novel immune-based therapies as alternatives to antibiotic treatment against *S. aureus* and potentially other bacterial infections.

Biofilm degradation in wound infections

Kendra Rumbaugh

Texas Tech University Health Sciences Center, Lubbock

Abstract: Wound infections are a major source of morbidity and mortality worldwide and exert a tremendous economic burden. These infections are typically comprised of complex, polymicrobial, biofilm-associated communities, which are exceedingly tolerant to antibiotic treatment and often result in amputation. Here I will discuss how the biofilm lifestyle of bacteria in wounds poses challenges to treatment and whether biofilm-degrading enzymes can be used to improve existing therapies.

Interspecies bacterial interactions impact selection for and gene expression in biofilm-optimised variants

Mette Burmolle

University of Copenhagen, Copenhagen

Abstract: Most bacteria in natural environments live in multispecies biofilms, featuring high diversity and chemical heterogeneity. The high cell-to-cell proximity found in these biofilms results in biotic interactions and niche-partitioning, facilitating co-existence of species that may otherwise out-compete each other. Additionally, due to fast generation rates of microbes and ceaseless biotic interactions, biofilms accelerate adaptation through the emergence of more fit genetic variants, most probably in response to niche-partitioning and local constraints.

Here I will present our ongoing work related to the characterization of a biofilm-optimised (wrinkled) variants of X. retroflexus. This variant emerged in biofilm co-cultures with P. amylolyticus and reinforced the original interspecific mutualistic interaction, due to altered c-di-GMP regulation and spatial organisation. When introduced to a four-species community, the variant dominated the more favourable biofilm top layers compared to the ancestral strain, indicating improved competitiveness. Interestingly, reduced abundance of the biofilm-optimised variant was detected in a three-species biofilm compared to a four-species one, which suggests that interspecies interactions impact selection for such variants.

To better understand dynamics between variants, ancestral strains and other community members, we also examined the impact on gene expression profiles (by mRNA sequencing) of either co-cultivation of the ancestor or the wrinkled variant X. retroflexus with P. amylolyticus or its supernatant. Unexpectedly, we found that the most marked difference in gene expression was observed when comparing mono-cultures of the ancestor and the wrinkled X. retroflexus, as approximately 500 genes were differentially expressed in these biofilms. Of these, 30 genes were predicted to encode biofilm-associated functions. When exposed to either live P. amylolyticus or its supernatant, expression profiles of the ancestor and the wrinkled variant were more similar, with the living partner P. amylolyticus being the key factor of this stabilization. Specifically, the stabilisation was caused by opposite regulation of specific genes in the wrinkled variant compared to the WT in mono- vs. co-culture conditions.

Nosocomial *P. aeruginosa* regulates alginate biosynthesis and T6SS during adaptive and convergent evolution for coinfection in critically ill COVID-19 patients

Liang Yang

Southern University of Science and Technology, Shenzhen

Abstract: COVID-19 pandemic has caused millions of death globally and caused huge impact on the health of infected patients. Recent studies have indicated that bacterial coinfection is an unignorable factor contributing to the aggravation of COVID-19 and posing great challenge to clinical treatments. However, there is still a lack of in-depth investigation on the coinfecting bacteria in COVID-19 patients for better treatment of bacterial coinfection. We sequenced and compared the genomes and transcriptomes of P. aeruginosa isolates longitudinally and parallelly for its evolutionary traits. P. aeruginosa overexpressed alginate and attenuated Type VI secretion system (T6SS) during coinfection for excessive biofilm formation and suppressed virulence. Results of bacterial competition assay and macrophage cytotoxicity test indicated that P. aeruginosa reduced its virulence towards both prokaryotic competitors and eukaryotic host through inhibiting its T6SS during evolution. P. aeuginosa T6SS is thus one of the reasons for its advantage to cause coinfection in COVID-19 patients while the attenuation of T6SS could cause a shift in the microecological composition in the lung. Our study will contribute to the development of therapeutic measures and the discovery of novel drug target to eliminate P. aeruginosa coinfection in COVID-19 patient.

Cyclic di-GMP diverges to control antibiotic synthesis in *Lysobacter*

Guoliang Qian
Nanjing Agricultural University, Nanjing

Abstract: *Lysobacter enzymogenes* is an environmental bacterium that secrets a heat-stable antifungal factor, HSAF, an antibiotic against crop fungal pathogens. Elevated levels of c-di-GMP inhibit HSAF synthesis. The transcription factor cAMP receptor-like protein Clp binds to two sites upstream of the promoter of the HSAF biosynthesis operon and activates gene expression. At elevated c-di-GMP levels, c-di-GMP binding to Clp compromises binding to DNA, particularly at the low-affinity binding site, which results in lower expression of the HSAF biosynthesis operon. Two c-di-GMP phosphodiesterases contribute the most to c-di-GMP-dependent regulation of HSAF production. One of them, the GGDEF-EAL protein, LchP, forms a protein complex with Clp. Such specificity of targeted action allows LchP that has relatively weak phosphodiesterase activity, to play an oversized role in Clp-dependent HSAF biosynthesis. The HD-GYP phosphodiesterase RpfG is another major phosphodiesterase, whose activity is increased at higher cell density via a quorum-sensing mechanism. Further, a common regulator of type IV pilus synthesis, PilR, modulates HSAF biosynthesis via an LchD-Clp complex, in which LchD acts as a c-di-GMP synthase and its gene transcript is inhibited by PilR phosphorylation. These findings represent novel insights into c-di-GMP-dependent antibiotic biosynthesis regulation in an agriculturally important bacterium.

To know and not just to believe regarding biofilms and the infectious microenvironment

Thomas Bjarnsholt Professor

Professor at the Costerton biofilm Centre, (SUND-UCPH), the head of Molecular Diagnostic Laboratory at Copenhagen University Hospital, Department of Clinical Microbiology (RH-KMA) and the founder of the "Biofilm Test Facility". His research output includes 215 scientific publications (published or In Press) from 2005 to present in peer reviewed international journals and books. Bjarnsholt is interested as to how bacteria initiate biofilms in the human body and why the immune defense seems to fail both in the initial infection and later in the chronic infection.

Abstract: Biofilms are increasingly associated with many chronic infections across the health field. The main problem with chronic infections is that biofilms are difficult to treat as bacteria in biofilms are tolerant to antimicrobials and the immune system.

In this presentation, I will highlight the challenge that biofilms pose in chronic, both in relation to treatment but also to the immune system. Furthermore, I will discuss the problems and pitfalls regarding diagnosis of these infections. However, what is a biofilm, do we all know what we talk about in vitro vs in vivo? Also, what is the infectious microenvironment of infections, and why do you need to know about this?

A critical role of biomaterial surface chemistry and environmental cues on biofilm formation of *Staphylococcus capitis*

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Abstract: *Staphylococcus capitis* has recently emerged as an important opportunistic pathogen implicated in hospital acquired infections associated with the usage of indwelling medical devices. A distinguishing pathogenesis attribute is its ability to form biofilms on medical implants. Prevention of biofilm formation on medical implants has become a great interest of many material engineers and microbiologists. This can only be achieved through a good understanding of the developmental pathway of microbial biofilms on biomaterial surfaces. How S. capitis forms biofilms on biomedical plastics and its regulation remains poorly understood.

We assessed different environmental conditions that might affect biofilm formation of S. capitis. High salinity was found to be essential for S. capitis to form biofilms in vitro. Transcriptional analysis and real time PCR suggested that high salinity stimulated the expression of icaADBC, genes encoding biofilm matrix polysaccharide intracellular adhesion (PIA) by 10-100 times. We also examined the impact of surface characteristics of biomaterials, by comparing biofilm formation on different commercially available surfaces. Regression analysis revealed that high percentage surface oxygen was required by S. capitis to form biofilm. Biomaterial surfaces with complimented oxygen element further confirmed our findings. Mechanistic studies showed oxygen function facilitated the “anchoring” of biofilm extracellular polymeric substances to the biomaterial surface and subsequent assembly of macrocolonies. This study offers a potential to control S. capitis biofilm infections by engineering biofilm-unfavourable biomaterial surface properties and environmental conditions.

Non-attached biofilm aggregates in chronic infections – and how to model them *in vitro*

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Abstract: It has become evident that the primary phenotype of biofilms in most chronic infections is not attached to any surface. Instead of the several hundred µm thick biofilms often found in many predominate in vitro biofilm model systems, biofilms found in infection, such as chronic wounds or cystic fibrosis-related pneumonia, are small non-attached aggregates. These aggregates are often seen as "floating" in a sea of inflammatory cells and mucus. The microenvironment and structure of these biofilm aggregates may differ significantly from the one found in a microtiter biofilm. This reality challenges us to incorporate the small non-attached biofilm aggregates phenotype into our in vitro biofilm models to further bridge the gap between our in vivo observation and our in vitro biofilm systems.

Sticking it to bacterial resistance using honey-inspired antimicrobial materials

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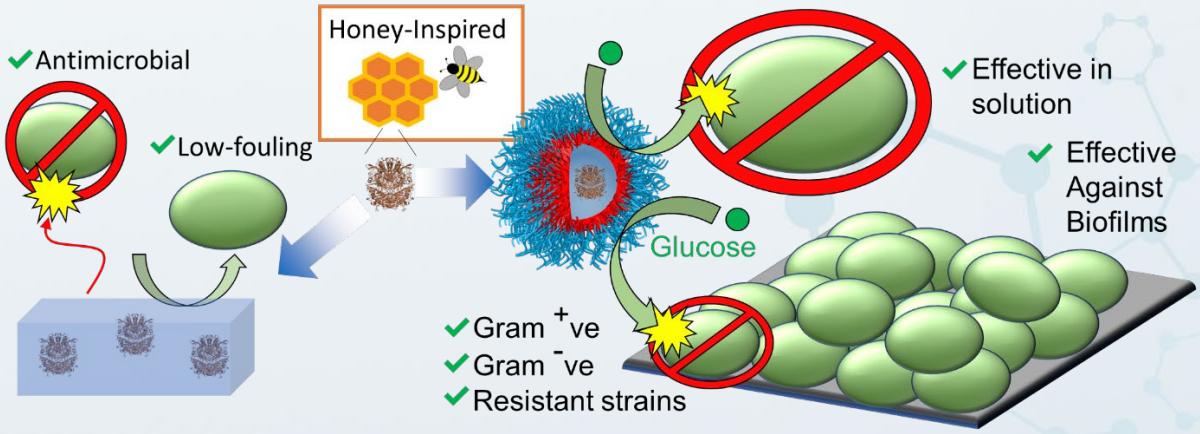
Abstract: The rise of antimicrobial resistance is at the forefront of global healthcare challenges, with antimicrobial infections on track to overtake cancer as a leading cause of death by 2050. Moving away from small molecule antibiotics, research attention has turned towards non-conventional approaches to fight drug-resistant bacteria, such as the use of antimicrobial polymers, carbon nanomaterials and metal nanoparticles. One relatively underexplored avenue is the use of antimicrobial enzymes, which when used in combination with the protective nature of inert polymer materials, represents a highly novel approach towards tackling microbial infections. Inspired by the antimicrobial properties of honey, herein we have developed a range of biohybrid enzyme-loaded materials and demonstrate their ability to "switch on" their antimicrobial activity in response to glucose, a ubiquitous environmental stimulus. Using polymerization-induced self-assembly, antimicrobial nanoreactors were prepared, which facilitated up to a seven-log reduction in bacterial viability at high glucose concentrations against a range of Gram-negative and Gram-positive bacterial pathogens, including drug resistant isolates and biofilms. Enzyme-loaded hydrogel materials were also prepared, which combined antimicrobial and low-fouling properties. It is envisaged that such biohybrid materials will become an important new class of antimicrobial biomaterials for overcoming antimicrobial resistance.



Prevention of oral diseases by anti-biofilm dental materials

Lei Cheng
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Abstract: Oral bacteria are one of the main key factors of oral diseases, like dental caries, periodontitis, Peri-implantitis. Therefore, it is necessary to develop novel anti-biofilm dental materials to prevent the biofilm-related oral diseases. Different dental materials were synthesized according to the specific features of different oral diseases. Herein, we reported tertiary amine (TA)–modified resin adhesives (TA@RAs) with pH-responsive antibacterial effect to reduce the occurrence of secondary caries. Besides, we also developed novel two-staged time-dependent materials for the prevention of implant-related infections. So more smart bioactive dental materials are widely needed to combat biofilm-related oral diseases.

Pleural empyema-related pathogens and biofilms

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Abstract: Pleural empyema is defined as frank pus in the pleural space, requiring immediate drainage. It is a serious illness and associated with high morbidity and mortality. The key factor leading to the lack of treatment progress may be that the pathogenesis that we do not fully understand, such as the causative microbiota, and the role of biofilm formation.

Firstly, we used next-generation sequencing based metagenomic method to detect microbiome in 45 patients with empyema. We found that Staphylococcus aureus is the most abundant species, and all empyema samples were mixed infection. Secondly, we used S. aureus and P. aeruginosa to construct two different rabbit pleural empyema models. We found that both S. aureus and P. aeruginosa can form biofilms on the surfaces of indwelling catheter, and in the fibrinous depositions of purulent exudate. Thirdly, we used PAO1, PAO1△wspF, and PAO1/plac-yhjH to investigate the effect of C-di-GMP on the biofilm formation in this pleural empyema model, and we found that C-di-GMP played an important role in biofilm formation in empyema.

Interactions between bacteria and their dead siblings

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Abstract: Biocides can effffectively kill bacteria; however, whether the dead bacterial cells left on the surface inflfluence the later growth of biofifilm is unknown. Utilizing a digital holographic microscopy (DHM) and a microrheometer based on magnetic force modulated atomic force microscopy (MF-AFM), the interactions between Pseudomonas aeruginosa (PAO1) and their dead siblings were examined. Firstly, we have cultured Pseudomonas aeruginosa (PAO1) biofifilm on their dead siblings and have investigated their evolution by using MF-AFM. The biofifilm growing on dead bacteria layers is softer in comparison with those upon alive siblings. The smaller population instead of the variation of extracellular polymeric substances (EPS) within the biofifilm upon the dead siblings is responsible for the softer biofifilm. On the other hand, the 3D motion behaviors and the underlying adaptation mechanism of planktonic PAO1 in response to the deposited dead siblings nearby were explored. The results showed planktonic cells near the surface covered with dead siblings have a lower density and a reduced 3D velocity compared with those upon viable ones. As a sign of chemosensory responses, bacteria swimming near the dead siblings exhibit increase in frequency of the 'flick' motion. RNA–seq reveals an upregulated expression of dgcM and dgcE inhibited the movement of PAO1, accompanied by increased transcriptional levels of the virulence factor related genes. Moreover, the decrease in L-glutamate and the increase in succinic acid in the metabolites of the dead bacteria layer promote the dispersion of planktonic bacteria. As a result, the dead siblings on a surface can inhibit the bacterial accumulation and colonization, by activating the adaptive defensive responses of PAO1 in the vicinity.

Mixed-species biofilm formation of *L. monocytogenes* in food processing plants and its inactivation by low-energy X-ray irradiation

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Abstract: *Listeria monocytogenes* cells readily attach to food contact surfaces and form biofilms, posing great risk for human health. The present study evaluates cell response of dual-species biofilms formed by *L. monocytogenes* and Pseudomonas fluorescens to food-related stress (desiccation or disinfection), transferability to salmon products as well as the inactivation kinetics by low-energy X-ray. Our results indicated that survival of *L. monocytogenes* under desiccation and disinfection could be enhanced in dual-species biofilms with P. fluorescens. Preformed P. fluorescens biofilms could enhance the survival of L. monocytogenes and reduce its transferability to food products, compared to other colonization sequences. In addition, low-energy X-ray could provide effective inactivation on mono-/dual-species *L. monocytogenes* and P. fluorescens biofilm cells on polyethylene, stainless steel, and acrylic acid surfaces. Co-culture enhanced the resistance of biofilm cells to low-energy X-ray irradiation, which also contributed to EPS removal. Besides, low-energy X-ray induce damages in glucose uptake system and changes in membrane potential and integrity in irradiated P. fluorescens cells. In conclusion, this study highlights the risk of *L. monocytogenes* contamination in pre-formed Pseudomonas biofilms and the efficacy of low-energy X-ray against biofilm cells in food processing facilities.

Strategies for structural characterization of EPS from biofilm

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Abstract: Polysaccharide molecules are constructed by long chains of monosaccharide units bound together via glycosidic linkages. Naturally occurring polysaccharides can be simply classified into four categories according to the source differences: plant polysaccharides, seaweed polysaccharides, animal polysaccharides and microbial polysaccharides. Each category has its own specific structural features.

The molecular structure offers the most fundamental knowledge for understanding the functional, conformational and physiological properties of polysaccharides. However, structural characterization of polysaccharides is a fairly challenging task due to the molecular complexity in terms of monosaccharide composition, glycosidic bonds (linkage patterns), degree of branching/branching position, α- or β-configurations, functional groups, molecular weight and molecular weight distribution.

Acquiring pure and narrow molecular weight dispersed polysaccharides samples are the prerequisites for their structural characterization; The controlled molecular chain degradation such as enzymatical hydrolysis or partial acid hydrolysis is normally required to convert large molecular weight polysaccharides into small fragments such as oligosaccharides; Monosaccharide composition analysis provides the first clue to the molecular structure; Methylation analysis combined with GC-MS uncovers the linkage patterns and molar ratios of the monomers while FT-IR can monitor and quantify the functional groups in the polysaccharide molecules; 1D &2D NMR spectroscopy, reveals structural features including linkage pattern, configuration, sequences as well as conformational properties of various sugar residues.

Novel drug delivery system against biofilms infections

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Abstract: The prevalence of infections with Helicobacter pylori (H. pylori) has progressively increased worldwide, which demonstrated to be closely correlated to its biofilm formation. H. pylori biofilms protect the bacteria by significantly decreasing their sensitivity to antibiotics. Moreover, H. pylori colonizes on the gastrointestinal tract epithelium which is covered by mucus layer, acting as another barrier to prevent antibacterial agents from reaching the colonization sites. Herein, we developed a classical four step-strategy to eradicate H. pylori biofilm including penetrating through mucus layer, destroying their extracellular polymeric substances (EPS) of biofilm, killing the exposed bacteria and inhibiting the re-adherence of H. pylori. In conclusion, the four-step strategy can eradicate H. pylori biofilms in vitro and in vivo, providing a novel strategy for clinical treatment of biofilm-related infections.

Biofilm Standard Methods: Enabling for innovation in the marketplace

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Abstract: Regulatory science is the development and standardization of laboratory tools to enable for informed decision making. These tools take the form of equipment, standard test methods, and guidance on pathways for registering products with regulatory bodies. Most current regulations base efficacy label claims on methods developed for suspended or dried surface bacteria. These methods are not appropriate for the determining the efficacy of biocides against biofilm bacteria and therefore provide a roadblock for getting anti-biofilm products into the marketplace. An exception in the lack of biofilm regulatory pathways is the US EPA guidance for products labeled with a "kills biofilm" claim for use on hard, nonporous surfaces. This presentation will identify new trends and technology aimed towards reshaping biofilm research in the context of regulatory science that will promote the advancement of innovation in the marketplace. An overview of the current biofilm standard test methods and the US EPA guidance for biofilm efficacy claims will be presented. A list of critical parameters to consider when drafting biofilm guidance documents will also be discussed.

Study on the regulation of multi-factor interactions during the *Vibrio parahaemolyticus* biofilm formation

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Abstract: Our work explored the regulation of Vibrio parahaemolyticus biofilm under abiotic and biotic factors. The result shows: The biofilms formation of V. parahaemolyticus on the three abiotic contact surfaces is glass>polystyrene>stainless steel. Based on proteomics, the mechanism of the difference in biofilm formation on different contact surfaces is revealed. In addition, we also found that under the influence of biotic factors, V. parahaemolyticus and Listeria monocytogenes have a competitive interaction when co-cultured with L. monocytogenes, and a synergistic interaction when co-cultured with Shewanella putrefaciens. The reasons for the different interactions are the heterogeneity between different strains, the specific spatial arrangement of biofilms, the differential expression of EPS and biofilm-related genes, and the differences in cell activity and metabolic activity. Our research is of great significance for the prevention and control of the hazards of V. parahaemolyticus biofilms and food safety.

Naftifine derivatives inhibit biofilm formation of multidrug-resistant *Staphylococcus aureus* and potentiate antimicrobials

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Abstract: The pathogenicity of Staphylococcus aureus biofilm coupled with an escalated antimicrobial resistance give prominence to the urgency for novel anti-pathogenic, and anti-biofilm compounds. Naftifine as a novel inhibitor effectively obstructs staphyloxanthin synthesis by hindering the expression of CrtN. In this study, 25 naftifine derivatives were synthesized and characterized, and their anti-biofilm activity against S. aureus were evaluated in vitro in order to identify the potential biofilm inhibitor. It was found that 11 of 25 naftifine derivatives (50 μM) could remarkably inhibit biofilm formation of S. aureus SJTUF21143, and the strongest naftifine derivative was JX08806 with the inhibition rate at 76.30%. A further assay revealed that the most notable inhibitory concentration of JX08806 on biofilm formation was 100 μM. Moreover, JX08806 at this concentration had no obvious impact on the growth of S. aureus. In addition, the combination therapy between JX08806 and antimicrobials on multidrug-resistant S. aureus was performed to evaluate the ability of JX08806 to increase the susceptibility of S. aureus to antimicrobials. The S. aureus strain SJTUF21143 showed resistance to 3 antimicrobials namely penicillin, linezolid, and chloramphenicol. Addition of JX08806 (100 μM), the MIC value of penicillin (2048 to 1024 μg/mL) to S. aureus SJTUF21143 exhibited a 2-fold decrease. However, the MIC value of other 2 antimicrobials had no significant changes. Thus, the present study exemplifies the potential use of naftifine derivatives, especially JX08806, against the biofilm mediated infection of multidrug-resistant S. aureus.

The removal mechanism of *Listeria monocytogenes* biofilm by combined effect of acidic electrolyzed water and alkaline electrolyzed water

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Abstract: The control against biofilm is a research focus in the field of food safety. The efficient and broad-spectrum abilities of electrolyzed water (EW) to kill the pathogens have been recognized, and the synergies with other processing methods to enhance its disinfection effect have also been demonstrated. Our previous studies found that the combination of acid electrolyzed water (AcEW) and alkaline electrolyzed water (AlEW) could result in the enhancement of disinfection effect and eliminate the biofilm effectively on the fruits and vegetables, but its mechanism needed further research. The current study will select Listeria monocytogenes （LM） biofilm as the research object. Combined with the process conditions, the influencing factors of the combination of AcEW and AlEW on the elimination of LM biofilm would be evaluated. Based on the research methods of molecular biology, the study on the elimination mechanisms against LM bilfilm by the combination of AcEW and AlEW would be performed as follows: 1.the removal mechanisms would be clarified from two aspects including elution-sterilization and resistance change-sterilization. 2.the effect of the combination of AcEW and AlEW on the changes of signal molecules in LM biofilm would be evaluated in view of the quorum sensing (QS). As a result, the implementation of this project will provide the theoretical and technical support to solve the problem of microbial pollution in the food processing.

Biofilm: What can we learn from *Bacillus cereus*

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Abstract: Bacillus cereus is a well-known foodborne pathogen around the world and can be found in a variety of foods. It is very difficult to eliminate B. cereus contamination in foods due to its spore-forming ability. Bacillus cereus can lead to gastrointestinal and non-gastrointestinal diseases, even result in death. In this study, over 4300 food samples in China were collected, and the residual B. cereus strains were isolated. The contamination rates of B. cereus were high in different kinds of food, which may be caused by the strong biofilm formation ability of this bacterium. Using phenotypic screening, we identified strains with different biofilm formation ability. Either by multi-omics analysis and genetic manipulation or by random mutagenesis, the mechanism of biofilm formation in B. cereus was further investigated.

Mechanism of natural clays against single biofilm formation of *Staphylococcus aureus* on stainless steel surface

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Abstract: In this study, natural clay (Clay A) samples were assessed for their efficacy against Staphylococcus aureus biofilm on stainless steel surface at different concentrations (12.5%, 25% and 50%) and exposure times (2, 4 and 6 hours). The mechanism on action of the samples was determined by scanning electron microscopy (SEM) and confocal laser electron microscopy (CLSM) visualization. The clay-suspension samples were more effective than the clay-leachate in removing the biofilm cells, with > 4 log reduction when exposed for 6 hours at 12.5, 25 and 50% concentrations. All clay samples were ineffective when exposed for 2 and 4 hours (< 3 log reduction). Scanning electron microscope images showed biofilm degradation, while the live/dead cells were revealed in the confocal laser scanning microscopy images after the exposure to the clay-suspension samples. These findings could provide an insight towards the potential application of natural clays as biofilm removal agent on food contact surfaces.

Keywords: Biofilm; stainless steel surface; natural clay, Staphylococcus aureus

Biofilm formation and control in the dairy industry

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Abstract: Microbiological contamination is the main constraint in the manufacture of dairy products. This contamination originates from biofilms that grow on the internal surfaces of manufacturing plant, releasing bacteria, spores and enzymes into milk resulting in poor product quality. Blockage and loss of efficiency in heat transfer are also attributed to biofilm development. Biofilms can develop many stages of dairy manufacture stainless steel surfaces where raw milk is handled through to the waste treatment plant. The type of biofilm varies depending on the stage of dairy product manufacture. Psycrotrophic bacteria dominate in the raw milk handling zones while thermophilic bacteria dominate in the zones such as plate heat exchanges and evaporators where milk is heat treated. We now have evidence from comparative genomic sequencing that some of these bacteria, such as G. stearothermophilus have become adapted to the dairy environment, acquiring the ability to use lactose as a carbon source while others, including Bacillus licheniformis appear to thrive without this capability. Factors contributing to biofilm development include the need for ions, especially calcium ions. Clean in place systems are used to maintain the hygiene of dairy manufacturing plant but they are often not completely successful in eliminating the biofilm, allowing rapid re-colonisation of the manufacturing plant. Smart engineering solutions to disrupt the conditions that favour microbial growth or reducing the surface area available for biofilm growth, have helped in controlling biofilm development. In products where the ions are removed some thermophilic bacteria will not grow and manufacturing run lengths can be increased. Attempts to develop surfaces that prevent microbial colonisation have had limited success. Research is now focussed on understanding the composition of the biofilm matrix in a dairy system with an aim to improve the cleaning systems used in dairy manufacture.

Molecular mechanism of membrane disintegration and dysfunction in foodborne pathogens with ultrasonic treatment

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Abstract: We aimed at providing new insights on the response of bacterial cell membranes to ultrasound exposure. Escherichia coli (E. coli) O157:H7 cells were exposed to different ultrasound treatments and the dynamic changes in cell viability were assessed over time. Significant increase in outer and inner membrane permeability, along with the membrane depolarization, membrane fluidity reduction, and membrane composition changes were investigated. Moreover, the alterations in the sensitivity of ultrasound-treated cells to antimicrobial compounds were evaluated by exposure to thyme essential oil nanoemulsion (TEON), indicating the effects of ultrasonic field on facilitating the antibacterial efficacy of TEON. In this perspective, the synergistic mechanisms of ultrasound with TEON against bacterial cells were investigated. Further, an integrated transcriptomic and proteomic analyses were conducted and a set of 59 genes or proteins that was differentially expressed in ultrasound-treated cells was uncovered, providing an overview of the cellular responses to ultrasonic field. From the obtained data, it was proposed that the metabolism disorder of cellular membrane lipids (lipopolysaccharide, phospholipid, and fatty acid included) was one of the main challenges for the bacteria upon ultrasonic stress. Collectively, we proposed a novel mechanism regarding the ultrasound-induced membrane disintegration from a multi-omics perspective, which may present an important step toward deciphering the molecular inactivation mechanism of ultrasonic field and provide a theoretical foundation for the application of ultrasound technology for the control of foodborne pathogens.

Cronobacter biofilm formation and control strategies in the food industry

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School of Food and Biological Engineering

Abstract: Cronobacter is a group of foodborne opportunistic pathogens that can cause life-threatening invasive diseases, such as necrotizing enterocolitis, meningitis, and sepsis in infants. The potential risk of Cronobacter contamination of powdered infant formula (PIF) is an issue that has attracted considerable attention from manufacturers, regulators, and consumers. Cronbacter biofilms on the surfaces of equipment and in diverse food-production environments constitute a mode of cell growth that protects the pathogen from hostile environments, and are an important source of persistent contamination of food products. Bacterial biofilms are difficult to remove due to their resistant properties. Conventional cleaning and sterilizing procedures may be insufficient for biofilm control, and may lead to further biofilm development and dispersal. Consequently, novel control strategies are being developed, such as nanotechnology-based delivery systems, interspecies interactions, antimicrobial molecules of microbial origin, natural extracts, and phages. This report focuses on describing the mechanisms underlying the biofilm formation and behavior of Cronobacter and discussing potential control strategies.

Influence of quorum sensing on formation of viable but nonculturable (VBNC) cells of beer-spoilage lactic acid bacteria growing in biofilms

Yang Deng

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Abstract: Lactic acid bacteria (LAB) are the most common beer-spoilage bacteria, regardless of beer type, and therefore pose significant problems for the brewing industry. Biofilm and planktonic cells of many beer-spoilage LAB can enter into a viable but nonculturable (VBNC) state when they were incubated at 4°C in commercial Chinese lager beer. VBNC cells can resuscitate when suitable condition arise, yet the molecular mechanisms facilitating resuscitation in most bacteria are not well understood. We discovered that bacterial cell-free supernatants (CFS) can awaken pre-existing VBNC cells, while CFS from a quorum sensing mutant was unable to produce the same resuscitative effect. Furthermore, the quorum sensing autoinducer, AI-2, could induce resuscitation of VBNC LAB cells, and VBNC cells of a mutant unable to produce AI-2 were unable to resuscitate unless the cultures were supplemented with exogenous AI-2. The quorum sensing inhibitor cinnamaldehyde delayed the resuscitation of wild-type VBNC cells, confirming the importance of quorum sensing in resuscitation. By monitoring AI-2 production by VBNC cultures over time, we found quorum sensing signaling to be critical for natural resuscitation process. This study provides new insights into the molecular mechanisms stimulating VBNC exit from dormancy, which has significant implications on microbial control in beer industry.

Discovery of anti-biofilm terpenoid compounds: (+)-nootkatone as an example

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Abstract: Natural products are widely used as anti-biofilm agents. Our study screened the anti-biofilm effect of 40 terpenoid compounds, and found that (+)-nootkatone, a sesquiterpene ketone mainly existing in the essential oils of grapefruits, exhibited good anti-biofilm efficacy against multidrug-resistant S. aureus. Crystal violet staining found that (+)-nootkatone inhibited S. aureus biofilm formation at a sub-MIC (50 μg/mL), but it did not affect the bacterial growth of planktonic cells. In addition, it reduced bacterial exopolysaccharide production, and significantly reduced the thickness of biofilms, supported by light microscopy and confocal laser scanning microscopy. Besides, it was found to influence the sliding motility of S. aureus. On the other hand, (+)-nootkatone at 200 μg/mL could reduce the preformed biofilm mass by 50% and bacterial cell death of 79%, accompanied with a reduction of exopolysaccharide. The anti-biofilm effects of (+)-nootkatone should be associated with the suppression of biofilm-related genes, including sarA, icaA, agrA, RNAIII, and spa. Finally, the cell toxicity experiment suggested that there was no evident toxicity of (+)-nootkatone to human normal cells. Therefore, (+)-nootkatone is a promising natural product against S. aureus biofilms, and has the potential to be used in the food industry to fight against S. aureus-induced safety issues.

Research on the role of quorum sensing in biofilm of Leuconostoc citreum and its application in dairy fermentation

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Abstract: Leuconostoc citreum, a type of food-grade probiotic bacteria, plays an important role in the food fermentation of milk, vegetable, meat, and wine. Recently, there has been growing research interest in the biofilm formation and quorum sensing (QS) system of probiotics. Biofilms help bacteria survive under adverse conditions. L. citreum 37 was a biofilm-forming strain isolated from dairy products in Inner Mongolia. The aim of this study was to clarify that luxS/AI-2 involved in the regulation of L. citrem 37 biofilm formation, to analyze the integrity of the luxS/AI-2 system based on whole-genome sequencing and indicated the key genes through homologous overexpression. Genome assembly yielded two contigs (one chromosome and one plasmid), and the complete genome contained 1,946,279 bps. The genome sequence analysis showed that there were several pathways such as the two-component system, QS, and seven other signal pathways, and 26 genes (including luxS, pfs, and 24 other genes) may participate in QS related to biofilm formation, showing that the LuxS/AI-2 system is complete in the genome of L. citreum 37. The qPCR of pfs, luxS genes, AI-2 production of L. citreum 37 in planktonic state and biofilm state, and expression of pfs, luxS genes in E. coli BL21 showed that the expression of luxS genes was consistent with the production of AI-2 and was positively correlated with biofilm formation. Therefore, luxS may regulate the biofilm formation of L. citreum 37 by participating in AI-2 synthesis. Then, luxS homologous overexpression strain was constructed. Although the growth of overexpression strains lags behind that of wild-type strains, AI-2 synthesis and biofilm are significantly higher than those of wild-type strains ($P<0.05$). therefore, LuxS/AI-2-dependent QS plays an important role in the regulation of biofilm-forming activities in L. citrem 37. Finally, L. citrem 37 was fixed on the nanofiber membrane to be a starter for milk fermentation. The survival rate of immobilized luxS-overexpressed L. citrem 37 in simulated gastrointestinal fluid was higher than that of wild-type strains. Moreover, the immobilized L. citrem 37 and luxS-overexpressed L. citrem 37 can ferment skim milk for 5 times, showing a good application prospect. The stress-tolerant biofilm-forming L. citreum 37 can be a food-grade microbial cell factory.

Biofilms in intermittent and continuous flow drinking water distribution systems

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Abstract: The presence of biofilms in drinking water distribution systems (DWDS) can affect both water quality and system integrity; yet these systems remain poorly studied due to a lack of accessibility of buried pipes. To learn about long-term effects of biofilms growing in systems under continuous operation (CWS) we have been operating two independent full-scale DWDS testbeds (A and B) equipped with online sensors that supply water to campuses situated in Singapore since 2018. In addition to such continuous operation, an estimated 309 million people worldwide are supplied annually with water via piped distribution networks that are operated intermittently. To study intermittent (IWS) alongside continuous (CWS) operation and their effects on water quality we constructed an additional DWDS testbed (C) situated above ground and amenable to experimental manipulation. Both low and high flow rates were used to investigate the formation of biofilms on pipe walls and their subsequent detachment and entry into the bulk water. We followed microbial community dynamics using the Phenoflow approach, which generates phenotypic fingerprints based on flow cytometry data that characterize microbial communities associated with routine operational conditions. Periods of intermittent and extended stagnation showed increased microbial loads derived from detached biofilms when compared to continuous flow conditions. We simulated daily flushing conditions associated with intermittent water supply (biofilm growth in partially filled pipes), compared with continuous supply (pressured water pipes). Finally, we observed effects of extended stagnation (filled pipes, no flow) after a nine-week lockdown. In each scenario, we collected biofilms on pipe-wall coupons and water samples. We used flow cytometry measurements to interpret microbial loads (at inlet and outlet conditions) and an array of physicochemical tests to monitor water quality. Flushing the system caused turbulent flow conditions inside the pipe and resulted in a transient release of bacteria (compared to inlet conditions) over a period of 60 seconds, characterized by a much higher proportion of live cells (60% vs 15% at the inlet), before reverting to previous conditions. Similar transient effects were observed for total dissolved solids and conductivity. These trends were not found in the continuous flow system (at similar flow rates). The results show that significant biofilm growth can occur between water supply periods in IWS systems (i.e., no flow in partially filled pipes), and will cause increased biomass production when the water supply is reconnected (initial flushing/re-filling). Similar transient conditions can occur in pressured water pipes after extended stagnation periods of low/no flow. While extended pipe flushing can restore residual disinfectant levels in CWS systems, IWS consumers often collect the first available water and risk becoming exposed to harmful pathogens.

Synergistic metabolism and community structure in algal-bacterial counter-diffusion biofilms for treating biogas slurry

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Keywords: algal-bacterial consortia; membrane-aerated biofilm; counter-diffusion;synergistic nitrogen metabolism; microbial community structure

Summary: Nutrient removal and microbial community structure in an algal-bacterial membrane-aerated biofilm reactor wereinvestigated for treating piggery biogas slurry. The NH4+-N removal load was 2.22 ±0.15 gN/(m2·d) at 54.1 ±3.2 mg/L of influent NH4+-N. NH4+-N removal unstabilized and effluent water quality significantly fluctuated at 113.4 ±5.0 mg/L of influent NH4+-N. NH4+-N removal efficiency and removal load recovered to 65.7% and 1.72 ±0.31 gN/(m2·d), respectively, after the influent NH4+-N concentration switched back to 60.5±7.3 mg/L. COD was negatively correlated with Nitrosomonasand Nitrobacterand positively correlated with Nitrospira, and Nitrosomonasand Nitrobacterwere negatively correlated with effluent nitrite and nitrate. Effluent nitrite, nitrate, chemical oxygen demand, and extracellular polymeric substances significantly affect microbial community structure of the algal-bacterial membrane-aerated biofilm, andmicroalgae inoculation improves the survival and activity of nitrite-oxidizing bacteriain membrane-aerated biofilms.

Electroanalysis of *Candida albicans* biofilms: a suitable real-time tool for antifungal testing

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Abstract: *Candida albicans* is a fungal pathogen that accounts for more than a million death annually. Analysis of *Candida* biofilms and rapid assessment of antifungal therapy is a critical challenge in clinical practice. Current biochemical methods are time-consuming and expensive or require expert interpretation of the results. Hence, there is the need for a cheap and rapid approach for antifungal drugs testing. While electroanalysis has been previously adopted to determine antibiotic susceptibility in bacterial biofilms, there are no previous studies on yeast and fungi. In this work, electroanalysis of *C. albicans* biofilm and evaluation of anti-biofilm activity of clinically relevant antifungal compounds using commercial screen-printed carbon electrodes (SPEs) is reported. Results are compared with standard biochemical and microscopic methods. The electrochemical output decreases by 47.5%, 73.4%, and 88.5% in biofilms treated with the antifungals fluconazole (Flz), amphotericin B (AmB), and complex Ag3, respectively. This study further reiterates the stance on the rapid development of resistance of *C. albicans* biofilm to existing drugs. Overall, chronoamperometry allows measuring *Candida* biofilm respiration rate as early as 10 h after inoculation, which show promises for the development of bioelectrochemical sensors for antimicrobial testing in clinical settings.

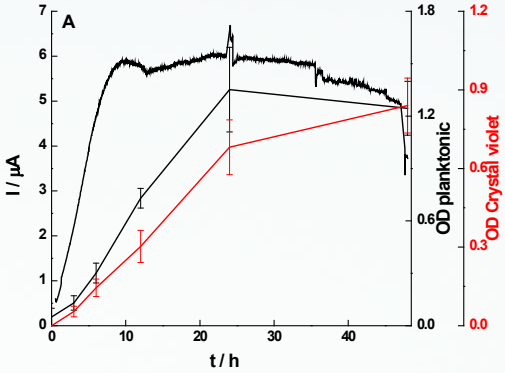

Figure 1: Planktonic and biofilm growth of *C. albicans* biofilm at different times, compared to the current output over 48 h. Planktonic cell count and biofilm crystal violet assays were performed in four

Keywords: Electroanalysis, *Candida albicans*, Fluconazole, Amphotericin B, Complex Ag3, Biofilm

The Biofilm way of thinking in bioremediation

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Abstract: The biofilm way of thinking was used as a basis for planning, design and implementation of remedies at a contaminated landfill in Maryland, USA. More than fifty years ago, mixed contaminants were deposited at an unlined landfill resulting in TCE (trichloroethylene) contaminating a nearby creek and surrounding groundwater sources. To prevent TCE from reaching the creek, a biowall was installed downstream from the landfill to remediate groundwater containing up to 600 ppb TCE. Breakthrough of TCE degradation products subsequently required a trench to be installed upgradient from the biowall to enhance microbial activity by increasing groundwater residence time, pH, and available organic carbon. The design of both biowall and the trench was performed while considering how biofilm formation and contaminant degradation activity can be promoted thus preventing the transport of TCE to the creek. Several bioremediation and bioaugmentation approaches involving biofilms such as biosolids addition and bioaugmentation with biofilm covered activated carbon particles will be discussed.

Industrial characterization system for biofilms on materials

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Abstract: Biofilms form on materials' surfaces and affect materials characteristic and performances in various ways, even though it might be positive or negative. The effect covers diversified areas in daily lives, industrial fields, medical fronts etc. The capability of biofilm formation and growth on materials can be understood as materials' infectious capability and surface characteristics of materials. It means how bacteria could survive in biofilms formed on materials. The information would lead to the new developments of anti-corrosive materials in marine and other humid environments, anti-infectious biomaterials and medical equipment/facilities used in hospitals, food industrial materials, various liquid pipes, air conditioners, wash machines, walls, floors, kitchen and washrooms, etc. However, the suitable evaluation methods for the materials' characteristics are still lacking. In Japan, SIAA, a big non-commercial organization composed of several hundred companies, is going to establish the evaluation standard for materials' anti-biofilm characteristics. In this presentation, I will mention some conventional research tools to visualize and evaluate biofilms qualitatively and also the SIAA proposal. And I will compare all of them and discuss on the efficiency, merits and demerits of new standards, looking for the future movement and its direction.

Motility selection contributes to collective antibiotic tolerance in bacterial swarms

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Abstract: Bacterial populations such as biofilms and swarming bacterial colonies often survive antibiotic treatment at concentrations lethal to individual cells. This phenomenon is known as non-heritable collective antibiotic tolerance. Understanding the mechanisms of collective antibiotic tolerance may help design novel antibacterial treatment strategies that could alleviate the effect of multidrug tolerance and prevent the selection of antibiotic resistant mutants. In our recent work we found that, when bacterial swarms encounter inhibitory concentrations of aminoglycoside antibiotics, the colony advancing edge is enriched with cells having transient and non-heritable drug tolerance. These less-susceptible cells sustain colony expansion into territories with lethal drug concentrations, thereby conferring antibiotic tolerance to the entire swarm. We used quantitative fluorescence imaging and single cell tracking to reveal that the enrichment of these drug-tolerant cells is not due to their growth advantage but results from a unique motion pattern of swarm cells, namely dynamic motility selection. As the dynamic motility selection is an inherent property of bacterial swarms, it may serve as a general means of adaptive stress-response for bacterial swarms to cope with environmental stresses that cause collateral effect on flagellar motility.

A variant *ECE1* allele contributes to reduced pathogenicity of *Candida albicans* during vulvovaginal candidiasis

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Abstract: Vulvovaginal candidiasis (VVC), caused primarily by the human fungal pathogen *Candida albicans*, results in significant quality-of-life issues for women worldwide. Candidalysin, a 31 amino acid peptide toxin derived from a 271 amino acid polypeptide (Ece1p) encoded by the *ECE1* gene, plays a crucial role in driving immunopathology at vaginal mucosa, including neutrophil recruitment, pro-inflammatory cytokine production, and tissue damage. The objective of this study was to determine whether expression and/or processing of Ece1p differs across *C. albicans* isolates and whether this partly underlies differential pathogenicity observed clinically. Using whole genome and targeted sequencing approaches, we determined that a number of clinical isolates, including strain 529L, harbor a similarly expressed, yet distinct Ece1p isoform variant that encodes for a predicted functional candidalysin. Transformation of the entire *ECE1* open reading frame (ORF) from 529L into an SC5314-derived *ece1*Δ/Δ strain results in significantly reduced vaginopathogenicity as compared to an isogenic control transformed with that derived from the reference isolate SC5314. However, *in vitro* challenge of vaginal epithelial cells with synthetic candidalysin demonstrated similar toxigenic activity amongst SC5314 and 529L isoforms. Creation of an isogenic panel of chimeric strains harboring swapped Ece1p peptides or HiBiT tags revealed a secretion defect encoded by the 529L ORF that was associated with reduced virulence. A genetic survey of over 100 clinical isolates showed a conserved linkage between Ece1p P2 and P3 sequences, suggesting that substrate specificity around Kex2p-mediated KR cleavage sites involved in protein secretion may contribute to differential pathogenicity amongst clinical isolates. Therefore, we present a novel mechanism for attenuation of *C. albicans* virulence at the *ECE1* locus.

New antibiofilm strategy against fungal/bacterial polymicrobial biofilms

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Abstract: In the past, biofilm-related research has focused mainly on axenic biofilms. However, in nature, biofilms are often composed of multiple species, and the resulting polymicrobial interactions influence industrially and clinically relevant outcomes such as performance and drug resistance. Especially, polymicrobial biofilm related infections have been a major threat in health care because of the increased resistance to antimicrobials and the critical biological differences between fungi and bacteria. In this report, we show that drug tolerance of bacteria is significantly increased in a fungal/bacterial polymicrobial biofilms compared to its tolerance in an axenic bacterial biofilm. And then, drug loaded chitosan nanoparticles were prepared and exhibited excellent antibiofilm activity against planktonic bacteria or fungi, mono- and polymicrobial biofilm formations and preformed biofilms. Finally, we report the co-immobilization of cellobiose dehydrogenase and deoxyribonuclease I on positively charged nanoparticles resulted in a bi-functional nanoparticle (CSNP-DNase-CDH) targeting both biofilm matrix and microorganisms. The CSNP-DNase-CDH could disrupt the biofilm formation through degradation of eDNA, reduce biofilm thickness, and kill microbial cells.

pH and light-responsive polycaprolactone/curcumin@ZIF-8 composite films with enhanced antibacterial activity

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Abstract: Food packaging materials, especially biodegradable polymer composites incorporated with natural antimicrobial agents with excellent antibacterial activities, are highly demanded and attracted immense attention. Herein, a polycaprolactone/curcumin@zeolitic imidazolate framework-8 (PCL/Cur@ZIF-8) composite film with enhanced antibacterial activity was developed. Curcumin, a natural photosensitizer, was loaded in the highly porous nanocrystals ZIF-8 to improve its poor water solubility and stability. The integral structure of Cur@ZIF-8 was maintained well in the PCL matrix even at the highest loading of 35% (w/w), and all composite films had good light transmittance at 420-430 nm. The PCL/Cur@ZIF-8 composite films responded to the acidic growth environment of bacteria by releasing zinc ions and curcumin molecules. Furthermore, upon blue light irradiation (420-430 nm, 2.2 mW/cm2), curcumin molecules generated singlet oxygen. With the synergistic effects of zinc ions and singlet oxygen, the composite films exhibited a 99.9% reduction of Escherichia coli and Staphylococcus aureus strains when the amount of Cur@ZIF-8 loading was more than 5% (w/w), as well as a strong anti-adhesion effect on bacteria. Moreover, Bacterial resuscitation tests indicated that the composite films exhibited 100% reduction in the adhered bacteria population by treating with photodynamic sterilization. This is the first study presenting that the incorporated-curcumin ZIF-8 nanoparticles in the matrix of polymer are pH and light responsive for anti-adhesion of bacteria, which is of great potential application as anti-bacterial packaging material for food industry.

Keywords: photodynamic therapy; polycaprolactone; bioactive compound; controlled release; antimicrobial.

Biofilm vs microbial inoculation in biofertilization

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Abstract: More than a century ago, scientists identified that inoculation of effective microbes, rhizobia in particular improved plant growth and yield. Based on the subsequent research, they developed biofertilizers for legumes and then for non-legumes. The theory behind this practice is that the inoculated microbes colonize the root system and provide nutrients and plant growth promoting substances via a range of mechanisms like biological nitrogen fixation, nutrient solubilization etc. However in the recent past, it has been proven that developed, beneficial microbial communities in biofilm mode can exert a better effect than the microbial inoculation on plant growth and yield, and also soil condition. Therefore, biofilms have now been formulated as biofertilizers which are known as Biofilm biofertilizers (BFBFs). Once applied to the soil with crop plants, the BFBFs release a wider range of biologically important biochemicals which are generally depleted under conventional agricultural practices. The most important process that takes place with the biochemicals is the breaking of dormancy of microbial seed bank that is developed to bypass the stresses of the agricultural practices like tillage, chemical inputs etc. This increases biodiversity and abundance of soil microbes and their communities. This is well-supported by root exudates of the growing crop plants, leading to a symbiotic interaction which is beneficial to both the microbes and the plant. Also that improves soil organic matter, and hence soil health, which has implications in crop production and the environment. In this manner, the BFBFs application increases functioning and sustainability of agroecosystems. The BFBFs are now being researched in several countries with promising results. In Sri Lanka, they are being used in thousands of hectares of paddy cultivation by cutting down farmers' chemical fertilizer use up to 50%, while increasing crop yields by ca. 20% on average.

Agriculturally beneficial biofilms as inoculants for sustainable and integrated nutrient and disease management: from lab to land

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Abstract: Microbial inoculants represent promising supplements to inorganic fertilizers and plant protection chemicals in agriculture, across crops and ecosystems, as they play a significant role in maintaining long-term soil productivity, ecological sustainability, and safer produce. In the last few decades, inoculants preparations have progressed from single culture, to uni-to multifunctional consortia, as a means of providing nutrients, plant growth enhancers, disease control and other useful attributes for the overall health of the plant. Naturally occurring biofilms represent microbial communities embedded in a matrix of polymer and attached to biotic or abiotic surfaces, which proliferate in a diverse range of environments; however, their utility as inoculants is less studied. Our investigations were aimed towards optimization of *in vitro* development of biofilms using two agriculturally important organisms- fungal mycelium of *Trichoderma viride* and a filamentous cyanobacterium (*Anabaena torulosa*) as matrices, with partners as beneficial bacteria/fungi endowed with biocontrol or nutrient mobilizing potential. Optimization of the medium, inoculation rate, sequence of addition and population counts of partners, PGP traits, including antifungal activity, Indole Acetic Acid (IAA) production were evaluated for efficient biofilm formation. Light, confocal and electron microscopic observations aided in analyzing the progress of biofilm development. Evaluation of biofilm formulations as inoculants in diverse crops-cereals, vegetables, legumes and flowers, under field and protected cultivation environments, revealed significant enhancement in growth, yields, and nutrient availability in soil, besides reduced disease severity. Such biofilms exhibited synergism in terms of the PGP traits and the capacity to maintain the metabolic activity, upto harvest stage and exhibit increased survival in the rhizosphere. Transcriptome analyses provided important clues regarding the regulation of genes involved in biofilm formation, while molecular tools illustrated the beneficial modulation of the rhizosphere microbiome. Our investigations reveal the promise of these biofilms as novel organic options for agriculture.

The application of biofilm biofertilizer to increase crop yield and soil fertility status

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Abstract: Biofilm biofertilizer was a biotechnology as an effort to increase the effectivity and consistency of biofertilizer in supporting plants growth so increase their yields. Although it has been developed for several decades in the world, but biofilm biofertilizer is relatively new and not develope yet in Indonesia. This presentation is written based on some reasearches to evaluate if the liquid biofilm biofertilizer inoculant formula (BiO2) we have developed is effective in increasing growth and yield of some plant crop as well as soil fertility status. Research experimentals were conducted seperately both in time (2015 – 2020) and location (in Surakarta region), with plant indicators of rice paddy on Entisols, soybean on Alfisols, shallot on Alfisols and Vertisols, egg plant on Vertisols, and mustard, kale and spinach on Lithosols. Liquid biofilm biofertilizer (BiO2) was sprayed to the palnt and soil with various doses of application. The application of this biofilm biofertilizer inoculant increase plant growth and yield with various enhancement but it ability to increase available soil nutrients was lower than chemical fertilizer. It may need to develope more formula with high capasity to increase both plant growth and yield as well as soil nutrients availability in various soil condition.

Keywords : biofilm, biofertilizer, plant growth, plant yield, soil nutrients

Enhanced recovery of biophotosensitizer from microalgal biofilm by photosyn thetic electrons extraction towards photolytic removal of antibiotic in wastewater

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Abstract: A novel approach by extraction of photosynthetic electrons from microalgae was used to enhance production of microalgae-originated photosensitizer for photolytic removal of antibiotic residues in effluents from conventional bio-treated wastewaters. Results showed that the accumulation of photoactive substances in extracellular polymeric substance (EPS) of *chlorella vulgaris* was positively related to the amounts of photosynthetic electrons extraction which is a potential-dependent process. The protein and humic acid which are considered two main photoactive substances in EPS produced at 0.6V accumulated to a high level of 320 and 24 μg/cm^3 which were 4.7 and 6.4-folds higher than that produced at potential free. The EPS produced at 0.6 and 0.8V led to 1.34 and 1.53-fold acceleration in photosensitized degradation of TC compared to that of EPS free. The complex heterocyclic ring structure of TC was broken down into simple monocyclic aromatic compounds, indicating a marked reduction in biotoxicity and recalcitrance. This technology provides a new alternative to conventional physicochemical treatment as complementary treatment processes for biological wastewater treatment in terms of antibiotics removal.

Keywords: Microalgae; biophotosensitizer; photosynthetic electron extraction; antibiotic; photosensitive degradation

Application of the kombucha biofilm for the development of functional beverages

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Abstract: Owing to increased awareness on the maintenance of health and wellness, the functional beverage sector is one of the fastest growing segments in the beverage industry. These drinks are popular because it is easy for a person to drink a beverage which contains purported health benefits, than consume an equivalent food product. Also, consumption of beverages is a habit which is applicable to many age groups and demographics than food products. As a result of these consumer behaviours, Kombucha tea has gained immense popularity with demonstrated health benefits such as anti-cancer, antioxidant, detoxification, anti-microbial and Angiotensin-Converting Enzyme (ACE) inhibitory activities. These effects have even been observed at clinical trials (Malbaˇsa, et al., 2011). This beverage has a history of several thousands of years in the East, while it soon became popular in the West as well owing to the palatability of flavour and taste (Mohammadshirazi and Kalhor, 2016).

The Kombucha beverage is produced by the fermentation of tea and sugar by a symbiotic association of bacteria and yeasts – primarily acetic acid bacteria and osmophilic yeast. These microbes collectively form a 'tea fungus', which is a name given because of the mushroom-like biofilm appearing in the fermented broth. The yeasts convert sugar added to tea into organic acids and ethanol. The enzymes, bacterial acids, and other secondary metabolites produced by the microbes impart the functional properties to the beverage. There are other kinds of bioactive metabolites produced as a result of the fermentation, especially through the biotransformation of polyphenols (Mohammadshirazi and Kalhor, 2016). Typically, black or green tea can be used as the medium of fermentation and the period of incubation may vary from a few days up to 2 weeks, albeit several unwanted metabolites might be produced in the broth if the fermentation is carried out for more than 2 weeks (Amarasinghe et al., 2018). The type of sweetening agent used for the preparation of the tea can be varied, and when subjected to the fermentation process, the metabolites formed from the breakdown of the sweetener can impart various functional properties (Watawana et al, 2016).

The Kombucha broth can be bottled for commercialization once the fermentation process is complete. The beverage can be easily prepared in households as well, given that the ingredients required for the production are simply tea, sugar and water. Once the biofilm is formed, it can be extracted / removed and applied into other types of liquid media as well. It has been used to ferment coffee (Watawana et al, 2015) and coconut water

(*Cocos nucifera* var. *aurantiaca*) [Watwana et al, 2016] resulting in two novel functional beverages of different flavor and taste. Recent studies have applied the biofilm to ferment fruit extracts as well and the substrates which have been used in the Kombucha biofilm-based fermentation process gets innovative every day.

Overall, the Kombucha beverage has demonstrated versatility and flexibility to be applied in various conditions and fermentation media, which has resulted in the development of novel beverages of varied disease preventive effects. Many of these can be seen in the current product market and consumers in several countries and regions have identified Kombucha as a go-to functional beverage for obtaining the necessary bioactives for health and wellness purposes.

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Fouling microorganisms in the reservoirs of the groundwater treatment system

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Abstract: The work discusses the features of the structure of fouling (biofilms) formed in the water treatment system of the iron-bearing groundwater of the Tungusskii deposit (Far East, Russia). The main factors affecting the composition of biofilms are trace amounts of organic substances, oxygen, different phylogenetic composition of microbial communities and their ability to form a polysaccharide matrix. The formation of structured communities in biofouling of the water treatment system can have a negative impact on the organoleptic characteristics of drinking water due to the formation of complex trophic connections.

2.Student Presentation

Application of transposon inserted mutant sequencing analysis in identifying genetic determinants of bacterial biofilms

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Abstract: As the ubiquitous mode of bacterial growth in nature,biofilm niches bacteria to survive in hostile environments; thereby, biofilm-associated infections are the leading cause of refractory chronic infections. It is considerably significant to investigate the molecular mechanism of biofilm formation, maintenance and virulence for a better insight in biofilm control strategies. Although several key factors such as extracellular polymeric substance synthesis, quorum sensing, and c-di-GMP signaling have been well documented, there is still a huge gap between the biofilm genotype and phenotype. In this work, we present a protocol for high-throughput screening of biofilm genetic determinants via transposon insertion sequencing (Tn-seq) approach in tubing biofilm cultivation systems. *Pseudomonas aeruginosa* transposon random insertion library were generated as a model input library. The genetic determinants of biofilm formation can be rapidly screened out by comparative Tn-seq analysis of input and output samples of *P.aeruginosa* Tn-library tubing biofilms. By employing transposon insertion sequencing, we discovered extensive potential biofilm determinators with unknown functions in PAO1 besides the well-known biofilm regulators. Overall, the integrated methods provide a novel perspective to study the correspondence between biofilm genotype and phenotype via one batch of experiment with strong reproducibility.

Low concentrate of antibiotics enhance biofilm formation in *Staphylococcus aureus*

Ziqi Liu

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Abstract: Background: In food system, foodborne microbes exist as complex polymicrobial community instead of single species. Their interaction will change the gene expression, metabolic mode and level of the microorganisms in the food system, and pressure response. Thus, understanding microbial behavior and polymicrobial interaction are of importance. **Materials/methods:**Saccharomyces cerevisiae were co-cultured with Lactobacillus plantarum in different proportions. The phenotypic changes of growth and morphology during the co-cultivation of L. plantarum and S. cerevisiae were detected by CFU method using selective medium and electron microscope. Both individually cultured and co-cultured samples are subjected to transcriptome sequencing using RNA-seq to obtain transcription profiles, perform gene expression differential analysis and functional enrichment analysis. **Results:**The interaction model between S. cerevisiae and L. plantarum with different growth patterns is similar. During co-cultivation, L. plantarum inhibits S. cerevisiae growth possibly through cell-cell aggregation and the inhibition rate is about 80%. L. plantarum inhibits S. cerevisiae with its own growth unaffected. L. plantarum activates lamBDCA QS system to enhance adhesion and adhere to yeast. Signal molecules are secreted to stimulate yeast activating stress response genes. However, the down regulating of basic DNA-RNA-protein process, cell cycle, meiosis, and mating MAPK signaling pathways lead to the lowered growth rate. The down-regulation of starvation adjustment, high-osmolality survival, cell wall remodeling pathways and cell integrity genes WSC1/2/3 lead to the death of some cells. The lowered growth rate and cell death cause the inhibited growth of S. cerevisiae. **Conclusions:**L. plantarum inhibits S. cerevisiae growth possibly through cell-cell aggregation and the inhibition rate is about 80%. L. plantarum might activate QS system to enhance adhesive ability. The down-regulation of yeast MAPK signaling pathway might result in the reduced growth rate.

Antibacterial self-assembled nanodrugs composed of berberine derivatives and rhamnolipids against *Helicobacter pylori* biofilms

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Abstract: Despite decades of efforts including the conventional triple therapy, Helicobacter pylori(H. pylori) infections remain drastically high recrudescence or reinfection. The prevalence of infections with H. pyloristay high worldwide, which was demonstrated to be closely correlated to its biofilm formation.H. pylori biofilms protect the internal bacteria by significantly decreasing their sensitivity to antibiotics. Moreover,H. pylori colonizes on the gastrointestinal tract epithelium which is covered by mucus layer, acting as another barrier to prevent antibacterial agents from reaching the colonization sites.Some attempted to disturb biofilms integrity and improve antibiotic diffusion by near-infrared photodynamic or photothermal therapy, the majority of which were restricted to superficial biofilm infections, thus were unacceptable in H. pylorirelated non-superficial infections.Herein,four kinds of lipophili calkyl berberine derivatives (BDs)were combined with rhamnolipids (RHL)to fabricate self-assembled nanodrugs (BD/RHLNDs)in one step to overcome the dual obstructions of both mucus layer and biofilms. Molecular dynamics simulations estimated that the driving forces for self-assembly of BD/RHLNDs were electrostatic and hydrophobic interactions. BD/RHLNDs, characterized by appropriate size(<200nm), negative charge and enhanced hydrophilicity, successfully penetrated through mucus layer without interacting with mucins. Subsequently, nanodrugs anchored in the H. pylori colonization sites and disrupted biofilms structure by destroying EPS components, followed by the eradication of the H.pylori.Moreover,BD/RHLNDs inhibited residual H. pylorire-adhesion, which was of great significance for preventing persistent infections caused by the re-formation of biofilms. In an H. pylori-infected mice model, C10-BD/RHLNDs group showed 40 folds less remnant H. pyloriand greater mucosal protection compared with the conventional clinical triple therapy. In conclusion, BD/RHLNDs could penetrate through mucus layer and effectively eradicate H. pylori biofilms in vitro and in vivo, providing a novel strategy for clinical treatment of biofilm-related infections.

Mucus penetration enhanced lipid polymer nanoparticles improve the eradication rate of Helicobacter pylori biofilm

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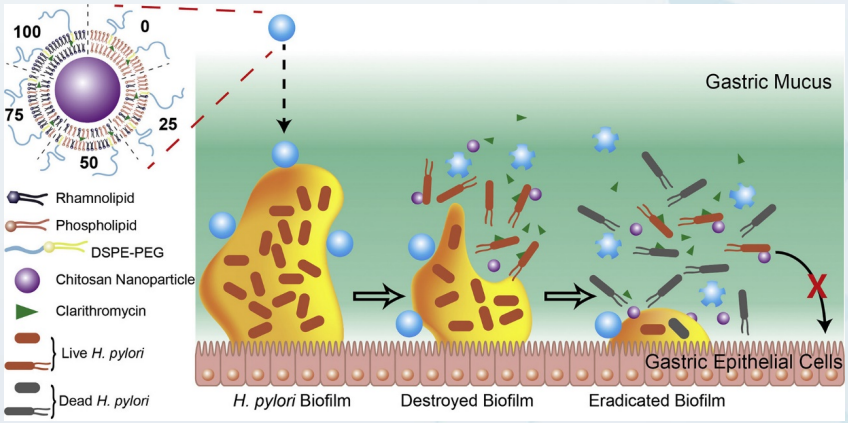
Abstract: The resistance of *Helicobacter pylori* (*H. pylori*) to conventional antibiotic treatments becomes prevalent and was found to be highly correlated with biofilm formation. Moreover, *H. pylori* colonizes on the digestive tract epithelium located under the mucus layers, which further reduces therapeutic efficacy as mucus layers trap and remove exogenous substances including drugs. Herein, we reported a novel lipid polymer nanoparticles (LPNs) to overcome both biofilm and mucus layers obstruction. LPNs employed chitosan nanoparticle (CS NPs) as the core, mixed lipid layer containing rhamnolipids (RHL) as the shell and the surface of LPNs was further modified with DSPE-PEG2000 to improve hydrophilicity. Clarithromycin (CLR), a first-line drug for *H. pylori* infection, was encapsulated in LPNs. LPNs, especially the formulation utilizing 100% of RHL as the lipid shell, exhibited excellent eradicating ability to *H. pylori* biofilm, which was mainly reflected in the significant reduction of biofilm biomass and viability, destruction of biofilm architecture and elimination of extracellular polymeric substances (EPS). The anti-biofilm activities of LPNs are related to: 1) the disrupting effect of RHL on biofilm matrix; 2) antibacterial effects of CLR and CS NPs on biofilm bacteria and 3) inhibitory effects of CS NPs and RHL on bacteria adhesion and biofilm formation. Furthermore, PEGylated LPNs could rapidly penetrate through mucus without interacting with mucins and effectively eradicate *H. pylori* biofilm under mucus layer. In conclusion, a novel approach of drug-containing LPNs that could penetrate through mucus layers and effectively eradicate *H. pylori* biofilm provides new ways to treat persistent *H. pylori* infections.



Schematic illustration of the structure of LPNs and the process of eradicating bacterial biofilm by LPNs. The numbers around LPNs represent the percentage of RHL contents.

Polymicrobial interaction between *Lactobacillus plantarum* and *Saccharomyces cerevisiae*

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Abstract: Background: In food system, foodborne microbes exist as complex polymicrobial community instead of single species. Their interaction will change the gene expression, metabolic mode and level of the microorganisms in the food system, and pressure response. Thus, understanding microbial behavior and polymicrobial interaction are of importance. **Materials/methods:**Saccharomyces cerevisiae were co-cultured with Lactobacillus plantarum in different proportions. The phenotypic changes of growth and morphology during the co-cultivation of L. plantarum and S. cerevisiae were detected by CFU method using selective medium and electron microscope. Both individually cultured and co-cultured samples are subjected to transcriptome sequencing using RNA-seq to obtain transcription profiles, perform gene expression differential analysis and functional enrichment analysis. **Results:**The interaction model between S. cerevisiae and L. plantarum with different growth patterns is similar. During co-cultivation, L. plantarum inhibits S. cerevisiae growth possibly through cell-cell aggregation and the inhibition rate is about 80%. L. plantarum inhibits S. cerevisiae with its own growth unaffected. L. plantarum activates lamBDCA QS system to enhance adhesion and adhere to yeast. Signal molecules are secreted to stimulate yeast activating stress response genes. However, the down regulating of basic DNA-RNA-protein process, cell cycle, meiosis, and mating MAPK signaling pathways lead to the lowered growth rate. The down-regulation of starvation adjustment, high-osmolality survival, cell wall remodeling pathways and cell integrity genes WSC1/2/3 lead to the death of some cells. The lowered growth rate and cell death cause the inhibited growth of S. cerevisiae. **Conclusions:**L. plantarum inhibits S. cerevisiae growth possibly through cell-cell aggregation and the inhibition rate is about 80%. L. plantarum might activate QS system to enhance adhesive ability. The down-regulation of yeast MAPK signaling pathway might result in the reduced growth rate.

C. *albicans* augments *S. aureus* quorum sensing during polymicrobial infections

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Abstract:*Candida albicans*, an opportunistic fungal pathogen, and *Staphylococcus aureus*, a ubiquitous pathogenic bacterium, are among the most prevalent causes of nosocomial infections and cause severe morbidity and mortality. Moreover, they are frequently coisolated from central venous catheters and deep-seated infections, including intra-abdominal sepsis. Relatively little is known about the complex interactions and signaling events that occur between microbes and even less so how microbial "cross-talk" shapes human health and disease.

Using a murine model of polymicrobial intra-abdominal infection (IAI), we have previously shown that coinfection with *C. albicans* and *S. aureus* leads to synergistic lethality whereas monomicrobial infection is nonlethal. Therefore, we aimed to identify staphylococcal virulence determinants that drive lethal synergism in polymicrobial IAI. Using the toxigenic *S. aureus* strain JE2, we observed that co-infection with *C. albicans* led to a striking 80-100% mortality rate within 20 h p.i while monomicrobial infections were non-lethal. Use of a GFP-P3 promoter *S. aureus* reporter strain revealed enhanced activation of the staphylococcal *agr* quorum sensing system during in vitro polymicrobial versus monomicrobial growth. *C. albicans*-mediated elevated toxin production and hemolytic activity was determined to be *agr*A-dependent and genetic knockout and complementation of *hla* identified α-toxin as the key staphylococcal virulence factor driving lethal synergism. we correlated α-toxin production with significant increases in biomarkers of liver and kidney damage during coinfection and determined that functional toxin was required for morbidity and mortality.

We next sought to determine the candidal effector(s) mediating this enhanced virulence by employing an unbiased screening approach. *C. albicans* transcription factor mutants were evaluated for their ability to induce *S. aureus agr* activation in polymicrobial culture. Incredibly, we identified several mutants that displayed defects in augmenting *S. aureus agr* activity in vitro. Two of the mutants failed to completely synergize with *S. aureus* in vivo and further analysis revealed the necessity of the uncharacterized *C. albicans* transcription factor, *ZCF13*, in driving enhanced toxin production both in vitro and in vivo. Collectively, we identified a novel effector by which *C. albicans* augments *S. aureus* virulence and identified a potential mechanism of fungal-bacterial lethal synergism.

Monitoring the 3D morphology of growing biofilms

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Abstract: The formation of biofilms facilitate the adherence of single cell and provide defence to harmful factors from outside environments. Herein, we set up an off-axis digital holographic microscopy (DHM) in reflection mode to monitor the 3D morphology of growing biofilms. Using off-axis DHM, the holograms of surface adhered bacteria are captured during colonization which can be reconstructed to obtain the phase differences among image pixels. The phase differences reflect the refractive index and morphology difference between the biofilms and the surrounding medium. As a result, the growth of *Lactobacillus rhamnosus* GG in MRS broth were monitored. After 8 h of cultivation, the biofilms grown on the substrate occupied about 25.4 % of view area and closely distributed. The thickest part of the biofilms was 2 μm, which equals to the thickness of two layers of bacterial cells. DHM was proved to be highly sensitive to the tiny variation in the biofilm morphology without any destruction to the sample in real time.

Molecular epidemiology characteristics of 146 CRE infections in Guangzhou, China

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Objectives: Infection by carbapenem-resistant Enterobacteriaceae (CRE) is a global public health problem. We aimed to investigate the molecular epidemiological characteristics of CRE in china and provide the evidence for the clinical rational use of antibiotics.

Methods: In this study, we performed whole-genome sequencing to investigate the genetic basis of the CRE-mediated nosocomial infections in China. Analysis of MLST, antibiotics resistance genes, plasmid replicons, virulence genes, and genetic environment was also performed.

Results: Klebsiella pneumoniae (89, 61.48%) was the most common CRE species, primarily prevalent in the ICU(36, 39.56). A total of 146 CRE strains showed high resistance rate to multiple antibiotics, especially cephalosporins and carbapenems. However, most of these isolates were susceptible to tigecycline (81.7%) and colistin (75.0%). The predominant sequence type (ST) of carbapenem-resistant K. pneumoniae (CRKP) isolates was ST11 (79.12%, 72 / 89). ST410 (21.4%, 6/28) was predominant in Escherichia coli isolates, and half of them carried blaNDM-5. In addition, 3 cases of ST88 Enterobacter cloacae isolates carried blaNDM-1 gene were also found. We identified Co-carring of NDM-5, KPC-2 in CRECO isolates with ST167. In addition, NDM-1 and KPC-2 co-carried by CREL with ST 594 were also found. Notably, the proportions of KPC-ST11 K. pneumoniae in CRKP isolates was 93.05%. In addition, we found three novel ST, which are ST5386 - 5388.

Conclusions: Overall, we have described CRE isolates' molecular epidemiological characterization over five years in a hospital from southern China. KPC-ST11 CRKP remains a significant threat in China. Strengthen further the precaution and monitoring of this kind of strains is necessary.

Keywords: carbapenem-resistant; Enterobacteriaceae; Molecular epidemiology; ST11

Molecular epidemiology and antibiotics resistance analysis of non-typing *H. influenzae* after the introduction of the Hib vaccine in Guangzhou, China

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Abstract: Aim: To investigate the molecular epidemiology and antibiotic resistance of *H. influenzae* after the introduction of the Hib vaccine in Guangzhou, China. **Method:** A total of 93 *Haemophilus influenzae* were collected from the first affiliated hospital of Guangzhou Medical University from June 2019 to June 2020. Species identification, susceptibility, molecular capsular typing, multilocus sequence typing and the clinical characteristics analysis of patients were performed. **Results:**The *Haemophilus influenzae* enrolled in our study were all confirmed as non-typable *Haemophilus influenzae* with a high ampicillin resistance rate (more than 70%). Most of the *Haemophilus influenzae* were isolated from children and elder patients with respiratory symptom. A total of thirty-six different NTHi sequence types (STs) were identified in our study, ST12 was the most popular sequence type in our study. **Conclusion:** Non-typable *Haemophilus influenzae* with diverse sequence types seems has become the prevalent capsular type in Guangzhou, China. It should rise our concern in preventing the transmission of non-typable *Haemophilus influenzae* with molecular diversity.

Keywords: Non-typing *Haemophilus influenzae*, molecular epidemiology, antibiotics resistance

SPR on biofilms: with biofilm associated genes screening in *S. aureus* as an example

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Abstract: The biofilm is a natural protection state formed during the growth of bacteria, and more than 90% of the microorganisms grow in the form of biofilm. The traditional culturing can only identify the species, not the biofilm formation. Thus, researchers detect biofilms at the genetic level. Polymerase Spiral Reaction (PSR) is a novel isothermal amplification method to detect biofilm at genetic level with easy operation, low cost and high throughput. This DNA amplification method also can combine with SPR biosensor. The PSR-SPR technology is able to convert the refractive index signal into reflective intensity for detecting the reaction. For now, we have detected the biofilm formation related genes of S.aureus by isothermal amplification methods with Nucleic acid dye. We conducted PSR assays with total 524 strains to recognize related genes for biofilm formation of S.aureus. 96.0% strains were positive to be carrying atl gene, which existed in high conserved sequence of S. aureus genetics. 36.0% strains were positive in carrying sasG gene, contributing to the regulation of biofilm formation. 80.0% strains were positive in agr gene, regulating the detachment and sloughing of biofilm. As for ica operon, 73% strains were positive in carrying icaA, icaD and icaBC.

As we can see, the isothermal amplification methods with color change are helpful to recognize the biofilm related genes achieving high-throughput rapid detection for biofilm related genes. And next, we will detect this biofilm related genes on SPR biosensor and explore its detection limit.

Control and impact of glycogen utilization and synthesis in *C. albicans* mediated vulvovaginal candidiasis

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Abstract: Vulvovaginal candidiasis (VVC) is a common disease of the lower reproductive tract of immunocompetent and otherwise healthy women and is most frequently caused by the opportunistic fungal pathogen Candida albicans (C. albicans). It's been well demonstrated that high level of reproductive hormones stimulates thick vaginal epithelium that increases glycogen availability, an excellent carbon source, for multiple vaginal organisms to thrive in this specific asymptomatic infection. However, it's still unknown whether *C. albicans* is able to utilize vaginal glycogen to promote its colonization and infection during VVC. Given above consideration, our results indicates that the ability of extracellular glycogen utilization is limited in *C. albicans* as well as other six Candida species that commonly mediate VVC. To verify potential intracellular glycogen metabolism genes listed on Candida Genome Database, use of a conventional auxotrophic strain validated that *gph1*, *gsy1* and *glc3* are fundamental genes on *C. albicans* glycogen metabolism pathway, which encode glycogen phosphorylase, synthase and branching enzyme accordingly. The increase and decrease survival rate of *glc3Δ/Δ* and *gsy1Δ/Δ* were confirmed with GFPy and dTomato tagged strains in a nutrient limited environment *in vitro*. Moreover, treatment of THP-1 monocytic cells with glycogen synthesis defect strain *gsy1Δ/Δ* induced significantly higher proinflammatory cytokine IL-1β response from both yeast and germ-tube cells in a dose-dependent manner. Our findings demonstrate that glycogen facilitates *C. albicans* long-term survival *in vitro*, and defect in intracellular glycogen synthesis promotes host immune response potentially due to cell wall exposure/unmasking and/or structural chang

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