Programme & Abstracts

BIOFILMS 8

27 - 29 May 2018

Aarhus University · Aarhus · Denmark

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BIOFILMS 8

Nordre Fasanvej 113, 2nd floor
2000 Frederiksberg C
Denmark

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SAVE THE DATE

FEMS 2019

7-11 July 2019

8th Congress of European Microbiologists
Glasgow, Scotland | www.fems2019.org

WELCOME

Dear participant,

It is a great pleasure to welcome you to the Biofilms 8 conference in Aarhus, Denmark.

During the 2,5 conference days, you will experience a diverse programme that includes high level scientific presentations and networking activities - an excellent opportunity to exchange knowledge and experiences within biofilms.

Biofilms 8 is the 8th conference in a series that cover the topic of bacterial biofilms in the broadest sense. The conference focus is on the basic scientific question of how biofilms form, grow and interact with their surroundings. You will meet researchers from the natural sciences, engineering, and health science to exchange their research on how biofilms develop, how they interact with their surroundings, and how they can be controlled in a natural, industrial, or clinical setting.

The main subjects of the conference are:

- Molecular mechanisms in biofilm formation
- The biofilm matrix
- Bacterial attachment
- Modelling biofilms
- Biofilm ecology
- Evolution in biofilms
- Biofilm control
- Novel methods for biofilm characterization

We hope you will enjoy the conference and your stay in Aarhus!

Kind regards from the Local Organising Committee,

Rikke Louise Meyer
Associate professor, Interdisciplinary Nanoscience Center and Department of Bioscience, Aarhus University (Conference Chair)

GENERAL INFORMATION

CONFERENCE VENUE

Aarhus University
Bygning 1412 (Building 1412)
Nordre Ringgade 4
8000 Århus, Denmark

CONFERENCE LANGUAGE

The conference will be held in English.

NAME BADGES

All participants and exhibitors must wear the name badge in the conference area at all times. The badge must be visible.

LUNCH AND COFFEE BREAKS

Lunch is available in the poster area. Coffee is available in the exhibition area. See programme for exact time of breaks.
Exhibition area: Vandrehallen
Poster area: Stakladen & Richard Mortensen room

SPEAKER INFORMATION

Please bring your presentation to the session room before your session starts. We recommend you upload your presentation at least 30 min before your session. A technician will be present to assist in the upload if necessary. Please bring your presentation on a USB.

Unless otherwise agreed all presentations will be deleted after the conference in order to secure that no copyright issues will arise at the end of the conference.

WIFI

Free WiFi is provided throughout the venue by logging on the network "AU Guest". Open an internet browser and log on through one of the accounts.

MOBILE PHONES

All mobile phones must be on silent mode during the sessions.

CLOAK ROOM

A manned cloak room located in the basement under the auditorium "Aula" will be available throughout the conference.

LOST AND FOUND

Found items should be returned to the registration desk. If you lose something, please report to this desk for assistance.

CONFERENCE SECRETARIAT

CAP Partner
Nordre Fasanvej 113
2000 Frederiksberg C, Denmark
Tel: +45 70 20 03 05

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SOCIAL MEDIA

Find Biofilms 8 on Facebook (Search for "Biofilms Conference Series") and Twitter (@Biofilms8)
Use #Biofilms8

CONFERENCE WEBSITE

www.conferences.au.dk/biofilms8

SOCIAL EVENTS

Welcome Reception (included in the registration fee)

Date:	27 May 2018
Time:	18.30 - 20.30
Place:	Poster area, Stakladen, Aarhus University

The welcome reception will take place in the poster area in Stakladen at Aarhus University from 18.30 - 20.30.

Conference Dinner (included in the registration fee)

Date:	28 May 2018
Time:	19.00 - 00.00
Place:	Turbinehallen, Kalkværksvej 12, 8000 Aarhus C

The conference dinner will take place from 19.00 - 00.00 in Turbinehallen. The Turbinehallen is a rustic and vibrant venue full of atmosphere and character, centrally located in Aarhus in the urban harbour area in the heart of the Aarhus Film Town.

Join us at the dinner and catch up with colleagues and friends, and make new acquaintances! Please note that the dinner is included in the registration fee, but registration is required.

HOW TO GET TO THE CONFERENCE DINNER VENUE:

To reach the dinner venue from the University, you can take bus 100, 200, 16 or 18 from the busstop "Aarhus Universitet. Randersvej/ Nordre Ringgade" at the intersection of Randersvej and Nordre Ringgade and get off at the Aarhus Central station. The dinner venue is located a 5-10 minutes walk from the central station.

ORGANISATION

Rikke Louise Meyer
Interdisciplinary Nanoscience Center and Department of Bioscience, Aarhus University (Chair)

Thomas Emil Andersen
University of Southern Denmark, Denmark

Mette Burmølle
Copenhagen University, Denmark

Matthew Fields
Center for Biofilm Engineering, Montana State University, USA

Ákos Kovács
Professor, Technical University of Denmark, Denmark

Per Halkjær Nielsen
Aalborg University, Denmark

Daniel Otzen
Aarhus University, Denmark

Trine Rolighed Thomsen
Aalborg University, Denmark

POSTER SESSIONS

The poster sessions are held during lunch breaks. Please be present at your poster during these times. See the exact time of your poster session here below:

	Categories	Presentation time	Poster number
Sunday 27 May	The biofilm matrix Molecular mechanisms in biofilm formation Bacterial attachment	12.00 - 13.00	Uneven numbers
		13.00 - 14.00	Even numbers
Monday 28 May	Biofilm ecology Modelling biofilms Evolution in biofilms Other	11.30 - 12.30	Uneven numbers
		12.30 - 13.30	Even numbers
Tuesday 29 May	Biofilm control Novel methods for biofilm characterization	10.50 - 11.40	Uneven numbers
		11.40 - 12.30	Even numbers

PRIZES AND AWARDS

Thanks to our 3 sponsors below, a number of prizes will be awarded during the closing ceremony on Tuesday 29 May 2018. The prizes will consist of 8 poster prizes and 1 Young Investigator Award. We deeply thank our sponsors for the support:

JOURNAL OF MEDICAL MICROBIOLOGY

Journal of Medical Microbiology provides comprehensive coverage of medical, dental and veterinary microbiology and infectious diseases, including bacteriology, virology, mycology and parasitology.

Articles are published in the following areas:
Pathogenesis, Virulence & Host Response; Clinical Microbiology; Microbial and Molecular Epidemiology; Microbiome and Microbial Ecology in Health; One Health - Emerging, Zoonotic & Environmental Diseases; Prevention, Therapy and Therapeutics; Antimicrobial Resistance; and Disease, Diagnosis and Diagnostics.

JOURNAL OF MEDICAL MICROBIOLOGY
The full breadth of clinical microbiology

NPJ BIOFILMS AND MICROBIOMES

The journal hosts cross-disciplinary discussions and allows for our understanding of mechanisms governing the social behaviour of microbial biofilm populations and communities, and their impact on life, human health, and the environment, both natural and engineered.

npj | Biofilms and Microbiomes

MICROORGANISMS JOURNAL

Microorganisms (ISSN 2076-2607) is an international, peer-reviewed open access journal which provides an advanced forum for studies related to prokaryotic and eukaryotic microorganisms, viruses and prions. Articles published in Microorganisms are indexed in PubMed (NLM).

microorganisms
an open access journal by MDPI

PROGRAMME SUNDAY 27 MAY

Time	Abs.	Title	Speaker	Area	Sponsored by
07.30		**Registration desk opens**			
09.00 - 09.15		**Welcome & Opening Ceremony** By Biofilms 8 Chair, Rikke Louise Meyer		**Auditorium Aula**	
9.15 - 10.00	O1	**Bird's Eye Lecture: The biofilm matrix: strategies for protection and exploitation**	**Nicola Stanley-Wall,** UK	**Auditorium Aula**	MICROBIOLOGY SOCIETY
10.00 - 10.40		**Coffee/Tea Break**		**Exhibition area**	
10.40 - 12.00		**Session 1: The biofilm matrix** **Chair: Per Halkjær Nielsen & co-chair: Daniel Otzen**		**Auditorium Aula**	THORLABS
10.40 - 11.00	O2	Glycosylated amyloid-like proteins in the structural extracellular polymers of aerobic granular sludge enriched with ammonium oxidizing bacteria	Yuemei Lin, The Netherlands		
11.00 - 11.20	O3	Formation of functional non-amyloidogenic fibres by recombinant Bacillus subtilis TasA	Elliot Erskine, UK		
11.20 - 11.40	O4	Insight into the RapA lectin and its use in the study of biofilm matrix formation by rhizobia	Patricia Abdian, Argentina		
11.40 - 12.00	O5	Secreted, Large-Scale, Extracellular Membrane Systems in Microbial Biofilms	Matthew Fields, USA		
12.00 - 14.00		**Lunch & Poster session**		**Poster & Exhibition area**	
14.00 - 14.30	O6	**Invited Lecture:** **Molecular interactions of staphylococcal biofilm forming proteins**	**Joan Geoghegan,** Ireland	**Auditorium Aula**	
14.30 - 15.50		**Session 2: Molecular mechanisms in biofilm formation** **Chair: Daniel Otzen & co-chair: Rikke Meyer**			Leica
14.30 - 14.50	O7	Cytochrome Bd-I is used for energy production in uropathogenic E. coli biofilms	Maria Hadjifrangiskou, USA		
14.50 - 15.10	O8	Heat activates cyclic diguanylate production in bacteria	Joe Harrison, Canada		
15.10 - 15.30	O9	Sortase-assembled pili of Enterococcus faecalis contribute to iron-mediated extracellular electron transfer and iron-augmented biofilm	Kimberly Kline, Singapore		
15.30 - 15.50	O10	Physical determinants of amyloid assembly in biofilm formation	Maria Andreasen, Denmark		
15.50 - 16.20		**Coffee/Tea Break**		**Exhibition area**	
16.20 - 16.50	O11	**Invited Lecture: How do bacteria respond to their adhering state?**	**Henny van der Mei,** The Netherlands	**Auditorium Aula**	
16.50 - 18.10		**Session 3: Bacterial attachment** **Chair: Rikke Meyer & co-chair: Thomas Andersen**			JPK Instruments
16.50 - 17.10	O12	Cell lysis prompts an early mechanical coupling and biofilm formation in dilute bacterial suspensions	Iztok Dogsa, Slovenia		
17.10 - 17.30	O13	Bone environment and its relationships with bacterial biofilm	Fany Reffuveille, France		
17.30 - 17.50	O14	The role of dynamic Tad pili in bacterial surface sensing	Yves Brun, USA		
17.50 - 18.10	O15	A role for two-component systems in bacterial attachment and antibiotic tolerance in Listeria moncytogenes	Hüsnü Aslan, Denmark		
18.10 - 18.30	O16	**Invited Lecture:** **Are biofilms really the dominant way of life for prokaryotes on Earth?**	**Hans-Curt Flemming,** Germany	**Auditorium Aula**	
18.30 - 20.30		**Welcome Reception in the poster area** **(included in registration fee)**		**Stakladen**	PERFECTUS BIOMED

PROGRAMME MONDAY 28 MAY

Time	Abs.	Title	Speaker	Area	Sponsored by
08.00		**Registration desk opens**			
09.00 - 09.40	O17	**Bird's Eye Lecture: Cooperation and competition in biofilms**	**Kevin Foster,** UK	**Auditorium Aula**	MICROBIOLOGY SOCIETY
9.40 - 10.10		**Coffee/Tea Break**		**Exhibition area**	
10.10 - 11.30		**Session 4: Biofilm ecology** **Chair: Mette Burmølle & co-chair: Ákos Kovács**		**Auditorium Aula**	UNISENSE
10.10 - 10.30	O18	Biofilm architecture confers individual and collective protection against phage infection	Lucia Vidakovic, Germany		
10.30 - 10.50	O19	Effect of fluctuating environmental conditions on the spatial self-organization and emergent properties of a synthetic microbial biofilm	Davide Ciccarese, Switzerland		
10.50 - 11.10	O20	AHL quorum sensing mediates species interactions in multispecies biofilms	Sujatha Subramoni, Singapore		
11.10 - 11.30	O21	Biofilm thickness controls the contribution of stochastic and deterministic processes in microbial community assembly	Jane Fowler, Denmark		
11.30 - 13.30		**Lunch & Poster session**		**Poster & Exhibition area**	
13.30 - 14.00	O22	**Invited Lecture:** **Multiscale analysis of microbial cross-feeding in biofilms: from Yellowstone hotsprings to chronic wounds**	**Ross Carlson,** USA		MICROBIOLOGY SOCIETY
14.00 - 15.20		**Session 5: Modelling biofilms** **Chair: Matthew Fields & co-chair: Rikke Meyer**		**Auditorium Aula**	ZEISS
14.00 - 14.20	O23	Developing a novel understanding of E. coli K-12 pellicle formation, morphology, and physiology	Stacey Golub, UK		
14.20 - 14.40	O24	Increasing the Space-Time Yield in Lactic Acid Production by the Use of Biofilms	Laure Cuny, Germany		
14.40 - 15.00	O25	Estimation of mechanical and hydraulic biofilm properties from optical coherence tomography measurements	Morez Jafari, The Netherlands		
15.00 - 15.20	O26	Optically patterned biofilms for synthetic microbial consortia	Xiaofan Jin, USA		
15.20 - 15.50		**Coffee/Tea Break**		**Exhibition area**	
15.50 - 16.20	O27	**Invited Lecture:** **Why evolution in biofilms is different, and a few remarkable consequences**	**Vaughn Cooper,** USA		
16.20 - 17.40		**Session 6: Evolution of biofilms** **Chair: Ákos Kovács & co-chair: Mette Burmølle**		**Auditorium Aula**	BioNordika
16.20 - 16.40	O28	Long-term co-adaptation of Pseudomonas aeruginosa biofilms with amoeba affects virulence traits	Diane McDougald, Australia		
16.40 - 17.00	O29	Evolution in changing environments: Specialist and generalist strategies during non-stable selection of the biofilm phenotype	Jonas Stenløkke Madsen, Denmark		
17.00 - 17.20	O30	Cheating promotes evolution of hyper-cooperators by shifting phenotypic heterogeneity in biofilms	Marivic Martin, Germany		
17.20 - 17.40	O31	Increased rate of mutation to antimicrobial resistance in polymicrobial biofilms	Jeremy Webb, UK		
19.00 - 00.00		**Congress Dinner at Turbinehallen, Aarhus** **(included in the registration fee, registration required)**		**Turbinehallen** Kalkværskvej 12, 8000 Aarhus	

PROGRAMME TUESDAY29 MAY

Time	Abs.	Title	Speaker	Area	Sponsored by
08.30 - 09.00	O32	Invited Lecture: **Tuning biofilms architecture to control their functions**	**Romain Briandet,** France	**Auditorium Aula**	
09.00 - 09.30		**Coffee/Tea Break**		**Exhibition area**	
09.30 - 10.50		**Session 7: Biofilm control** **Chair: Thomas Andersen & co-chair: Trine Thomsen**		**Auditorium Aula**	AH diagnostics
09.30 - 09.50	O33	Characterization of anti-curli antibody based approaches to eradicate Salmonella Typhimurium biofilms	Sarah Tursi, USA		
09.50 - 10.10	O34	A New Strategy for Biofilm Control Using Bioinspired Dynamic Surface Topography	Dacheng Ren, USA		
10.10 - 10.30	O35	Biofilm control in cooling towers: the effect of biodispersants on freshwater biofilms developed in flow lanes	Luciana Di Gregorio, Italy		
10.30 - 10.50	O36	Substrate Mediated Enzyme Prodrug Therapy (SMEPT) to combat implant-associated biofilms	Signe Maria Nielsen, Denmark		
10.50 - 12.30		**Lunch & Poster session**		**Poster & Exhibition area**	
12.30 - 13.00	O37	Invited Lecture: **Interrogating the interplay of metabolism and structure in bacterial communities**	**Lars Dietrich,** USA		
13.00 - 14.00		**Session 8: Novel methods for biofilm characterization** **Chair: Trine Thomsen & co-chair: Per Halkjær Nielsen**		**Auditorium Aula**	FLUXION
13.00 - 13.20	O38	Novel uses for Synchrotron Radiation in the study of Biofilms	Ben Libberton, Sweden		
13.20 - 13.40	O39	Introducing a novel, fully-automated cultivation and screening tool for the structural and mechanical investigation of biofilms by means of optical coherence tomography	Luisa Gierl, Germany		
13.40 - 14.00	O40	Nanoparticle-based chemical imaging in biofilms and tissues	Michael Kühl, Denmark		
14.00 - 14.30		**Awards Ceremony, Introducing Biofilms 9 & Closing Session**		**Auditorium Aula**	

EXHIBITORS AT BIOFILMS8

Exhibition will be in ”Vandrehallen” located near the entrance to the venue and next to the auditorium “Aula”.

- AH Diagnostics
- BioNordika
- Fluxion
- JPK Instruments
- Leica
- Perfectus Biomed
- Thorlabs
- Unisense
- ZEISS

ORAL ABSTRACTS

BIOFILMS 8

ORAL ABSTRACTS

[O1] Bird's Eye Lecture: THE BIOFILM MATRIX: STRATEGIES FOR PROTECTION AND EXPLOITATION

Nicola Stanley Wall[1]

[1]*University of Dundee, Scotland, United Kingdom*

Biofilms are defined as self-organised multicellular aggregates in which the resident cells are embedded in a self-produced extracellular polymeric matrix. Production of the biofilm matrix fulfils a variety of functions from scaffolding and nutrient acquisition to protection and signalling, thereby allowing microorganisms to survive in diverse, and often hostile habitats. Composition of the biofilm matrix varies with species but typically comprises extracellular proteins, polysaccharides, lipids and extracellular DNA (eDNA). Much attention has been paid to how production of the molecules in the matrix is controlled, with more recent work expanding our understanding of the biochemical and biophysical properties of matrix components. Using the Gram-positive bacterium *Bacillus subtilis* we will take a closer look at the biofilm matrix, examining how the molecules function and how, in the future, we might be able to capitalise on the intricate systems that have evolved to structure biofilms.

[O2] GLYCOSYLATED AMYLOID-LIKE PROTEINS IN THE STRUCTURAL EXTRACELLULAR POLYMERS OF AEROBIC GRANULAR SLUDGE ENRICHED WITH AMMONIUM OXIDIZING BACTERIA

Yuemei Lin[1], Clara Reino[2], Julián Carrera[2], Julio Perez[2], Mark van Loosdrecht[1]

[1] *Department of Biotechnology, Delft University of Technology, Netherlands*
[2] *Universitat Autònoma de Barcelona, Spain*

A new type of structural extracellular polymers (EPS) was extracted from aerobic granular sludge dominated by ammonium oxidizing bacteria. It was analyzed by Raman and FTIR spectroscopy to characterize specific amino acids and protein secondary structure, and by SDS-PAGE with different stains to identify different glycoconjugates. Its intrinsic fluorescence was captured to visualize the location of the extracted EPS in the nitrifying granules, and its hydrogel-forming property was studied by rheometry. The extracted EPS is abundant with cross ß-sheet secondary structure, which is one of the typical properties of amyloids; the proteins/polypeptides are glycosylated. In addition, the EPS forms hydrogel with high mechanical strength. The extraction and discovery of glycosylated amyloid-like proteins further shows that conventionally used extraction and characterisation techniques are not adequate for the study of structural extracellular polymers in biofilms and/or granular sludge. Confirming amyloids secondary structure in such a complex sample is challengeable due to the possibility of amyloids glycosylation and self-assembly. A new definition of extracellular polymers components which includes glycosylated proteins and better approaches to studying glycosylated amyloids are required to stimulate biofilm research.

ORAL ABSTRACTS

[O3] FORMATION OF FUNCTIONAL NON-AMYLOIDOGENIC FIBRES BY RECOMBINANT BACILLUS SUBTILIS TASA

Elliot Erskine[1]

[1] *University of Dundee, Dundee, United Kingdom*

Amyloid fibres are most infamous for their involvement in the neurodegenerative illnesses' Alzheimer's disease and the infectious Creutzfeldt-Jakob but the ultra-stable cross-β form has recently found association with biological function. These 'functional amyloids' have now been identified across the Kingdoms. In bacterial biofilms these proteinaceous fibres are exploited to provide structure to the community and protection to the resident cells. *Bacillus subtilis* is a ubiquitous soil-dwelling organism whose biofilm is important for agricultural purposes. The predominant protein in the matrix is the fibre-forming TasA, previously classified as a functional amyloid. We wished to further characterise the structure and function of this protein and therefore developed a recombinant system to allow an interdisciplinary analysis. We show that the biologically active recombinant fibres are predominately α-helical and do not have the canon cross-β structure suggestive of amyloid fibres. We demonstrate that the N-terminus is key for fibre polymerisation, generating a monomeric form that lacks activity and is correspondingly susceptible to proteolysis. Investigation of the biological activity of fibrous TasA *ex vivo* revealed that in concurrence with self-assembly *in vitro*, the biological activity was independent of the TapA accessory protein in the biofilm. Instead the extracellular polysaccharide (EPS) proved an essential partner for rugosity of the biofilm. The specificity of the molecular mechanism by which TasA fibres interact with the other matrix molecules was probed by examining the biological activity of orthologous TasA. Looking beyond the significance of the *B. subtilis* biofilm matrix, TasA has been used as the basis of amyloid-inhibitory drug-screening therefore it is imperative that this fibre-forming protein (amongst others) is not mischaracterised.

[O4] INSIGHT INTO THE RAPA LECTIN AND ITS USE IN THE STUDY OF BIOFILM MATRIX FORMATION BY RHIZOBIA

María Soledad Malori[1], Daniela Russo[1], Julio Caramelo[1], Laura Navas[2], Marcelo Berretta[2], Graciela Benintende[2], Patricia Abdian[2]

[1]*Fundación Instituto Leloir, Conicet, Buenos Aires, Argentina*
[2]*Instituto Nacional de Tecnología Agropecuaria (Inta). Instituto de Microbiología Y Zoología Agrícola, Conicet, Hurlingham, Buenos Aires, Argentina*

Despite the importance of the matrix in the biofilm mode of life, little is known about the mechanisms leading to matrix assembly and the extracellular proteins involved in this process. We have previously characterized the RapA lectin secreted by *Rhizobium leguminosarum* and other rhizobia, and showed it has a profound impact in the organization of the biofilm matrix. The RapA lectin interacts specifically with the acidic polysaccharides (exo- and capsular polysaccharides) produced by *R. leguminosarum* in a calcium-dependent manner. The protein is solely composed of two homologous CHDL (cadherin-like) domains that adopt β-sheet conformation, a common fold in carbohydrate binding proteins. Given the large amount of available sequences, in this work we performed a phylogenetic analysis of CHDL domains in Raps (*Rhizobium* adhering proteins) secreted by rhizobia as a base to predict their functional properties. Then we dissected the RapA protein in its two halves, and studied the properties of the individual CHDL domains. The domains were PCR-amplified, cloned as 6xHistag fusions and purified from the soluble fraction of *Escherichia coli* cell cultures. The individual CHDL domains of RapA were structurally analyzed by circular dichroism (CD) spectroscopy. A functional test by means of a binding inhibition assay (BIA) was carried out with the acidic exopolysaccharide, and also with the lipopolysaccharide (LPS) to determine if RapA could act as an anchor for the capsular polysaccharide to the cell surface. No LPS binding by RapA or by the individual CHDL domains were detected under the assay conditions. However, our results show that the lectin activity is confined to the carboxy terminal CHDL domain (Cter-CHDL), which folds in response to calcium addition and is able to bind to the exopolysaccharide, although with less affinity than the entire RapA lectin. Moreover, the green fluorescent protein (GFP) was fused to RapA and to its Cter-CHDL, and the performance of the purified fusion proteins to target the exopolysaccharide was assessed, showing they could be useful as novel fluorescent probes for acidic exopolysaccharides produced by several rhizobia of agronomical interest.

[O5] SECRETED, LARGE-SCALE, EXTRACELLULAR MEMBRANE SYSTEMS IN MICROBIAL BIOFILMS

Lauren Franco[1], Armaity Siva Wu[2], Michael Joo[2], Nassim J Mancuso[2], Amita Gorur[2], Ambrose Leung[2], Danielle M Jorgens[2], Jonathan Remis[2], Joaquin Correa[2], Julijana Ivanisevic[3], Gary E Siuzdak[3], Manfred Auer[2], Matthew Fields[1]

[1]*Montana State University, Center for Biofilm Engineering, 366 Barnard Hall, Bozeman, United States*
[2]*Lawrence Berkeley National Laboratory, United States*
[3]*Scripps Research Institute, United States*

The biofilm matrix is increasingly being realized to contain a variety of intra- and inter-matrix interactions that contribute and control biofilm behavior; however, extracellular membranes have not been previously reported despite the occurrence of membrane vesicles in many types of microorganisms. *Desulfovibrio vulgaris* biofilms exhibited extracellular, elongated structures that in cross section appeared membranous, or as complex geometrical enclosed shapes devoid of cells. Non-osmicated, UAc only stained biofilm sample revealed an unstained thin core structure that upon osmication became black, indicating the thin structure was lipid-based. Serial section lipophilic dye FM1-43 in cryostat-sections revealed that the membrane structures persist for tens of micrometers, and metal precipitation occurred predominantly on the extracellular structures. 3D renderings after Serial Block Face Scanning Electron Microscopy (SBF/SEM) demonstrated long lamellar structures associated with metal deposits that extended up to 100 µm, essentially the entire length of imaged biofilm. Due to involvement with metal precipitates, biofilms were grown under electron-acceptor limitation (EAL), and the distribution of extensive membrane structures increased. Quantification of total fatty acids as FAMEs indicated that the EAL-biofilm had 3-fold increased FAME content, and untargeted metabolomics experiments indicate the increased occurrence of twelve long-chain fatty acids that included methyl-hexanoic and methyl-heptanoic acid. EAL-biofilms exposed to Cr(VI) showed increased viability compared to biofilms grown under a balanced condition, and these results suggested a protective role for the membrane structures during metal exposure. To our knowledge, this is the first report of secreted, large-scale, extracellular membrane systems in microbial biofilms (SLEMS), and the described structures have implications for microbial biofilms and the evolution of biological systems.

[O6] Invited Lecture: MOLECULAR INTERACTIONS OF STAPHYLOCOCCAL BIOFILM FORMING PROTEINS

Joan Geoghegan[1]

[1]*Trinity College Dublin, Ireland*

Our understanding of the molecular interactions underlying biofilm development in staphylococci has advanced remarkably over the past decade. *Staphylococcus aureus* forms biofilms on indwelling medical device surfaces and in host tissues, often leading to persistent and destructive infections. Cell to cell interactions during staphylococcal biofilm accumulation rely either on the production of a matrix of poly-N-acetyl-β-(1–6)-glucosamine or on cell surface proteins. In *S. aureus*, the cell wall-anchored (CWA) surface proteins FnBPA, FnBPB, SasG and SdrC extend from the bacterial surface and mediate cell to cell interactions during biofilm accumulation. Each protein is capable of forming homophilic interactions with an identical partner protein on a different cell linking the bacteria to each other. Alternatively CWA proteins promote biofilm formation through their interactions with host-derived molecules. Fibrinogen and fibronectin-binding proteins tether bacteria to tissues or to indwelling medical devices that have been conditioned with host plasma proteins. Fibrin-binding CWA proteins promote bacterial clumping in fibrin-rich fluids leading to the formation of biofilm-like aggregates of bacteria. Recent insights into the molecular basis of the protein-protein interactions underlying biofilm development in staphylococci have revealed potential new targets for biofilm-targeting agents.

ORAL ABSTRACTS

[O7] CYTOCHROME BD-I IS USED FOR ENERGY PRODUCTION IN UROPATHOGENIC E. COLI BIOFILMS

Maria Hadjifrangiskou[1]

[1]*Vanderbilt University Medical Center; Department of Pmi, Nashville, United States*

Significance: *Escherichia coli* is a very diverse species, encompassing 5 well-characterized phylogroups, A, B1, B2, D and E. Of these phylogroups, *E. coli* in clades B2 and D are considered to be the most adapted to the human host and are known to persist for years. These same B2 and D strains have also evolved to colonize extra-intestinal niches, such as the urinary tract, where they can cause infection. Uropathogenic *E. coli* (UPEC), which cause the majority of urinary tract infections (UTIs), form biofilms on catheters, as well as on the bladder and kidney surface. The molecular mechanisms driving biofilm formation by UPEC strains are not well defined. We lead studies to bridge this gap in knowledge.

Rationale and Hypothesis: Although UPEC are facultative anaerobes, our studies have demonstrated that UPEC rely on components of aerobic respiration for energy production in the bladder and for biofilm formation. Cytochrome *bd* acts as the primary oxidase in low oxygen concentrations, which are encountered by UPEC in the bladder and deep within biofilms. ***We hypothesized that cytochrome bd expression and function regulates UPEC biofilm architecture and generation of heterogeneity that in turn leads to a robust biofilm community.***

Results: We demonstrate that mutants deleted for Cytochrome Bd lose biofilm rugosity and architecture, suggesting a role for cytochrome Bd-I in energy production within biofilms. Biofilms formed by cytochrome Bd-I mutants appeared flat and compact and displayed higher sensitivity to antibiotics. These results could be the result of increased penetrance (due to reduced extra-cellular matrix production) and unperturbed energy-dependent transport across the inner membrane, suggesting that in the absence of cytochrome Bd-I, bacteria are somehow maintaining proton motive force. Subsequent analyses probed at: a) the abundance and composition of the extracellular matrix; b) the levels of cytochromes Bo and Bd-II and c) the role of oxygen sensors upstream of cytochrome Bd-I in the wild-type parent and the cytochrome Bd-I mutant.

[O8] HEAT ACTIVATES CYCLIC DIGUANYLATE PRODUCTION IN BACTERIA

Joe Harrison[1], Henrik Almblad[1], Trevor Randall[1], Jacquelyn Rich[1], Fanny Liu[1], Katherine Leblanc[1], Ryan Groves[1], Tara Winstone[1], Nicolas Fournier[1], Yuefei Lou[1], Ian Lewis[1], Justin MacCallum[1], Bryan Yipp[1]

[1] *University of Calgary, Calgary, Canada*

The second messenger cyclic diguanylate (c-di-GMP) in the opportunistic bacterial pathogen *Pseudomonas aeruginosa* regulates biofilm formation, which may be key for survival in a host. C-di-GMP is produced by a variety of intracellular diguanylate cyclases (DGCs), and can be degraded by c-di-GMP specific phosphodiesterases. However, the stimuli and mechanisms of DGC activation are in many cases unknown. Here we identify a thermosensing diguanylate cyclase (*tdcA*) that enables *P. aeruginosa* to increase biofilm formation as temperature rises from 25 to 37 °C. Recombinant TdcA displays thermostatted activity: it is inactive at 25 °C, but displays linearly increasing reaction rates between 28 and 42 °C, allowing for a large change in intracellular c-di-GMP over a narrow range of temperatures. Heat-sensing is enabled via a Per-Arnt-SIM type III (PAS_3) domain, which is a previously undescribed function for this widespread family of protein domains. Drawing on principles from statistical mechanics, the Boltzmann equation can be used to model TdcA activity, which cannot be accurately approximated by existing textbook models of temperature-dependent enzyme kinetics alone. Using molecular genetic methods, mouse infection models and intravital microscopy, we observed that a functional copy of *tdcA* in *P. aeruginosa* mediated the expression of c-di-GMP-regulated extracellular polysaccharides that were critical for innate immune suppression. Finally, bioinformatics analyses indicated that *tdcA* orthologues are widespread in both environmental and pathogenic bacteria. We propose that *tdcA* exemplifies a new class of heat-sensing enzymes that behave like molecular thermostats, allowing for the rapid change of cellular c-di-GMP over a narrow but physiologically relevant range of temperatures.

ORAL ABSTRACTS

[O9] SORTASE-ASSEMBLED PILI OF ENTEROCOCCUS FAECALIS CONTRIBUTE TO IRON-MEDIATED EXTRACELLULAR ELECTRON TRANSFER AND IRON-AUGMENTED BIOFILM

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Enterococcus faecalis are ubiquitous members of the human gut microbiota as well as significant opportunistic pathogens in biofilm-associated infections. We have discovered that the *E. faecalis* biofilm matrix can immobilize iron, which promotes extracellular electron transfer (EET) and increased ATP production leading to augmented biofilm growth. In addition, excess dietary iron is associated with increased *E. faecalis* colonization in the lower GI tract of mice. Because microbial nanowires contribute to electron transfer in other bacteria species, we hypothesized that *E. faecalis* sortase-assembled pili might contribute to iron-associated EET and iron-augmented biofilm. Here we present data showing that pilus gene expression is induced in increasing iron concentrations and that these induced pili are essential for iron-mediated biofilm augmentation. *E. faecalis* pilus null mutants are unable to sustain current production showing that pili are necessary for EET. Finally, using a combination of imaging and inductively coupled plasma mass spectrometry (ICP-MS), we show that pili co-localize and co-purify with iron. Together these findings support a previously unappreciated role for *E. faecalis* sortase-assembled pili, in addition to their role in adhesion, in which they bind iron within the biofilm matrix to promote EET, augment biofilm formation, and GI colonization.

[O10] PHYSICAL DETERMINANTS OF AMYLOID ASSEMBLY IN BIOFILM FORMATION

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Bacterial biofilms are of central importance in the context of a number of human diseases and amyloid fibrils represent one of the major components of the extracellular matrix of bacterial biofilms. However, the mechanisms and role of the fibril assembly kinetics in biofilm formation are not yet understood. Through detailed kinetic analysis of the aggregation of two proteins, FapC from *Pseudomonas fluorescens* and CsgA from *Escherichia coli*, we find that, despite the low sequence identity, the two proteins share a common aggregation mechanism, involving nucleated growth of linear fibrils that are unable to self-replicate. The measured rates of elongation for the two systems are very similar and comparable to the rates of formation of biofilms *in vivo*, indicating amyloid assembly as a possible control factor in biofilm formation. The observed convergent evolution suggests that only a narrow window of mechanisms and rates of assembly allows for successful biofilm formation.

ORAL ABSTRACTS

[O11] Invited Lecture: HOW DO BACTERIA RESPOND TO THEIR ADHERING STATE?

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Bacteria adhere to surfaces in most industrial and natural environments, regardless of whether the surfaces are of synthetic or biological origin, including the surfaces of prokaryotic and eukaryotic cells. Bacterial adhesion is the start of biofilm formation, but it still remains a challenge to define the end of biofilm formation. Biofilms are defined as surface-adhering and surface-adapted communities of microorganisms, that grow embedded in their self-produced matrix of extracellular polymeric substances. Interesting enough biofilm phenotypes do not emerge homogeneously across a biofilm. Heterogeneous micro-environments with different microbial composition, pH, live-dead ratios of bacteria, extracellular polymeric substances production, including eDNA-rich or -poor domains, differential penetrability, density, water content and channelization have been observed in biofilms using fluorescent probes or optical coherence tomography. The development of heterogeneous phenotypes at the level of biofilm communities, as well as at the level of single-bacteria, has been amply studied and reviewed with respect to gene expression and genotypic changes in planktonic bacterial aggregates and biofilms grown in well plates or on agar. However, the question of what actually triggers the emergence of heterogeneous micro-environments in biofilms remains unanswered. This leads to the hypothesis that phenotypically heterogeneous, emergent micro-environments in biofilms develop as a response of bacteria to their adhering state and are governed by the local properties of the substratum surface. No surface is entirely homogeneous, while adhering bacteria can substantially contribute to stochastically occurring surface heterogeneity. Accordingly, bacterial adhesion forces sensed by initial colonizers differ across a substratum surface, leading to differential mechanical deformation of the cell wall and membrane, where many environmental sensors are located. Bacteria directly adhering to heterogeneous substratum domains therewith formulate their own local responses to their adhering state and command non-conformist behavior, leading to phenotypically-heterogeneous micro-environments in biofilms.

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[O12] CELL LYSIS PROMPTS AN EARLY MECHANICAL COUPLING AND BIOFILM FORMATION IN DILUTE BACTERIAL SUSPENSIONS

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We have demonstrated that the individual cells in dilute bacterial cultures, which were previously thought to be purely planktonic, mechanically couple to each other as determined by optical tweezers and TEM[1]. The mechanical coupling in dilute bacterial cultures was observed in *Bacillus subtilis*, *Pseudomonas* spp., *Escherichia coli* and *Staphylococcus aureus*. Our research brakes with the central assumption that cells are not mechanically coupled with other bacteria in dilute bacterial suspensions. We have for the first time measured viscoelasticity of the single bacterial cell in dilute suspensions. In addition, we were able to support our prediction that the observed mechanical coupling is partially due to existence of weak entangled polymer extracellular network and not simply a consequence of long range hydrodynamic effect or direct linking between bacterial pairs. Although the mechanical coupling of the mutant *B. subtilis* strain lacking flagella (Δhag) was significantly reduced the coupling was still present. We have obtained data that indicate that the long range interactions could be mediated by eDNA released by cell lysis. When shaken cultures of *B. subtilis* were put to rest the cell lysis increased. Consistently, the coupling distance between bacterial pairs increased as well. Using ultra-sensitive fluoresce probes for nucleic acids we were able to monitor cell lysis in real time and observed the leakage of eDNA from individual cells. The eDNA has been implicated in an early biofilm formation. One could argue, however, that the observed cell lysis was due to rapid and unexpected change of the environmental conditions during the growth and may not be relevant for the formation of biofilms. To answer this we have developed a protocol to monitor in real time the formation of *B. subtilis* biofilm under the confocal laser microscope. We have observed that significant number of cells lysed soon after the inoculation. Furthermore the maturation of the pellicle was accompanied by additional cell lysis, supporting our hypothesis that cell lysis is important in early bacterial mechanical coupling leading to biofilm formation.

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[O13] BONE ENVIRONMENT AND ITS RELATIONSHIPS WITH BACTERIAL BIOFILM.

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The impact of bacteria on immune system or host cells described some of the interactions between bacteria and host. In this study, we studied the impact of bone cells and their microenvironment on *Staphylococcus aureus* capacity to form biofilm. Indeed, we hypothesized that the identification of signals that could induce irreversible bacterial adhesion in bone context, would help to target and to develop a strategy preventing biofilm-associated infections. Biofilms represent a major threat in public health due to their high capacity of antimicrobial resistance. We showed that various bone environment factors such as the presence of Ca^{2+} or Mg^{2+}, lack of oxygen and starvation, increased bacterial adhesion. In addition, we tested the impact of human osteoblast-*like* cell culture supernatants on biofilm formation and observed an increase in bacterial adhesion capacity by 2-fold (p=0.015) compared to control. Moreover, we stimulated osteoblast-*like* cells with TNF-α, mimicking inflammatory conditions. An increase of bacterial adhesion by almost 5-fold (p=0.003) was observed. However, the activity of synthetic antibiofilm peptides counteracted the induction signal(s) for bacterial adhesion present in the supernatants. Thus, antibiofilm peptides represent good candidates for developing a prevention strategy. In conclusion, the bone environment and bone cells derived-products could influence *S. aureus* biofilm formation, which demonstrates a direct impact of our body on bacterial behavior.

[O14] THE ROLE OF DYNAMIC TAD PILI IN BACTERIAL SURFACE SENSING

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The attachment of bacteria to surfaces provides advantages such as increasing nutrient access and resistance to environmental stress. Attachment begins with a reversible phase, often mediated by surface structures such as flagella and pili, followed by a transition to irreversible attachment, typically mediated by polysaccharides. How cells sense their contact with a surface and transduce this information to mediate the transition to permanent adhesion and to initiate biofilm formation is a matter of great interest. We have used single cell approaches to dissect the mechanisms of adhesion in *Caulobacter crescentus*. Surface contact by motile swarmer cells bearing a flagellum and pili at the same pole rapidly stimulates the biosynthesis of the holdfast adhesive polysaccharide. In contrast to a prevalent model, the flagellum is not required for surface contact stimulation of holdfast synthesis, but pili are critical for this process. *Caulobacter* swarmer cells harbor Tad pili, which are widespread across the bacteria and are often involved in adhesion. In contrast to Type IVa pili (T4P), Tad pili lack a retraction ATPase, and whether they retract like T4P has remained elusive. We have developed a broadly applicable pilus labeling method that enables real-time observation of pilus dynamics and targeted physical obstruction. We find that Tad pili undergo dynamic cycles of extension and retraction that cease within seconds of surface contact, and this arrest of pilus activity coincides with surface-stimulated holdfast synthesis. Physically blocking pilus retraction is sufficient to stimulate holdfast synthesis in the absence of surface contact, in a process that involves cyclic-di-GMP signaling. Thus, resistance to type IV pilus retraction upon surface attachment is used for surface sensing.

ORAL ABSTRACTS

[O15] A ROLE FOR TWO-COMPONENT SYSTEMS IN BACTERIAL ATTACHMENT AND ANTIBIOTIC TOLERANCE IN LISTERIA MONCYTOGENES

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Listeria monocytogenes is a foodborne pathogen that survives as biofilm on food-processing equipment. Stress affecting the cell membrane activates several two-component systems (TCS) that trigger a genetic response, which involves modulation of the cell envelope. We therefore hypothesized that this response also affects cell attachment, leading to promotion of biofilm formation under stressful conditions.

We tested this hypothesis by investigating the role of the TCS LisRK in attachment of *L. monocytogenes*. LisRK is activated in all cells in stationary-phase cultures, hence we compared attachment under flow of stationary-phase cells from a wildtype and a Δ*lisK* mutant strain. Significantly more wildtype cells attached, suggesting that activation of LisRK made cells more adhesive. Adhesion forces can be quantified with high sensitivity and spatial resolution using atomic force microscopy. We conducted nanomechanical mapping and force spectroscopy on the wildtype and Δ*lisK* mutant strains. Surface topography, and mechanical- and physicochemical properties of the two strains were probed simultaneously in physiological buffer. Force-distance curves and adhesion maps indicate that the wildtype cells were significantly more adhesive than the Δ*lisK* mutant.

Activation of LisRK allows cells to grow at slightly elevated antibiotic levels. In biofilms however, the extreme antibiotic tolerance ascribed to non-growing persister cells. We therefore investigated a possible link between LisRK activation and persister cell formation in planktonic and attached populations. In planktonic cultures, persister cells were significantly more abundant in the wild-type strain, suggesting that LisRK activation promotes formation of persister cells. After attachment to polystyrene for 5 minutes, we determined the antibiotic tolerance of the attached population by measuring the minimal biofilm eradication concentration against β-lactam antibiotics. The antibiotic tolerance of the Δ*lisK* mutant strain was similar for the attached and planktonic populations, but for the wildtype strain, the antibiotic tolerance was >1000 fold higher in the attached population, and indicated the antibiotic tolerance of persister cells. In conclusion, our study shows that cell envelope stress in *L. monocytogenes* can trigger attachment and persister cell formation via the two-component system LisRK. These finding imply that two-component systems are important for triggering the survival mechanisms that are at the core of biofilms.

[O16] Invited Lecture: ARE BIOFILMS REALLY THE DOMINANT WAY OF LIFE FOR PROKARYOTES ON EARTH?

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In many publications on biofilms, it is presumed that the majority of prokaryotes on Earth exist in the form of biofilms, providing ecosystem services on this planet. Biofilms are considered as a form of collective life and in biofilms the cells develop emergent properties, different from single organisms, and represent a much more complex, higher level of organization. Belief in dominance of the biofilm mode of life comes from the observation that the cell density in biofilms is several orders of magnitude higher than in the surrounding water phase, and that the cells enjoy crucial advantages in the ubiquitously existing biofilms. However, to date no global data analysis supporting this view exists. We offer a critical discussion of the definition of biofilms, comprising many different manifestations of microbial aggregates of various size. We compile the current state of estimates of global cell numbers in major microbial habitats. Most microorganisms on Earth ($3.4 - 16 \times 10^{30}$) live in the "Big Five": oceans (1.2×10^{29}), the upper (2.4×10^{30}) and the deep sediment ($0.25\text{-}5 \times 10^{30}$), soil ($2.6\text{-}2.9 \times 10^{30}$) and deep terrestrial subsurface $0.5\text{-}2.5 \times 10^{30}$). All other habitats, e.g, neuston, atmosphere, animals, phyllosphere etc. inhabit only up to 10^{25} cells or less each. While the majority of cells in the oceans arguably seem to live a solitary life (flocs, marine snow, and colonized particles are not accounted for), upper sediments and soil are clearly dominated by biofilms. Deep seafloor sediments and terrestrial subsurface are implicitly considered to be colonized by solitary cells without taking biofilms into account. However, biofilms will prevail when cells divide (even after extremely long generation times) and form microcolonies. Thus, at least three quarters of all prokaryotes on Earth live in biofilms, and this census contours the global relevance of this form of life.

ORAL ABSTRACTS

[O17] Bird's Eye Lecture: COOPERATION AND COMPETITION IN BIOFILMS

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Since Darwin, evolutionary biologists have been fascinated by cooperative behaviour. Honeybee workers labour their whole life without reproducing, birds make alarm calls, and humans often help each other. In recent years, it has also become clear that microbes are also capable of cooperation. They commonly live in densely-interacting biofilms that can have major effects on animals and plants. But what determines if microbes are cooperative towards each other and their hosts? We study this question by combining theory with experimental systems, including pathogenic bacteria, budding yeast and the mammalian microbiome. We find that single-genotype patches naturally emerge in biofilms, which favours strong cooperation by kin selection. By contrast, interactions *between* genotypes can be strongly competitive. Bacteria strains are often at war and we find that they can rapidly detect incoming attacks and respond in kind. Interactions within microbial biofilms then follow the same evolutionary principles that were first understood through the study of animal behavior. However, one unusual and fascinating property of microbes is that an entire ecosystem can lie within another evolving organism: a host. This raises the possibility that hosts will act as ecosystem engineers that change the rules of microbial interaction for their own benefit.

[O18] BIOFILM ARCHITECTURE CONFERS INDIVIDUAL AND COLLECTIVE PROTECTION AGAINST PHAGE INFECTION

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In their natural environments biofilms regularly encounter the presence of bacteriophages (or simply: phages) which use bacteria as their host for self-replication. In order to understand the interaction of phages and biofilms, as well as their coexistence, we developed a method to visualize phage spread inside living bacterial communities. By insertion of *sfgfp* into the T7 phage genome, the conversion of susceptible to infected cells can be monitored in space and time. We discovered that biofilm susceptibility to phage infection is dependent on the stage of biofilm development and the production of biofilm matrix. *E. coli* biofilms that were grown for 48 h or less were rapidly eradicated due to phage infection. By contrast, biofilms grown for 60 h and more experienced no biomass reduction in the presence of phages. The removal of curli fibers, a major component of the *E. coli* matrix, generated biofilms that were susceptible to phage infection, regardless of the age of the biofilms. Visualization of curli fibers within growing biofilms further demonstrated a dynamic change in matrix composition. The development of phage tolerance in biofilms was synchronous with the production of curli fibers. We further discovered that curli fibers protect bacterial communities via two mechanisms: (1) Curli prevent phages from diffusion inside biofilms, and (2) curli fibers protect individual cells from phage infection. Our results demonstrate that a single component of the biofilm matrix can provide individual as well as collective protection against phage infection.

ORAL ABSTRACTS

[O19] EFFECT OF FLUCTUATING ENVIRONMENTAL CONDITIONS ON THE SPATIAL SELF-ORGANIZATION AND EMERGENT PROPERTIES OF A SYNTHETIC MICROBIAL BIOFILM

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Background: Every natural microbial community is exposed to temporal fluctuations in their local environment. The mechanisms that provide stability to a microbial community during environmental fluctuations, however, are unclear. Environmental fluctuations can change how microorganisms interact, which in turn can change how they arrange themselves in space. Spatial self-organization, as well, can be an important determinant of the stability of the whole microbial community. This then raises two interrelated questions: 1) How do temporal fluctuations in environmental conditions affect spatial self-organization? 2) Do these fluctuations affect the long-term stability and the survival of the microbial community?

Objectives: We addressed these questions using a two-strain synthetic microbial cross-feeding community. We can experimentally fluctuate the environment between conditions that promote mutualism or competition between the microbial strains. We hypothesized that such fluctuations should destabilize the community; the patterns of spatial self-organization that emerge under competition conditions (strain segregation) should be detrimental under mutualism conditions (strain intermixing). How then could the microbial community persist under such fluctuations?

Methods: We investigated this question using a synthetic cross-feeding community composed of two strains of the bacterium *Pseudomonas stutzeri*. Under anaerobic conditions, one strain consumes nitrate to nitrie while the other consumes nitrite. Under aerobic conditions, in contrast, both strains compete for oxygen. Thus, we can fluctuate the environment between aerobic (competition) and anaerobic (mutualism) conditions and measure the consequences on spatial self-organization and community stability.

Results: We found that fluctuations between mutualism and competition conditions do indeed reduce the stability of the microbial community as a whole. Interestingly, the effect depends on the strength of the mutualistic interaction. When the mutualistic interaction is strong, the two strains maintain more intermixing, thus mitigating the segregating effects of competition. In contrast, when the mutualistic interaction is weak, the two strains intermix less, thus amplifying the segregating effects of competition.

Conclusion: We found that complex spatial self-organization is important to provide community stability during temporal fluctuations in environmental conditions. Spatial self-organization is thus impacted by temporal fluctuations, but also affects stability to temporal fluctuations, highlighting its role as an emergent property of the microbial community.

[O20] AHL QUORUM SENSING MEDIATES SPECIES INTERACTIONS IN MULTISPECIES BIOFILMS

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A major concern is to understand processes that govern functional synergy and dynamics of multispecies bacterial biofilms that are important medically, environmentally and industrially. Here, we have used a model mixed species biofilm community comprising *Pseudomonas aeruginosa* PAO1, *Pseudomonas protegens* PF5 and *Klebsiella pneumoniae* KP1 with higher biomass, lower levels of genotypic variants and higher levels of resilience to antimicrobial stress conditions such as SDS and tobramycin, compared to monospecies biofilm populations. In order to understand these properties further, in this study we evaluated the function of N-acyl homoserine lactone (AHL)-dependent quorum sensing (QS) system of *P. aeruginosa* PAO1, which is known to regulate group behavior including biofilm formation and production of effector molecules, in determining the structure and function of this mixed species biofilm community. Mixed species biofilms containing either wild type PAO1, *lasIrhlI* (QS signal negative) or *lasRrhlR* (QS signal receptor negative) mutants were grown under continuous flow conditions and their composition was analyzed by confocal laser scanning microscopy. We observed that mixed species biofilms containing *P. aeruginosa* QS mutants had significantly altered proportions of *K. pneumoniae* and *P. protegens* populations compared to mixed species biofilms with the wild type *P. aeruginosa*. Surprisingly, the proportions of *P. aeruginosa* were the same for both the wild type and the QS mutants. Moreover, dual-species biofilms of *P. aeruginosa* wild type, *lasIrhlI* or *lasRrhlR* mutant with either *K. pneumoniae* or *P. protegens* revealed that the absence of *P. aeruginosa* QS affects both species interactions in different ways. Initial experiments suggest that the *P. aeruginosa-P. protegens* interaction is competitive and likely to be mediated by contact dependent mechanisms. We also show that the *P. aeruginosa* QS system, through the regulation of downstream target genes, alters the stress resistance of the whole community. These observations suggest that QS plays an important role in modulating community biofilm structure and physiology, affecting both interspecific interactions and community level protection provided by species specific functions.

ORAL ABSTRACTS

[O21] BIOFILM THICKNESS CONTROLS THE CONTRIBUTION OF STOCHASTIC AND DETERMINISTIC PROCESSES IN MICROBIAL COMMUNITY ASSEMBLY

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Niche and neutral theories provide diverging viewpoints on the importance of selection and neutral processes in community assembly. In practice, both deterministic and stochastic factors play a role in microbial community assembly, though little is known about whether the relative importance of these processes can be managed. In this study, we examined the relative contribution of stochastic and deterministic processes in the assembly of biofilms of different thicknesses. This was achieved using Z-carriers®, biofilm carriers with a grid with controlled wall height that determines maximum biofilm thickness. Duplicate Z-carriers of each thickness (50, 200, 300, 400, 500 um) were sampled 107 days apart from nitrifying reactors during steady state operation. Influent and effluent were also sampled at intervals. DNA was extracted and subjected to 16S rRNA gene amplicon sequencing and qPCR for total Bacteria. Beta-diversity analysis shows that communities on biofilm carriers were distinct from those in the influent and effluent and exhibited less temporal variation in composition than both influent and effluent communities. Variation in microbial community composition over time was greatest in thin biofilms and decreased with thickness. Overall, the biofilm communities were strongly influenced by deterministic processes as only a small number of sequence variants (SVs) were shared between the carriers and influent. The number of shared SVs between the influent and carriers increased with biofilm thickness. Neutral community modelling showed that a greater percentage of these shared SVs were neutrally assembled with increasing thickness, corresponding to a linear relationship between biofilm thickness and migration rate. Together, these observations suggest that biofilm thickness influences the relative importance of neutral and deterministic processes on community assembly. Although selection was important in all biofilm communities, stochastic factors play a greater role in the assembly of thicker biofilms. In addition, the biofilm community composition was stable once established, with increasing stability with biofilm thickness. We propose that in the thin biofilms, the small, active volume is subject to greater competition for space and resources, while in the thicker biofilms, the greater volume and presence of less active lower layers increase the contribution of neutral processes in community assembly.

[O22] Invited Lecture: MULTISCALE ANALYSIS OF MICROBIAL CROSS-FEEDING IN BIOFILMS: FROM YELLOWSTONE HOT SPRINGS TO CHRONIC WOUNDS

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Interactions among microbial community members can lead to emergent properties, such as enhanced productivity, stability, and robustness. Iron-oxide mats in acidic, high-temperature hot springs of Yellowstone National Park contain relatively simple microbial communities and are well-characterized geochemically. Consequently, these communities are excellent model systems for studying the metabolic activity of individual populations and key microbial interactions. The current study integrates data collected *in situ* with *in silico* calculations across process-scales encompassing enzymatic activity, cellular metabolism, community interactions, and ecosystem biogeochemistry. For instance, metagenomic and transcriptomic data are used to reconstruct carbon and energy metabolisms of an important autotroph (*Metallosphaera yellowstonensis*) and heterotroph (*Geoarchaeum* sp. OSPB). *In situ* geochemical analyses, including oxygen depth-profiles, Fe(III)-oxide deposition rates, stable carbon isotopes and mat biomass concentrations, are combined with the cellular models to explore autotroph-heterotroph interactions important to community structure-function. The integration of metabolic modeling with *in situ* measurements demonstrates that the mat communities operate at their maximum total community growth rate as opposed to net community growth rate, as predicted from the maximum power principle. In a complimentary biofilm study, chronic wound pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* are analyzed using genome-scale metabolic reconstructions integrated within a reaction-diffusion framework to identify resource usage strategies as a function of time, space, species and phenotypic strategy. Together, these studies integrate multiscale data with practical ecological theory to provide a basis for predicting and interpreting microbial interactions and community-level cellular organization.

ORAL ABSTRACTS

[O23] DEVELOPING A NOVEL UNDERSTANDING OF E. COLI K-12 PELLICLE FORMATION, MORPHOLOGY, AND PHYSIOLOGY

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During work to generate and optimize a robust, recombinant *Escherichia coli* K-12 biofilm capable of biocatalysis, formation of biofilms floating on the air-liquid interface, commonly referred to as pellicles, were observed. Unlike other well-characterized, pellicle-forming bacteria, such as *Bacillus subtilis*, there are few reports of *E. coli* K-12 pellicles in the literature. In order to study pellicle formation and physiology, a growth model was developed and pellicle matrix, maturation, and morphology were monitored over time and compared to development of solid surface-attached (SSA) biofilms. Pellicles and SSA biofilms were analyzed by confocal microscopy using lectins to visualize extracellular polymeric substances and DNA stains. This work revealed substantial differences in their development, architecture, and composition. Furthermore, pellicle structure was shown to differ between the air-facing and liquid-facing surfaces. By use of reporter plasmids, *csgB* (a component of the protein adhesin curli critical in SSA *E. coli* K-12 biofilm formation) expression was compared between pellicles, SSA biofilms, planktonic cells, and sedimented biomass. Although curli production is induced in both pellicles and SSA biofilms, there were temporal differences in expression in the two systems. While literature reviews have not revealed any published data recording these observations in *E. coli* K-12, research into other strains have noted that upregulation of curli in pellicles results in a more stable floating biofilm that has an enhanced resistance to strain. The sturdiness of the floating pellicle in addition to its proximity to both liquid and air phases make *E. coli* K-12 pellicles an exciting and innovative prospect in the biotechnology industry. In addition to potential industrial applications, the matrix composition, structure, and morphology of pellicles versus SSA biofilms will be discussed in *E. coli* K-12 laboratory strains for the first time.

[O24] INCREASING THE SPACE-TIME YIELD IN LACTIC ACID PRODUCTION BY THE USE OF BIOFILMS

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The application of biofilm technology for the production of both low-cost and valuable compounds allows the white biotechnology to benefit from the advantages of biofilms compared to suspended cultures. In this study, we focus on the production of optically pure lactic acid. Recently, the interest in lactic acid has greatly increased, especially due to the synthesis of polylactic acid, a biodegradable and biocompatible polymer. However, the high costs of lactic acid are responsible for its low competitiveness to petroleum-derived plastics.

For the production of lactic acid, we cultivate a monoseptic biofilm consisting of *Lactobacillus* bacteria with a strong preference for planktonic growth (provided by BASF SE). Biofilm growth is achieved in a glass tube reactor. The biofilm system can be cultivated in a continuous mode and kept monoseptically for at least 3 weeks. It was shown that higher cell densities lead to a significantly increased space-time product yield compared to the planktonic culture. The productivity reached a constant value after only 3 days of cultivation and no decline was observed during the rest of the cultivation period, confirming thus the suitability of the system for long term production. Thus, the use of biofilms proves to be a promising method to increase the productivity and thereby reduce production costs of lactic acid.

The analysis of biofilm performance at different flow conditions revealed that productivity increases with the flow velocity. This is explained by the decreased retention time of the liquid phase in the tube reactor and, thus, a minor pH drop caused by the released lactic acid. At low flow velocities, the pH drops to a value where growth and production are significantly inhibited.

During the cultivation, the biofilm (thickness and structure) was analyzed by Optical Coherence Tomography (OCT), a non-invasive imaging tool that can be applied in-situ during reactor operation without disturbing system or process. At the end of the experiment, the biofilm was additionally visualized by Nuclear Magnetic Resonance (NMR) to investigate the biofilm coverage over the cross-sectional area of the tube reactor.

ORAL ABSTRACTS

[O25] ESTIMATION OF MECHANICAL AND HYDRAULIC BIOFILM PROPERTIES FROM OPTICAL COHERENCE TOMOGRAPHY MEASUREMENTS

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Biofilm presence induces additional resistance to water permeation and causes increased cross-flow pressure drop in membrane processes affected by biofouling. There is a reciprocal biofilm/flow interaction. The flow deforms the biofilm by shear forces when there is flow over the biofilm or by flow-driven compression due to pressure forces in filtration cases. Biofilm deformation, in turn, causes a decrease in porosity and elevated hydraulic resistance, with less water flow through the biofilm. Moreover, biofilm deformation leads to material stiffening and mechanical consolidation, probably with negative effects on biofilm removal. It has become therefore evident that *in-situ* studies are needed on how biofilm hydraulic resistance is affected by biofilm mechanical response to the applied forces. For this aim, optical coherence tomography (OCT) proves to be an excellent *in-situ* and real-time non-invasive measurement/visualization technique.

A computational model was applied to several experimental datasets obtained in two conditions: (i) flow-through with step-wise changes of permeate flux and pressure in dead-end ultrafiltration; (ii) flow-over with changes of flow velocity in a rectangular mini-fluidic channel. Two-dimensional biofilm geometries were extracted from cross-sectional OCT scans to access the un-deformed and deformed structures as a result of hydrodynamic loading. The OCT images of biofilm structure were implemented in fluid-structure interaction computer simulations using a poroelasticity model coupling fluid dynamics with solid mechanics. The simulation results were compared with the real deformed geometries. Mechanical (e.g., elastic modulus, Poisson ratio, viscoelastic relaxation time, critical plastic stress) and hydraulic (e.g., permeability coefficient, porosity exponent) biofilm properties were assessed by parameter estimation routines.

Biofilm elastic moduli of 70-500 Pa were found (in agreement with other measurement techniques), with clear biofilm stiffening and consolidation at increased flow rates and applied forces. The small irreversible deformation of biofilms grown on membranes could be explained by both elastoplastic and viscoelastic models with short relaxation times (~ 20 s). Biofilm porosity variation due to compression was also evaluated and decreased permeability by pore closure upon compression was quantified. The importance of this newly developed method lies in providing more accurate biofilm parameters to be used in numerical models targeting both filtration and biofilm removal strategies.

[O26] OPTICALLY PATTERNED BIOFILMS FOR SYNTHETIC MICROBIAL CONSORTIA

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Naturally existing terrestrial bacteria primarily live in surface-attached biofilm communities. Within these biofilms, cooperative behaviours such as metabolic division of labour exist due to coupling between ecological interactions and spatial community structure. However, this coupling remains poorly understood, making it challenging for synthetic biologists to engineer microbial consortia that take advantage of community division of labour. Using optogenetic tools, we have developed a biofilm culture platform with spatiotemporal control over cell-surface attachment, which patterns bacterial biofilm communities in a light-regulated manner. With this platform, we are able to seed biofilms with ecologically interacting strains in arbitrary, non-naturally occurring patterns at a spatial resolution on the order of 25 microns. In doing so, we decouple spatial structuring in biofilms from native ecological interactions. This platform can be used (1) to investigate how patterning and cooperation develop in bacterial communities, and (2) to engineer synthetic microbial consortia incorporating biological division of labour. An accompanying Monte-Carlo model provides insight on the biophysical formation mechanism for the engineered light-regulated biofilms.

[O27] Invited Lecture: WHY EVOLUTION IN BIOFILMS IS DIFFERENT, AND A FEW REMARKABLE CONSEQUENCES

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Opportunists such as *Burkholderia and Pseudomonas* have extremely low genome-wide mutation rates and among the largest effective population sizes of any organism measured. These two properties provide clear evidence that the power of natural selection to refine any trait important for fitness in Bcc is almost unimaginably strong. Consequently, the inherent capacity of these bacteria to grow on a variety of biotic surfaces in aggregates or larger biofilms is likely governed by well-coordinated regulatory circuits. A powerful approach to discover these circuits is the genomewide surveillance of evolution in action under conditions that select strongly for biofilm production, and hence alterations in these pathways. Theory predicts that the structured environment of biofilms facilitates the simultaneous rise of multiple adaptive lineages, which could reveal different adaptations or parallelism in the same pathways. Here, we report results from many laboratory evolution experiments and longitudinal surveys of isolates from patients with cystic fibrosis (CF) that reveal repeated adaptive mutations in two interrelated systems: *rpfF/rpfR* in *Burkholderia* and *wsp* in both *Burkholderia* and *Pseudomonas*, which increase attachment and aggregation by regulating polymer and lectin production. Evolved nonsynonymous mutations in different protein domains of these clusters generate different biofilm phenotypes and thus inform the function of these pathways. Remarkably, mutations affecting the same protein residues evolve repeatedly in independent experiments, yet these sites are not hypermutable, which demonstrates extraordinarily strong selection for the adaptive traits generated by these mutations. This parallelism is remarkable because genomewide knockout screens have identified many more genes that affect biofilm traits. The key insight is that evolutionary dynamics in biofilms are superpowered to select for the very best new mutations that produce superior attachment or stress avoidance, often by disrupting negative regulators of pleiotropic circuits in optimal ways. Ultimately, such mutations should be very rare in nature, where the conserved "wild-type" allele represents the regulator that maintains an optimal range of function.

[O28] LONG-TERM CO-ADAPTATION OF PSEUDOMONAS AERUGINOSA BIOFILMS WITH AMOEBA AFFECTS VIRULENCE TRAITS

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Pseudomonas aeruginosa is an opportunistic pathogen that is a major cause of death in sufferers of cystic fibrosis (CF). Compared to environmental and clinical *P. aeruginosa* isolates, CF lung- adapted *P. aeruginosa* demonstrate altered phenotypes in traits associated with virulence. The reduction of virulence trait expression is proposed to reduce clearance of the bacterium by host immune cells, thus supporting intra-lung persistence. To further understand how intra-host survival affects *P. aeruginosa*, we investigated the phenotypic traits possessed by *P. aeruginosa* after long-term co-adaptation with the amoeba, *Acanthamoeba castellanii,* a common soil and water protist.

Co-cultures were established in low nutrient media within culture flasks (3 × independent populations), where naive *A. castellanii* were re-infected with amoeba-adapted *P. aeruginosa* every 3 days for 24 days. *P. aeruginosa* were liberated from *A. castellanii* and isolates collected for further analysis. Populations of *P. aeruginosa* not exposed to amoeba were simultaneously established and passaged as above.

Results show that *P. aeruginosa* isolates co-adapted with *A. castellanii* demonstrate decreased planktonic growth rates, reduced pyoverdine production, a reduction in twitching, swarming and swimming motilities, production of pyoverdine and pyocyanin and a reduction in virulence when tested in a *Caenorhabditis elegans* assay. These differences were supported by single nucleotide polymorphism analysis that revealed mutations in genes associated with those traits.

The phenotypic traits demonstrated by amoeba-adapted *P. aeruginosa* have similarities to *P. aeruginosa* isolated from CF lungs. This suggests that intracellular survival in amoeba results in parallel evolution whereby the adapted *P. aeruginosa* cells resemble those from long term colonisation of lungs. Furthermore, data from this study suggests that the alteration of virulence may be a ubiquitous response of *P. aeruginosa* to survive the intra-host environment.

ORAL ABSTRACTS

[O29] EVOLUTION IN CHANGING ENVIRONMENTS: SPECIALIST AND GENERALIST STRATEGIES DURING NON-STABLE SELECTION OF THE BIOFILM PHENOTYPE

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The environment of most bacteria changes continuously and it is therefore important to understand how such changes affect bacterial evolution. Here, we studied which genetic and selective determinants promote phenotype specialization versus responsive switching during non-stable selection. This question is of major interest in concern with sessile (e.g. biofilms) and planktonic (e.g. swimming) phenotypes as they are fundamental to the fitness of bacteria.

We evolved *Pseudomonas aeruginosa* PA14 in both constant and changing conditions where biofilm formation and/or swimming motility were selected for. In changing environments with global competition, a more heterogeneous population evolved, where both specialists and generalists co-occurred: Interestingly, over time, generalists were outcompeted by the specialists; although, the generalists became better at both phenotypes compared to the ancestor. Further experiments revealed that generalists were more likely to prevail when local competition could occur subsequent to resettlement.

Genome sequencing and measurements of cyclic di-GMP, a second messenger that facilitates the shift between biofilm-formation and swimming motility, revealed that mutations that effected this system was key for the adaptation of the phenotypes. Yet, neither specialists nor generalists lost their ability to regulate c-di-GMP levels. However, generalists had acquired mutations that optimized the bacterium's phenotypic response to both types of environments by more efficiently shifting the intracellular level of cyclic di-GMP.

We conclude that environmental change is critical for the adaptation of bacteria that ably shift between opposing physiological states; sessile and planktonic phenotypes. Yet, resettlement and local, as opposed to, global competition were key factors that stabilized the evolution of generalists.

[O30] CHEATING PROMOTES EVOLUTION OF HYPER-COOPERATORS BY SHIFTING PHENOTYPIC HETEROGENEITY IN BIOFILMS

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In *Bacillus subtilis*, the biofilm matrix is primarily composed of exopolysaccharide (EPS) and amyloid fiber (TasA) synthesized by enzymes or composed of proteins coded in the *epsA-O* or *tapA-sipW-tasA* operons, respectively. In response to various signals, these operons are heterogeneously expressed, yielding only a subpopulation of cells expressing the *eps* and *tasA* genes. Our previous study shows that the cells lacking both *eps* and *tasA* are excluded from the pellicle formed by the wild-type cells (Martin, *et.al.*, 2017 *Nature Commun*). Contrary, single mutant strains are able to mix with wild-type *B. subtilis* and act as cheaters by increasing their relative frequencies in the pellicles. However, it is uncertain how the existence of cheaters alters the bi-stability of matrix gene expression in producer strains. Several examples have been shown to describe microbial cooperation and the remarkable population dynamics between producers and non-producers (Nadell *et.al.*, 2008 *J Biol*, West *et.al.*, 2006 *Nat Rev Microbiol*). In the case of *B. subtilis* biofilm, it is unknown yet how such heterogeneous matrix expression will be influenced by the presence of cheaters and how such mixed population behaves in an evolutionary time scale.

In this study, pellicles formed by the co-culture of producer (wild-type) and cheaters (*eps* mutant) were repetitively re-inoculated every two days to follow alterations in biofilm gene expression heterogeneity and population ratio dynamics. Interestingly, selected populations of the co-cultures showed the evolution of hyper-cooperators exhibiting increased number of cells expressing the matrix gene, so-called hyper-cooperators. In addition, the evolved co-cultures containing hyper-cooperators allowed cheaters to increase in number, eventually resulting to population collapse. In contrast, when producers were evolved alone, most isolates produced biofilms with reduced matrix gene expression. This study points towards the alteration of phenotypic heterogeneity when cheaters invade the biofilms of *B. subtilis*.

ORAL ABSTRACTS

[O31] INCREASED RATE OF MUTATION TO ANTIMICROBIAL RESISTANCE IN POLYMICROBIAL BIOFILMS

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Using a dual species co-culture of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, we have investigated how biofilm growth, either within single species or dual species co-culture, can affect the rate of bacterial mutation, and how biofilm growth can contribute to bacterial variability and the emergence of AMR mechanisms.

Mutation rates of *P. aeruginosa* PA21 and *S. aureus* UAMS-1 were measured using Luria-Delbrück fluctuation analysis following growth as both planktonic and biofilm cultures, either alone or in co-culture. Mutants were determined based on their ability to grow on agar plates containing an inhibitory concentration of rifampicin. Mutation rates were calculated using the Ma-Sandri-Sarkar Maximum Likelihood Estimator and 94% confidence intervals compared for significance. Colony variants of both *P. aeruginosa* and *S. aureus* were isolated following biofilm co-culture and isolates were subjected to whole genome sequencing.

Mutation rates of *P. aeruginosa* and *S. aureus* increased 10-fold when grown as monospecies biofilms compared to monospecies planktonic cultures ($P \leq 0.01$). A similar increase was seen for both species when grown as a planktonic co-culture, and for *P. aeruginosa* when grown as a co-culture biofilm. However, when in a co-culture biofilm, the mutation rate for *S. aureus* increased over 100-fold compared to the rate observed following monospecies planktonic culture. Phenotypic and genetic analysis (whole genome sequencing) of colony variants from biofilm co-culture are revealing how mutation events relate to survival and adaptation under co-culture.

This work shows that biofilms promote mutation rates and genomic variation in bacteria which can accelerate the evolution of AMR. We also demonstrate that inter-species interactions during growth can have an important influence on bacterial mutation rates.

[O32] Invited Lecture: TUNING BIOFILMS ARCHITECTURE TO CONTROL THEIR FUNCTIONS?

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Biofilms are dynamic habitats which constantly evolve in response to environmental fluctuations and thereby constitute remarkable survival strategies for microorganisms. The modulation of biofilm functional properties is largely governed by the active remodeling of their three-dimensional structure and involves an arsenal of microbial self-produced components and interconnected mechanisms. The production of matrix components, the spatial reorganization of ecological interactions, the generation of physiological heterogeneity, the regulation of motility, the production of actives enzymes are for instance some of the processes enabling such spatial organization plasticity. Manipulating biofilm spatial organization represents an attractive approach to control their functional properties, whether beneficial or deleterious.

[O33] CHARACTERIZATION OF ANTI-CURLI ANTIBODY BASED APPROACHES TO ERADICATE SALMONELLA TYPHIMURIUM BIOFILMS

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Amyloids are naturally occurring fibrillary proteins defined by their conserved cross-beta sheet secondary structure. Difficult to eradicate, bacterial biofilms are the often associated with infections and medical complications. Amyloids are often produced as an essential component of the bacterial biofilm matrix. One of the most studied bacterial amyloids is curli, specifically produced by members of Enterobacteriaceae. Here, we propose a novel method to inhibit biofilm formation by targeting the amyloid component, curli, of the biofilm matrix produced by *Salmonella enterica* serovar Typhimurium (STM). Four human monoclonal antibodies (mAb), ALZ.4GI, ALZ.3H3, ALZ.2C10, ALZ.4A6, that have high affinity against human amyloid beta that also exhibit activity against curli formation thereby inhibiting STM biofilm formation. Anti-amyloid mAbs have the ability to inhibit biofilm formation by preventing curli fibrillization as monitored by Thioflavin T binding to synthetic CsgA peptides. Additionally, treatment with anti-amyloid mAbs reduce biofilm mass and reduce curli content within the biofilm matrix as examined by crystal violet assays and confocal microscopy. Monoclonal antibody treatment also altered biofilm architecture. By creating 3D reconstructions of biofilms grown with mAb, we observed that mAb treated biofilms exhibited a more diffuse biofilm. By tracking the movement of fluorescently labeled beads through biofilms treated with mAb, an increase in bead movement throughout the biofilm matrix indicated a loss of biofilm rigidity in comparison to untreated biofilms. The associated loss of biofilm integrity resulted in an increased recovery of bacteria within biofilm supernatant and an enhanced ability of macrophages to phagocytose biofilm associated bacteria. As biofilms are a significant complication associated with implanted medical devices, we explored the ability of the mAbs to be utilized as an anti-biofilm therapeutic *in vivo.* Catheters colonized with STM were implanted into the back flanks of mice in the presence or absence of mAbs. After 72 hours of STM growth, catheters were removed and resulting biofilm growth and curli content was assessed. As amyloids are produced by bacteria across four phyla including Bacteroidetes, Proteobacteria, Firmicutes and Thermodesulfobacteria, these studies will provide a potential therapy strategy to prevent biofilm formation by targeting the amyloid component of biofilms using amyloid specific monoclonal antibodies.

[O34] A NEW STRATEGY FOR BIOFILM CONTROL USING BIOINSPIRED DYNAMIC SURFACE TOPOGRAPHY

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Biofilms are surface-attached communities of microorganisms that are highly resistant to antimicrobials and disinfectants, causing persistent problems such as chronic infections in humans and biofouling in industrial settings. The adverse effects of biofilms on both the economy and public health have stimulated extensive biofilm research. However, long-term biofilm control especially the removal of established biofilms remains difficult. To address this challenge, we created micron scale topography using biocompatible shape memory polymers (SMPs). Our results demonstrated that mature biofilms can be effectively removed (up to 99.9% in minutes) by on-demand triggering of changes in surface topography. This method was found effective against biofilms of both Gram-negative and Gram-positive bacteria, demonstrating the potential of dynamic topography in biofilm control. However, the shape change of most biocompatible SMPs can only occur once, limiting the long-term application of this technology. To further engineer materials with repeating dynamic changes in surface topography, we were inspired to mimic beating cilia that protect higher organisms from infection by bacterial pathogens. We developed a platform technology that can create micron scale pillars on essentially any polymer of choice with synchronized movement at a desired frequency. Specifically, systematically designed poly(dimethylsiloxane) (PDMS) surface topographies filled with biocompatible superparamagnetic Fe_3O_4 nanoparticles were actuated with a programmed external magnetic field (5 mT) to mimic the beating of human motile cilia. These dynamic surface topographies were embossed onto PDMS surfaces to engineer a “living” surface with long-term antifouling effects. The antifouling properties of these surfaces were first validated against 48 h *Pseudomonas aeruginosa* PAO1 biofilms preformed before actuation. By actuating the pillars with a height of 10 μm, side width of 2 μm, and distance of 5 μm between adjacent pillars for 3 min, more than 99.9% biofilm cells were detached and removed by gentle rinsing. Release of nanoparticles from the pillars was negligible and no cytotoxicity to mammalian cells was observed. The dynamic topography of these surfaces can be induced remotely through wireless coupling. This unique feature along with the biocompatibility and potent antifouling effects make these surfaces promising candidates for future biomedical applications.

ORAL ABSTRACTS

[O35] BIOFILM CONTROL IN COOLING TOWERS: THE EFFECT OF BIODISPERSANTS ON FRESHWATER BIOFILMS DEVELOPED IN FLOW LANES

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Cooling towers of industrial systems can be affected by the development of substantial amounts of microbial biomass, including phototrophic biofilms. This requires cost intensive regular cleaning to avoid performance loss and equipment damages. The conventional control practice is hypochlorite treatment of circulating water, but biofilms are often recalcitrant owing to protection provided by their extracellular matrices. In this study, the effect of dispersants on biofilm cultures obtained from a natural inoculum (Elbe river) was investigated using a flow-lane incubator. The setup was fully controlled for irradiance, temperature and flow velocity. Ten incubator lanes, each accommodating 90 polycarbonate microscope slides positioned vertically, were inoculated and followed over 30 days. Two surface-active dispersants, one anionic and one non-ionic, were added separately at on-site concentrations to the circulating water at the beginning, after one, two and three weeks. Mineral particles (reflection), biofilm phototrophs (autofluorescence differentiating cyanos and algae), bacteria (SybrGreen) and glycoconjugates (AAL-lectin) were detected by multi-channel Confocal Laser Scanning Microscopy. Digital image data sets were assessed after thresholding for semi-quantitative analysis. Both dispersants affected biofilm growth, with a minor effect at day 8 but major differences evidenced after 15 day treatment. This was especially observed for the non-ionic dispersant, which appeared to primarily reduce the biofilm glycoconjugates. The results suggest that the non-ionic biodispersant has to be used in a different application strategy or in combination with a second chemical in order to push the biofouling below the “pain” threshold.

[O36] SUBSTRATE MEDIATED ENZYME PRODRUG THERAPY (SMEPT) TO COMBAT IMPLANT-ASSOCIATED BIOFILMS

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Staphylococcus aureus is a major causative agent of orthopedic implant infections. Conventional treatment often fail, resulting in implant replacement or lifelong treatment and resistance development.

Substrate mediated enzyme prodrug therapy (SMEPT) is a novel approach that allows for controlled, localized drug synthesis. In SMEPT, an externally administered, non-therapeutic prodrug converts to its corresponding active drug when encountering an immobilized enzyme imbedded within a thin polymer coating.

Our development of a novel synthesis method for generating antimicrobial glucuronide-prodrugs of moxifloxacin and ciprofloxacin have opened the door for using SMEPT in the field of medical microbiology. Upon administration of the antimicrobial prodrug, β-glucuronidase embedded in a multi-layered polyelectrolyte coating converts the prodrug to the active antimicrobial agent, enabling controlled local administration of the antimicrobial agent; change of treatment dose and type of antimicrobial. This project aims to provide proof-of-concept for the effect of our novel antimicrobial prodrugs on *Staphylococcus aureus* biofilms.

We proved enzymatic conversion of moxifloxacin-prodrug to active moxifloxacin by showing a reduction in in viable colony forming units from *S. aureus* biofilm following prodrug treatment, comparable to conventional moxifloxacin. We also showed the inhibitory effect of prodrug conversion on *S. aureus* grown on moxifloxacin-prodrug infused agar. Placing a surface-coated metal disk on the agar enabled conversion of prodrug into active moxifloxacin, resulting in complete clearance of bacterial growth. To investigate if surface-release of moxifloxacin prevents biofilm formation, we grew *S. aureus* in the presence of moxifloxacin-prodrug in a microfluidics device mounted on a coated glass surface. Imaging by confocal laser scanning microscopy showed that the conversion of moxifloxacin-prodrug under continuous flow prevented formation of *S. aureus* biofilm by greatly reducing the amount of surface attached bacteria.

In conclusion, we have shown proof-of-concept for the effect of our novel antimicrobial glucuronide-prodrugs on treatment of *S. aureus*. Our work paves the way for advances in the use of locally induced antimicrobial treatment and further research into novel ways of utilizing local conversion of antimicrobial glucuronide-prodrugs.

ORAL ABSTRACTS

[O37] Invited Lecture: INTERROGATING THE INTERPLAY OF METABOLISM AND STRUCTURE IN BACTERIAL BIOFILMS

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Though studies of signaling cascades can reveal important mechanisms driving multicellular development, the models that emerge often lack critical links to environmental cues and metabolites. We study colony biofilm formation in the bacterium *Pseudomonas aeruginosa*, with a focus on the effects of extra- and intracellular chemistry. *P. aeruginosa* produces oxidizing pigments called phenazines that affect biofilm morphogenesis. While wild-type colonies are relatively smooth, phenazine-null mutant colonies are wrinkled. Initiation of wrinkling coincides with a maximally reduced intracellular redox state, suggesting that wrinkling is a mechanism for coping with electron acceptor limitation. Mutational analyses and in situ expression profiling have revealed roles for redox-sensing regulatory proteins and respiratory enzymes, as well as genes involved in matrix production, in the morphogenetic responses of biofilms to redox conditions. To characterize endogenous electron acceptor production, we have developed a novel chip that serves as a growth support for biofilms and allows electrochemical detection and spatiotemporal resolution of phenazine production in situ. We are further developing this chip for detection of various redox-active metabolites. We have also begun to apply a new technique for monitoring phenazine-dependent metabolism within a colony biofilm that relies on stable isotope labeling and stimulated Raman scattering (SRS) microscopy. Through these diverse approaches, we are developing a broad picture of the mechanisms and metabolites that exert an integrated influence over redox homeostasis in *P. aeruginosa* biofilms.

[O38] NOVEL USES FOR SYNCHROTRON RADIATION IN THE STUDY OF BIOFILMS

Ben Libberton[1]

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The field of biofilm research has pioneered the use of new technologies to study both fundamental and applied aspects of biofilm. Biophysics, microfluidics and advanced microscopy techniques have significantly changed the landscape of biofilm research and increased our understanding of how microorganisms live in complex, structured communities. The more we learn about these communities, the greater the need for technology that can deliver high spatial and temporal resolution data to help us unravel the complexity within them. Recent developments in synchrotron technology has dramatically expanded the potential uses in biological sciences. Traditionally, synchrotron X-rays have been used mainly by structural biologists to obtain the structures of proteins but now there is a wider scope for them to be applied to whole cells or tissues to provide atomic scale resolution over vastly larger areas. These advancements make X-rays useful additions to the biofilm researchers toolbox. On the one hand, synchrotron facilities are complex and require a lot of specialised knowledge to operate, however, they are also user facilities. This means that most synchrotrons are set up with user offices to help new users start experiments and interpret results. At the newly opened MAX IV Laboratory in Lund there are several beamline experimental stations that lend themselves to the study of biofilms.

BioMAX – state of the art structural biology experimental station
SoftiMAX – X-ray microscopy generating confocal-like images with element distribution
CoSAXS – X-ray scattering to analyse the dynamics of micro- and nanoscale structure
HIPPIE – A spectroscopy technique that can be performed at ambient pressures on living samples

The use of synchrotron radiation in microbiology is just beginning and there has never been a better time to start using this powerful technology to study biofilms.

[O39] INTRODUCING A NOVEL, FULLY-AUTOMATED CULTIVATION AND SCREENING TOOL FOR THE STRUCTURAL AND MECHANICAL INVESTIGATION OF BIOFILMS BY MEANS OF OPTICAL COHERENCE TOMOGRAPHY

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In order to profit from beneficial biofilms or get rid of harmful ones, there is a need for better understanding biofilm development and structure. In addition to structural parameters the determination of material properties and resulting mechanical behavior (e.g., shear stress induced deformation) are also important. Hence, capturing the dynamics of material properties and mechanical behavior throughout a biofilm cultivation is of great interest and importance.

Optical coherence tomography (OCT) is an imaging modality ideal for biofilms since it allows for the monitoring of structure and deformation in real-time. Consequently, the mechanical characterization of biofilms can be performed *in situ* and non-invasively during biofilm cultivations. To gain a fundamental understanding of the relation between cultivation conditions and biofilm characteristics (e.g, structural, mechanical) a monitoring setup is required which allows for standardization of methods and analyses.

We thus developed a fully automated screening setup for lab-scale experiments. It is a robotic platform with an experimental layer on which mini-fluidic flow cells are operated in parallel (n ≤ 40). Visualization if biofilm developing is achieved by OCT imaging. The entire system is fully automated - incl. OCT positioning, focusing and dataset acquisition - allowing for direct inspection of biofilms in real-time and 3D. This methodology enables a valid description of the underlying interactions including a statistic treatment. Hard- and software are open source and can easily be adapted to specific experimental requirements.

Initial experiments investigated the effect of Fe^{2+} in the cultivation medium on the stability of *Bacillussubtilis* biofilms. Therefore, biofilms were cultivated in mini-fluidic flow cells (n = 8; sticky slides 0.4, ibidi GmbH, Martinsried, Germany) and exposed to different shear stress levels. Biofilm development was monitored daily applying OCT without user actions.

[O40] NANOPARTICLE-BASED CHEMICAL IMAGING IN BIOFILMS AND TISSUES

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Biofilms exhibit variable structural complexity, which modulates the chemical microenvironment and can lead to pronounced heterogeneity in the distribution and activity of microbial populations. This complicates the mapping of microbial activity in biofilms. Microsensors only provide a limited amount of detailed point measurements not fully accounting for the heterogeneous structural and chemical landscape in biofilms. Planar optical sensors can map the chemical conditions at the base of biofilms, while the chemical conditions at the flow-exposed upper biofilm surface cannot be mapped by planar optode based imaging. Here we report on novel chemical imaging approaches for mapping O_2 and pH at high spatio-temporal resolution over biofilm, coral and plant tissue surfaces coated by luminescent sensor nanoparticles. Furthermore, we present first data on intra-tissue/biofilm O_2 measurements with nanoparticles perfused into the sample. We give an overview of relevant instrumentation, calibration and application of such nanoparticle-based chemical sensing in different natural and constructed biofilms.

BIOFILMS 8

[P1] RSMA+HFQ BINDING TO VFR MRNA CAUSES CASCADE REGULATION VIA FLEQ TO CONTROL PSEUDOMONAS AERUGINOSA BIOFILM POLYSACCHARIDE PEL

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Expression of biofilm extracellular polysaccharide is often controlled by multiple regulatory elements. The PEL polysaccharides are one of the major biofilm matrix components produced by *Pseudomonas aeruginosa*. The post-transcriptional regulator RsmA has been implicated in the control of *pel* expression, but the only evidence to-date originates from transcriptomic studies. The RsmA protein binds to target mRNAs to modulate ribosomal access and thereby translation. RsmA is also known to de-stabilize some target mRNAs by unknown mechanisms. Here we show that RsmA is not a direct regulator of PEL. Rather, we trace the role of RsmA in PEL expression to the inhibition of the virulence transcription factor Vfr required for efficient transcription of *fleQ*. FleQ in turn, is a known transcription factor of the *pel* operon. Furthermore, we show that RsmA cannot bind *vfr* mRNA alone, but requires the RNA chaperone protein Hfq. This is the first example where an RsmA protein family member is demonstrated to require another protein for RNA binding.

[P2] STAPHYLOCOCCUS EPIDERMIDIS DOES NOT RELY ON POLYSACCHARIDE PRODUCTION WHEN FORMING BIOFILM IN HUMAN PLASMA

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Background: *Staphylococcus epidermidis* is predominate in implant-associated infections as it is excellent at forming biofilms. The extracellular matrix of *S. epidermidis* was first thought to consist mainly of polysaccharide intercellular adhesin (PIA), encoded by the *icaADBC* operon. However, many clinical isolates do not contain this operon. A giant surface-bound extracellular matrix-protein (Embp) may be relevant for *in vivo* biofilm. *S. epidermidis* may depend on either PIA or Embp and eDNA in forming biofilms. This study aims to determine the importance of Embp versus PIA for biofilm formation in standard laboratory media and in *in vivo*-like media with human plasma, and to learn how these matrix components affect mechanical properties and antibiotic resistance of biofilms.

Materials/methods: The strains included were *S. epidermidis* 1585WT (deficient of *icaADBC)*, and derivative strains that either lacked Embp, expressed Embp, or expressed *icaADBC* from a plasmid when induced. Strains were grown statically or under flow in standard laboratory media (BHI or TSB) or BHI supplemented with 10% or 50% human plasma. Confocal laser scanning microscopy determined biofilm structure and biovolume, and antibiotic binding was visualised with bodipyFL-vancomycin. Atomic force microscopy determined mechanical properties by nanoindentation.

Results: Three-dimensional imaging revealed that *S. epidermidis* only require polysaccharides for biofilm formation in absence of human plasma. Embp-expressing, PIA-negative strains that were biofilm-negative in standard laboratory media formed substantial biofilm amounts if human plasma was present, and there were no visible differences between PIA-producing and non-producing strains. Vancomycin penetrated all biofilms regardless of biofilm architecture, however, not all cells bound vancomycin, suggesting cell-to-cell variation in activity and susceptibility. PIA-producing biofilms were softer and more viscoelastic than biofilms relying on Embp (Welch's t-test, mean±SD: 0.8763±0.5206, n=3, $p<0.05$; and 1.263±0.4033, n=3, $p<0.05$; vs. 2.616±0.2433, n=3).

Conclusions: We propose that the assumed importance of polysaccharides for biofilm formation is an artefact from growing biofilms in standard laboratory media void of human matrix proteins. *S. epidermidis* employse diverse mechanisms for biofilm formation, which can be activated under different circumstances. We must therefore back to the drawingboard to study how *S. epidermidis* incorporates selv-produced and host-derived matrix components to successfully form antibiotic-resistant biofilms *in vivo*.

[P3] EFFECT OF SUCROSE ON BACTERIAL GROWTH, EXTRACELLULAR POLYMERIC SUBSTANCES PRODUCTION AND BIOFILM FORMATION IN TWO BACILLUS SUBTILIS STRAINS

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We studied the influence of growth conditions on bacterial growth, extracellular polymeric substances production and biofilm formation of the two *Bacillus subtilis* strains CIP52.65T (Marburg type) and CIP102517 (clinical origin).

The effects of Trypticase Soy (TS) supplementation with sucrose (20% w/v, TSS) were studied on planktonic growth, colony growth on agar plates, extracellular polymeric substances production and biofilm formation. Biofilm formation was obtained on filters deposited on agar plates, on immersed glass slides, and on stainless steel petri dishes, depending on the experiments. The generation time of planktonic cells increased from 31 to 59 min for CIP52.65T and from 25 to 46 min for CIP102517 in TS and TSS broth, respectively. The diameter of a macro-colony on the agar medium surface increased from 1.2 (+/-0.1) to 8.4 cm (+/-0.1) for CIP52.65T and from 2.1 cm (+/-0.1) to 7 (+/-0.2) for CIP102517 after 24h in TS and TSS agar, respectively. Confocal scanning laser microscopy analysis of biofilms formed on glass slides and infrared spectroscopy of biofilms formed on filters on agar plates showed an increase in the matrix content in proteins and polysaccharides in the presence of sucrose. The rheological analysis of biofilms formed in stainless steel cups showed that the presence of sucrose induced huge increases in conservation and loss moduli values for the two strains: G' increased from 10 to 208 Pa for CIP52.65T and from 3.5 to 82.5 Pa for CIP102517; G'' increased from 2 to 53 Pa for CIP52.65T and from 1.5 to 26 Pa for CIP102517, respectively.

All these results showed that the presence of sucrose at high concentration induced an increase in the production of extracellular matrix polymers at the expense of cell growth, leading to a different organization of the biomass and to a modification of the corresponding mechanical properties.

Keywords: *Bacillus subtilis*, extracellular polymeric substances, matrix, biofilm, mechanical properties

[P4] THE DUAL ROLE OF PSEUDOALTEROMONAS PSCICIDA BIOFILM FOR THE CORROSION AND INHIBITION OF CARBON STEEL ALLOYS

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It is well-known that bacteria and their metabolic byproducts can attach to the surface and induce the corrosion acceleration or inhibition. The microbiologically influenced corrosion is closely related to the biofilm formation. Generally, the biofilm is composed of bacterial cells, extracellular polymeric substance (EPS) secreted by bacteria, corrosion products, and so on. While some components can promote the steel corrosion, some have a protective effect for the steel. The study of biofilm community structure may provide information on the interactions of biofilm populations with metal alloys at different conditions. In this work, two different culturing conditions, shaker incubator and flow cells, were used to incubation of *P. pscicida* isolates on carbon steel (CS) surfaces and then the corrosion behavior of metal alloys and biofilm structure were studied. It is found that, a heterogeneous and patchy biofilm was formed on the CS in flow cell; while the biofilm covered homogenously whole CS surfaces incubated in shaker and reduce the corrosion rate after 2 weeks. CLSM images (Fig. 1) also confirmed the presence of homogenous biofilm on the surface after 2 weeks which almost included died cells, but in the flow cells a patchy biofilm is composed of died and live cells. When the biofilm was removed from CS surfaces, a lot of cracks were examined on the surface exposed to *P. pscicida* in flow cells for 2 weeks. However, intergranular corrosion was observed on the samples were incubated in shaker, but the surface was almost intact against of localized corrosion. So, it can be concluded that the biofilm structure will give information about the corrosion process of alloys.

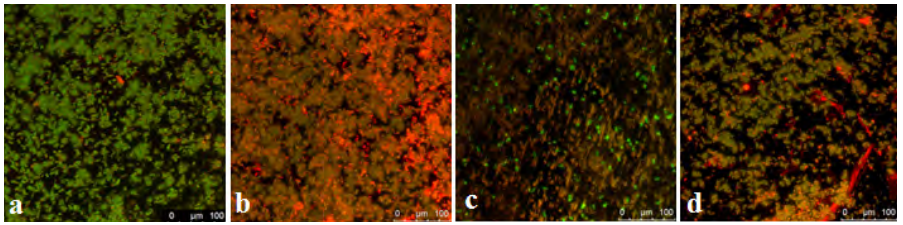

Fig. 1 CLSM images of CS samples exposed in artificial seawater containing *P. pscicida* in shaker incubator (a,b) and flow cells (c,d) for one and 2 weeks, respectively.

[P5] MEMBRANE DISTILLATION BIOFOULING: IMPACT OF FEED WATER TEMPERATURE ON BIOFILM CHARACTERISTICS AND MEMBRANE PERFORMANCE

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Reclamation of highly contaminated water has prompted the need for novel sustainable membrane technologies, such as membrane distillation (MD). Water flux in MD is driven by a water vapor pressure gradient between heated feed and cold distillate water separated by hydrophobic porous membranes. The greatest challenge for membrane-based wastewater reclamation is microbial fouling (biofouling), the establishment of a bacterial consortium within self-produced extracellular substances on the membrane and spacer surfaces. The goal of this study is to determine the effect of feed water temperature on biofilm characteristics and MD system performance. Dynamic biofouling experiments with thermophilic *Anoxybacillus gonensis*, isolated from municipal wastewater, were conducted in a bench-scale system with a cross-flow membrane cell. Feed temperature was set to 45, 55 or 65°C, while distillate temperature was adapted to reach an initial distillate water flux of 25±1L/m^2/h. System performance was continuously monitored via distillate water flux and conductivity. Biofilm was analyzed using total organic carbon, optical coherence tomography, confocal laser scanning microscopy (CLSM) and flow cytometry. Membrane performance was strongly impaired by biofouling, with best performance at 45°C. Flux decline was highest (87%) at 55°C, while at 65°C distillate conductivity increased more than 100fold and bacteria were observed in the distillate due to severe membrane wetting. Biomass and cell number on the membrane were highest at 55°C, while no significant differences between temperatures were observed on the spacer. Flow cytometry as well as CLSM showed more dead than live bacteria at all temperatures. Our results indicate that feed water temperature can be a determining factor for wastewater reclamation with MD. The impact of other parameters, such as flow conditions and bacterial community composition remain to be determined in future studies in order to maximize the potential of MD for wastewater reclamation.

[P6] A STUDY OF THE INTERACTION BETWEEN FLUORESCENTLY LABELLED SILICA NANOPARTICLES AND BIOFILMS OF P. FLUORESCENS AND P. PUTIDA

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The surface associated communities of microorganism in biofilms are encased in a matrix of extracellular polymeric substances (EPS). The EPS is made up of mainly polysaccharides, proteins, nucleic acids and lipids and plays an important role in maintaining the integrity of the biofilm[1]. Although the general composition of the EPS is known, it can be highly variable among strains and also among different growth conditions for the same strain. Due to the large variety of biopolymers in nature and the difficulty in their analysis, EPS has been called 'the dark matter of biofilms'[2].

The difficulty in removing unwanted biofilms using conventional approaches has prompted the development of new technologies. Amongst these, engineered nanoparticles show some promise. It has been previously established that nanoparticles possess anti-bacterial properties but there is little understanding of the mechanisms involved in these actions[3], particularly from the point of view of the role of the EPS. The complexity of the biofilm matrix together with the complexity of the nanoparticles within the environment of the matrix has hampered our fundamental understanding of the nanoparticle-biofilm interaction.

In order to develop a comprehensive understanding of the matrix of the biofilm and the interaction with nanoparticles, a range of experiments were carried out using two engineered bacterial strains: mCherry-expressing *Pseudomonas fluorescens* and GFP-expressing *Pseudomonas putida*. The biofilms were grown in both well-plates and on glass slides and were exposed to fluorescently labelled silica nanoparticles with different size and surface charge. Through UV-VIS and fluorescent spectroscopy and confocal scanning laser microscopy, it was possible to study the interaction between the nanoparticles and the biofilm and gain useful information on the uptake and diffusion of the nanoparticles within the biofilm matrix.

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[P7] BIOFILM FORMATION AND DISPERSAL OF KOCURIA VARIANS G23

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Kocuria varians is a Gram-positive bacterium belonging to the family *Micrococcineae*. The bacteria are predominantly isolated from biofilms in food-processing plants. Even though *K. varians* is considered to be non-pathogenic, studies have shown that native bacteria in these environments, such as *K. varians*, can promote the growth of potential pathogens, e.g. *Listeria monocytogenes*. A better understanding of biofilm growth of naturally occurring bacteria in food processing environments may therefore reduce the risk of product contamination by potential pathogens.

Here we report a study of biofilm formation of *K. varians* G23, originating from a Danish slaughterhouse under flow and static conditions. In the biofilm, *K. varians* G23 formed rigid cube-shaped structures followed by bursting and mass bacterial dispersal in both static and flow conditions. Comparison of five closely related *Kocuria* species revealed that the cubic biofilm structure was unique for *K. varians* G23.

The *K. varians* G23 biofilm was subjected to Confocal Laser Scanning Microscopy (CLSM) and component-specific stains to elucidate the unique biofilm structure. The fluorescent dye CDy11, which has particular affinity for amyloid structures, was localized on the surfaces of cells, consistent with earlier reports of amyloid-coated bacteria from other Gram-positive species[1]. Addition of the amyloid inhibitor epigallocatechin-3-gallate (EGCG) to the media in the BioFlux flow system strongly impeded *K. varians* G23 biofilm formation and ultimately stopped it at 100 µM EGCG. The effect was reversed when EGCG was removed from the system. Addition of EGCG did not affect the growth rate of planktonic *K. varians* G23 cultures. Currently, biofilm samples from *K. varians* G23 are being analyzed by label-free quantitative proteomics which hopefully will help identify potential amyloid proteins.

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[P8] HOST PROTEINS DETERMINE MRSA BIOFILM STRUCTURE AND INTEGRITY

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Human extracellular matrix (hECM) proteins aids the initial attachment and initiation of an infection, by specific binding to bacterial cell surface proteins. However, the importance of hECM proteins in structure, integrity and antibiotic resilience of a biofilm is unknown. This study aims to determine how specific hECM proteins affect *S. aureus* USA300 JE2 biofilms.

Biofilms were grown in the presence of synovial fluid from rheumatoid arteritis patients to mimic *in vivo* conditions, where bacteria incorporate hECM proteins into the biofilm matrix. Difference in biofilm structure, with and without addition of hECM to growth media, was visualized by confocal laser scanning microscopy. Two enzymatic degradation experiments were used to study biofilm matrix composition and importance of hECM proteins: enzymatic removal of specific hECM proteins from growth media, before biofilm formation, and enzymatic treatment of 24-hour-old biofilms.

hECM addition changed the overall biofilm structure, with larger dispersion of cells within the biofilm matrix. Fibrin, elastin, and collagen were important in forming and maintaining the biofilm structure. Their absence, from growth media, reduced biofilm formation 5-fold, indicating their importance in biofilm initiation. Their enzymatic degradation, in an established biofilm, caused dispersal, showing that these proteins are critical for structural integrity. A combination of antibiotics with hECM degrading enzymes did not improve the treatment outcome.

We conclude that while hECM proteins are an integral part of the biofilm matrix, we find no evidence that these matrix components are directly responsible for the biofilm's unique antibiotic resilience. The hECM proteins are however highly important in determining biofilm structure and initiation. When utilizing *in vitro* biofilm infection models, we therefore recommend addition of hECM proteins to standard growth media, in order to mimic biofilm properties and structure seen *in vivo*.

[P9] BIOFILM MATRIX FUNCTIONALITY

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The extracellular matrix represents an essential part of microbial biofilms and bio-aggregates. The matrix acts as a multi-functional structure and is considered to be of high importance in different stages of biofilm development. Nevertheless the matrix remains a challenge in many respects. The issues comprise the (1) biosynthesis, release and identity; (2) structural and biochemical analysis; (3) characterisation by -omics; (4) interactions and reactivity; (5) mechanics and dynamics; and (6) control and degradation. Focus of this discussion poster will be on matrix functionality. Several functions of the biofilm matrix are known and established, however there are emerging functions which are not readily known. The poster will present a comprehensive list of functions including: architecture, cryo-/osmo-protection, sorption, precipitation, adhesion, repellent, cohesion, connectivity, activity, surface-activity, information, competition, nutrition, motility, transportation, communication, conduction, dispersion, translocation (up till 2016) as well as more recently fractal formation and aggregation, toxin, microgel formation, hydrophobicity and mechanical properties, maturation, mineral scaffold, floating biofilm architecture, stress response (host, nutrition, desiccation, toxic compounds), cooperation-competition, protein scaffold, micro-domain production, avalanche motion, micro-channel formation, liquid crystal formation, growth on hydrocarbons, community positioning-evolution, detachment regulation, stratification, intercellular interaction, controlled bi-functionality, electron transfer, redox-activity, phage protection, socio-microbiology, ecosystem service. These functions will be linked to biochemical identity and briefly commented upon. The biofilm matrix functionality key-words listed will provide the basis for intense discussions of matrix function in different biofilm systems and various habitats.

[P10] EXTRACELLULAR DNA IN ANOXYBACILLUS AND GEOBACILLUS BIOFILMS

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Some of thermophilic bacilli members, such as *Anoxybacillus* and *Geobacillus* are common contaminants of dairy products. The biofilm matrix are encased in polysaccharides, protein, and extracellular DNA. The eDNA in the matrix of biofilms is required for the structural integrity of many biofilms. The current study presents the role of eDNA in biofilms of thermophilic biofilms for the first time. *G. thermodenitrificans* DSM 465^T and *A. flavithermus* DSM 2641^T strains were preferred. Tyriptic Soy Broth with appropriate pH value and salinity for each strain was used to maintain optimum conditions for assays. DNase I treatment was performed for both pretreatment and post treatment points. The broth cultures were prepared at appropriate conditions (without NaCl TSB, pH 7.5, 55°C for DSM 2641^T; 1.0% NaCl containing TSB, pH 7.5, 65°C for DSM 465^T) for 18 h and folllowing the incubation, the cultures were treated with 100 µg/mL DNase I (as final concentration) for 60 min at 37°C. The inactivation of the enzyme was also carried out before assays. 140 µL of the enzyme-treated and not treated samples were placed into 96-well polystyrene plates and the plates were incubated at appropriate conditions for 24, 48 and 72 hours. After the incubation, crystal violet assay was performed to determine biofilm yields. 48 h-old mature biofilm samples were also prepared for DNase I treatment. The mature biofilm samples were treated with 100 µg/mL enzyme for 1, 2, 4 and 12 h. The eDNA and genomic DNA of the strains were also purified and compared by electrophoresis. The mature biofilms samples (treated and control samples) were also stained with DEAD/LIVE BacLight kit and analysed by CLSM.. The adhesion was drastically effected by enzyme treatment. The mature samples were dispersed effectively and these results were also confirmed with CLSM analysis. The molecular weight patterns of gDNA and eDNA were also found to be different. The sanitation procedures based on enzymatic treatment can be considered.

Key words: Thermophilic bacilli, Anoxybacillus, Geobacillus, biofilm, eDNA

[P11] SEARCHING FOR NOVEL FUNCTIONAL AMYLOIDS IN BIOFILMS

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The identification of novel amyloids is key to understand the many roles of amyloids in biofilm structure and function. Unfortunately, isolation of amyloids is a challenging and not always successful process. However, by taking advantage of the structural stability and the polymeric nature of the amyloids, compared to most other proteins, a new method has been developed to screen for potential amyloids. Utilizing formic acid's ability to depolymerize an amyloid fibril, the samples were freeze-dried and dissolved in increasing concentrations of formic acid (0-100%) and then analyzed for a characteristic increase in protein abundance at a higher percentage of formic acid, where they are dissolved. The proteins were identified using label-free quantitative LC-MS/MS, and plots of all identified proteins were studied for potential amyloid patterns. The method has successfully been evaluated on two established Gram-negative amyloid systems and used to find a novel amyloid candidate in the potential pathogenic *Aeromonas* sp. Proof of concept for the application to complex microbial biofilms was done using activated sludge, where amyloid producing E. coli was added and subsequently traced. In addition, another potential novel amyloid candidate was found in the activated sludge biofilm.

[P12] MECHANISMS OF EXTRACELLULAR DNA INCORPORATION WITHIN THE SALMONELLA TYPHIMURIUM BIOFILM

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Salmonella enterica serotype Typhimurium is a Gram negative, motile bacterium that causes infection via the fecal oral route. These bacteria, as well as other *Enterobacteriaceae,* produce amyloid proteins called curli as a major proteinaceous component of their biofilm. Amyloids, like curli, can be produced by bacteria as well as humans. Accumulation of amyloids in humans can be found in complex human diseases such as Alzheimer's Disease. It is thought that the amyloids seen in humans are the result of misfolded aggregates, however bacterial amyloids have been found to be functional. Amyloid curli complexes with extracellular DNA within the biofilm. When purified from the matrix, these complexes are recognized by a variety of receptors. Previous studies have shown that the amyloid portion of these curli complexes is recognized by the Toll Like Receptor 2 (TLR2)/TLR1/CD14 complex as well as the NLRP3 inflammasome, leading to the production of proinflammatory cytokines such as interleukin-1 beta (IL-1β). Additionally, the eDNA within the complex has been shown to induce type I Interferon (IFN) production and autoimmunity through activation of TLR9. Recently, we determined variable amounts of DNA within the curli prepared in the lab. We have been testing two protocols and were able to manipulate the amount of DNA in the curli complexes. Controlling the variable of high and low amounts of DNA within the curli would be invaluable in future studies to delineate the role of eDNA in the autoantibody responses to curli treatments. To test this idea, we will treat groups of mice with high DNA and low DNA curli preps at the same amyloid concentration and determine autoantibody production. We expect that the autoantibody production will correlate with the amount of DNA present in the preps i.e., low DNA preps will induce little to no autoantibodies while high DNA preps will induce high autoantibodies. This will also provide insight into the role of curli in the autoimmune response seen in these mice. Additionally, moving forward we plan to test the role of NLRP3 and STING in their interactions with the high and low DNA content preps.

[P13] MOLECULAR MOTORS GOVERN LIQUID-LIKE ORDERING AND FUSION DYNAMICS OF BACTERIAL COLONIES

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Bacteria can adjust the structure of colonies to enhance their survival rate under external stress. Here, we explore the link between bacterial interaction forces and colony structure. We show that the activity of extracellular pilus motors enhances local ordering and accelerates fusion dynamics of bacterial colonies. The radial distribution function of mature colonies shows local fluid-like order. The degree and dynamics of ordering are dependent on motor activity. At a larger scale, the fusion dynamics of two colonies shows liquid-like behavior whereby the ratio of surface tension over viscosity decreases with decreasing motor activity.

[P14] THE TRANSCRIPTIONAL REGULATION OF PSEUDOMONAS PUTIDA BIOFILM ADHESIN GENE LAPA

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Pseudomonas putida forms biofilms on the roots of agriculturally important plants, protecting the plant from pathogens. *P. putida*'s biofilm is proteinaceous and known to contain two large adhesion proteins, LapA and LapF. LapA is the larger of the two (it is the largest protein in *P. putida* at 888 kDa) and seems to be essential for biofilm formation in all conditions. The presence of LapA on the cell surface is controlled by a well-described mechanism of cutting LapA off the cell.

We are investigating how the production of LapA is controlled on the transcriptional level. As the promoters of *lapA* were undescribed, we took it upon ourselves to search for them. Transcription of *lapA* in LB-grown bacteria is initiated from a surprisingly large number of promoters – six – and three of those display a moderate RpoS-dependence.

Secondly, we studied if and how *lapA* transcription is regulated by the global regulator protein Fis that we have previously linked to biofilm formation. We show that, indeed, Fis controls the transcription of *lapA*. *In vitro,* Fis binds the *lapA* promoter area in six specific positions and *in vivo* Fis activates the transcription of *lapA* while overexpressed in the cells. Two of the Fis binding sites, Fis-A7 and Fis-A5, are responsible for the positive effect of Fis on the transcription of *lapA*. Fis binding to the Fis-A7 site increases the level of transcription from the most distal promoter of *lapA*, whereas Fis binding to the Fis-A5 site could be important for modifying the promoter area topology.

These results shed light on the complex transcriptional regulation of *lapA* with its six promoters and show a potential mechanism how Fis regulates biofilm formation via LapA.

[P15] FIS' INVOLVEMENT IN PSEUDOMONAS PUTIDA BIOFILM FORMATION

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The regulators involved in the formation of *Pseudomonas putida* biofilm have been studied quite thoroughly. It is mostly described as a FleQ and c-di-GMP dependent biofilm, but little attention is paid to other potential regulators.

A global transcriptional regulator Fis participates in the biofilm formation of *P. putida*. We have shown that *fis*-overexpression enhances *P. putida*'s biofilm formation and have ascertained some mechanisms how Fis can participate in this process.

P. putida's biofilm matrix in LB medium is proteinaceous and two large adhesive proteins, LapA and LapF, are known to play a significant role in the attachment of the cells to surface and cell-cell interaction, respectively. Therefore the regulation of these genes seems to be crucial for understanding the biofilm formation in *P. putida*.

We have shown that Fis binds the upstream DNA of *lapA* and *lapF* genes *in vitro*, and respectively activates and represses the transcription of these genes according to β-galactosidase assay. Two such Fis-binding sites were identified for *lapA* and one, overlapping with the promoter, for the *lapF* gene. The mutation in these specific binding-sites abolished Fis regulation of transcription.

Additionally, Fis influences *P. putida* surface hydrophobicity that can be crucial for cell to cell aggregation or nonspecific adhesion to the surface. We have shown that cells lacking LapF are less hydrophobic than wild-type cells in stationary growth phase. The hydrophobicity of cells is restored by expression of *lapF* from IPTG-controlled promoter P_{tac} or mutating Fis-binding site Fis-F2 in front of the *lapF* gene.

In sum, Fis has a dual role in biofilm formation. It induces the expression of *lapA* and thereby enhances biofilm formation, and reduces hydrophobicity of cell surface that can weaken the cell-cell interaction in mature biofilm.

[P16] THE ROLE OF BIOFILMS IN THE PATHOLOGY AND TRANSMISSION OF THE HUMAN PATHOGENIC YERSINIAE

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There are three species of human-pathogenic Yersiniae; two which cause gastrointestinal disease (*Yersinia pseudotuberculosis* and *Yersinia enterocolitica*) and one which causes plague (*Yersinia pestis*), one of the deadliest diseases in human history. All are capable of a method of bacterial messaging known as Quorum sensing (QS), which allows individual cells to communicate in a density dependent manner and has been shown to regulate several virulence phenotypes, including biofilm formation.

Biofilms are especially important for *Y. pestis*, as transmission of plague relies on the blockage of the vector insect's digestive tract. This occurs when the bacteria attach to the chitinous gut lining, forming biofilms that can then be regurgitated with subsequent blood meals. Understanding the mechanisms of biofilm development is therefore important, as recent research has suggested a more prominent role in the spread of plague for human insect pathogens, such as fleas (*Pulex irritans*) and body lice (*Pediculus corporis*), compared to the traditional view of rats being the main transmission sources.

In the Yersiniae, biofilms consist of aggregations of bacteria embedded in a protective extracellular polymeric substance (EPS) matrix, composed primarily of the polysaccharide *N*-acetyl-D-glucosamine (GlcNAc). The production of GlcNAc, is regulated by NagC. NagC has been well studied in a number of bacteria, including *E. coli* and *Y. pseudotuberculosis* (which is genetically very similar to *Y. pestis*), and it is known to have various functions, including the regulation of GlcNAc production.

Caenorhabditis elegans are used as a model for biofilm formation, as bacteria are capable of establishing biofilms on the outside of the worms. By deleting *nagC*, we aim to ascertain the role of NagC in the Yersiniae's ability to regulate GlcNAc and therefore their ability to produce biofilms. This project aims to establish the role of NagC in the formation of Yersiniae biofilms in both *C. elegans* and *in vitro*, with a view to extending this research into flea and human body lice models.

[P17] THE ROLE OF THE STRESS SURVIVAL ISLET 2 IN BIOFILM FORMATION OF LISTERIA MONOCYTOGENES

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Listeria (*L.*) *monocytogenes* is the causing agent of listeriosis, a rare but severe disease associated with high mortality rates in humans. *L. monocytogenes* is able to survive a variety of stress conditions and to form biofilms leading to the colonization of different niches like the food processing environment. Recently we showed that the stress survival islet 2 (SSI-2), consisting of the genes *lin0464*, encoding a transcription factor, and *lin0465*, encoding a PfpI protease, is beneficial for survival under alkaline and oxidative stress conditions, which are routinely encountered in food processing environments. Strains harboring SSI-2 mainly belong to sequence type 121 (ST121). ST121 strains are predominantly isolated from food and food processing environments and known to persist for months and even years. The aim of this study is to investigate the role of SSI-2 in adhesion and biofilm formation.

Therefore we generated deletion mutants of *lin0464* and *lin0465* and the corresponding complemented strains using the persistent strain 6179. To determine adhesion, bacteria were grown in polystyrene wells at 25°C and 37°C for 24 hours and at 10°C for four days using two different media (Brain-Heart-Infusion supplemented with yeast extract (BHI-Y) and BHI-Y supplemented with 4% NaCl), respectively. Adhesion was determined using the crystal-violet binding assay. To study biofilm formation we plan to establish a continuous biofilm model using flow chambers.

All strains showed adhesion in polystyrene wells. The addition of 4% NaCl to the growth medium resulted in a decreased adherence in all used strains. When the gene *lin0465* encoding the protease was constitutively expressed, we observed a significant increase ($p<0.05$) of adhesion at all investigated temperatures (10°C, 25°C and 37°C). This effect was most drastic at 10°C which is the usual temperature in the food processing environment. In conclusion these preliminary results indicate that the protease Lin0465 has a role in adhesion, potentially resulting in increased biofilm formation.

[P18] AEROBIC VITAMIN B12 BIOSYNTHESIS IS ESSENTIAL FOR PSEUDOMONAS AERUGINOSA DURING BIOFILM GROWTH

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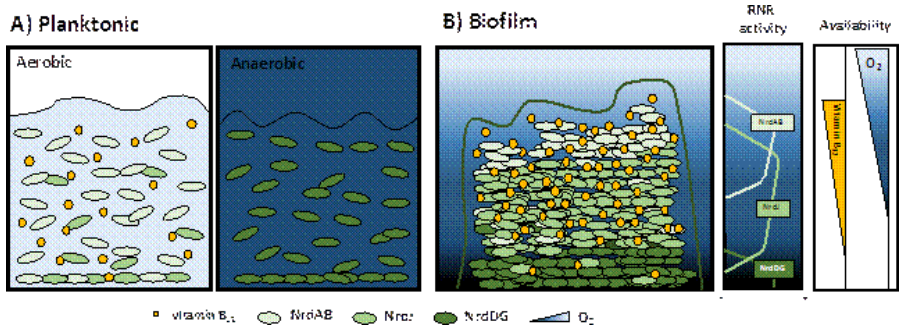
P. aeruginosa is a major pathogenic bacterium in chronic infections and is a model organism for studying biofilms. *P. aeruginosa* is considered an aerobic bacterium, but in the presence of nitrate, it also grows in anaerobic conditions. Oxygen diffusion through the biofilm generates metabolic and genetic diversity in *P. aeruginosa* growth, such as in ribonucleotide reductase activity. These essential enzymes are necessary for DNA synthesis and repair. Oxygen availability determines the activity of the three-**r**ibo**n**ucleotide **r**eductase (RNR) classes. Class II and III RNRs are active in the absence of oxygen; however, class II RNRs, which are important in *P. aeruginosa* biofilm growth, require a vitamin B_{12} cofactor for their enzymatic activity.

In this work, we elucidated the conditions in which class II RNRs are active due to vitamin B_{12} concentration constraints (biosynthesis or environmental availability). We demonstrated increased vitamin B_{12} levels during aerobic, stationary and biofilm growth that activate class II RNR activity. Our results unravel the *P. aeruginosa* mechanisms for dNTP synthesis during biofilm growth.



[P19] DIVERSE LIGAND-BINDING DOMAIN COMBINATIONS AT THE DISTAL END OF BACTERIAL RTX ADHESINS ARE ZIP CODS FOR BIOFILM FORMATION

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Many Gram-negative bacteria produce Repeats-In-Toxin adhesion proteins (RTX adhesins) to facilitate microbial adhesion to a variety of biotic and abiotic substrates. These large, multi-domain proteins anchored on the outer membrane share a common architecture comprised of three regions: 1) an N-terminal cell-membrane-anchorage region; 2) a large extension region consisting of tens to hundreds of tandem Bacterial Immunoglobulin-like (BIg) domains; and 3) a C-terminal region differing widely in structure, with domains responsible for adhesion and cohesion. Bioinformatic analyses of putative RTX adhesins suggest that many of their C-terminal regions contain currently unidentified ligand-binding domains, or domain homologues whose ligands are not yet known. Here we illustrate the variability in C-terminal regions having solved the structure of several RTX adhesin ligand-binding domains from diverse bacteria. The oil-eating bacterium *Marinobacter hydrocarbonoclasticus* and the opportunistic pathogen *Aeromonas hydrophila* both have calcium-dependent ligand-binding domains, specific for sugar and protein, respectively. The *M. hydrocarbonoclasticus* sugar-binding domain is of the type used by another bacterium (*Marinomonas primoryensis*) to adhere to diatoms to form a multispecies biofilm, while that of *A. hydrophila* is similar to integrin and other extracellular-protein-binding domains. Using the structures of these recombinantly-expressed proteins in concert with a series of biochemical assays and binding arrays, we have begun to identify the ligands for each domain, and understand how differences in domain complement reflect the organism-specific substrate-binding needs.

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[P20] QUORUM SENSING MOLECULES REGULATE EPITHELIAL CYTOKINE RESPONSE AND BIOFILM-RELATED VIRULENCE OF PREVOTELLA SPECIES

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Activation of the host immune system by bacterial burden is critical to trigger the inflammatory response. Quorum sensing (QS) signaling regulates host inflammatory modulators against bacterial pathogenicity, and at the same time, regulates adhesion, motility and biofilm-related virulence of several bacterial species. We previously demonstrated that the QS molecule, dihydroxy-2,3-pentanedione (DPD) and its analogs, significantly inhibited the biofilm-related virulence of *Prevotella aurantiaca*. Here, we examined the effects of estradiol and QS signaling on 1) cytokine response of human gingival keratinocytes (HMK) against whole cell extract (WCE) of other *Prevotella intermedia* group organisms (*P. intermedia*, *P. nigrescens*, and *P. pallens*), and 2) biofilm formation of *P. intermedia*, *P. nigrescens*, and *P. pallens*. All experiments, which included 2 strains of each species, were performed in the presence or absence of estradiol, and different concentrations of DPD and its analogs (ethyl-DPD, butyl-DPD, and isobutyl-DPD). Concentrations of interleukin (IL)-1β, -6, and -8 were determined by a Luminex multiplex immunoassay, biofilm levels were examined by the Bradford assay, and the microtopography of the same biofilms were assessed by scanning electron microscopy (SEM) imaging. Concentrations of IL-6 and IL-8 were elevated when HMK cells were incubated with estradiol and WCE of *P. intermedia* and *P. nigrescens*, and were reduced when incubated with estradiol and *P. pallens*. Butyl-DPD neutralized the estradiol- and WCE-induced cytokine expression of HMK cells. In the absence of estradiol, biofilm formation was significantly inhibited with increasing concentrations of DPD and analogs. This inhibition was less obvious after the addition of estradiol to growth media. SEM micrographs revealed a decrease in biofilm mass most detectable among the strains *P. intermedia* ATCC 25611 and *P. nigrescens* ATCC 33563 and AHN 8293. Our results indicate that the QS molecule butyl DPD could significantly neutralize the epithelial cytokine response, and at the same time, inhibit biofilm-related virulence of the *Prevotella* species in strain- and dose-dependent manners.

[P21] THE ROLE OF THE PHOSPHODIESTERASE NBDA IN BIOFILM DISPERSAL OF PSEUDOMONAS AERUGINOSA

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The opportunistic human pathogen *Pseudomonas aeruginosa* causes a broad range of acute and chronic infections often involving the formation of biofilms. *P. aeruginosa* is able to switch between the sessile and the planktonic lifestyle by a process that is called biofilm dispersal. Several environmental cues have been identified to trigger biofilm dispersal. One of these cues are non-lethal concentrations of nitric oxide (NO). The activation of the dispersal process involves the degradation of the second messenger c-di-GMP (bis-(3'-5')-cyclic dimeric guanosine monophosphate) [1]. The protein NbdA (NO-induced biofilm dispersion locus A) was found to play a major role in the NO-induced biofilm dispersal response of *P.aeruginosa* [2]. The tripartite protein NbdA consisting of the conserved domains MHYT-AGDEF-EAL was postulated to be directly involved in NO sensing and c-di-GMP degradation. Biochemical analyses of recombinant protein variants lacking the transmembrane MHYT-domain revealed NbdA being an active c-di-GMP degrading phosphodiesterase (PDE). Functional complementation of an *E. coli* phosphodiesterase deficient strain with a truncated NbdA variant confirmed PDE activity *in vivo*. In order to study the impact of NO sensing on the enzymatic activity, full-length protein NbdA was recombinantly produced and subsequently purified via affinity chromatography.

While the main hypothesis for *nbdA*-mediated dispersal of biofilm postulates direct NO sensing via the MHYT-domain, initial experiments also suggest a transcriptional regulation of *nbdA* expression by NO [2]. In order to study the putative NO-responsive transcriptional network we are using promoter *lacZ*-fusions in the background of several mutant strains. The obtained data will be presented and discussed with respect to the current model of NO-induced biofilm dispersal in *P. aeruginosa*.

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[P22] MOLECULAR MECHANISM OF NITRIC OXIDE-MEDIATED REGULATION IN PSEUDOMONAS AERUGINOSA BIOFILMS DISPERSAL

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Previous studies have shown that low dose nitric oxide (NO) can trigger *Pseudomonas aeruginosa* biofilm dispersal by modulating the level of the intracellular secondary messenger cyclic dimeric guanosine monophosphate (c-di-GMP). Diguanylate cyclase (GGDEF motif) and phosphodiesterase (EAL/HD-GYP motif) activities are responsible for the synthesis and hydrolysis of c-di-GMP, respectively. Various sensor domains have been found to link environmental cues to GGDEF and EAL/HD-GYP activities modulation, of which PAS and MHYT domains were of our interest due to their potentials to bind NO. In *P. aeruginosa* PAO1, a total of 13 proteins containing either PAS-DGC+/PDE or MHYT-DGC+/PDE were thought to be responsible for the NO-induced biofilm dispersal and were selected as our targets for investigation of their relationships between NO responses and biofilm phenotypes.

A range of NO donors were first tested for their efficacies on dispersing *P. aeruginosa* biofilms, of which 250μM Spermine NONOate (S150) showed outstanding results in dispersing ~60% batch cultured PAO1 biofilms within only 2 hrs. Gene deletion was then applied to 13 candidates for phenotypic analysis of mutants. Phenotypic assays for 13 mutants suggested that PA0861 and PA5017 play essential roles in *P. aeruginosa* PAO1 for reducing intracellular c-di-GMP levels, enhancing swarming motility and triggering biofilm dispersal in response to NO. PA4959 is responsible for both twitching and swimming motility, which contribute greatly to the 3D structures of the biofilms formed. Deleting PA4959 led to much enhanced biofilm dispersal upon NO treatment, providing insight that the deficiency in both Type IV pili and flagella functions may facilitate the elimination of *P. aeruginosa* biofilms. This work has enhanced our understanding of the NO-c-di-GMP-swarming-dispersal pathway and its regulators in PAO1, shedding light on NO signaling mechanisms and providing potential new targets for therapeutic drug design.

[P23] GROWTH MODE AND CARBOHYDRATE SOURCE INDUCE SIGNIFICANT SURFACE PROTEOME CHANGES IN PROBIOTIC LACTOBACILLUS RHAMNOSUS GG

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Engineering bacterial cells for bio-industrial processes and applications has primarily been based on the use of planktonically growing cells. Yet, majority of the bacteria prefer to grow as biofilms, which clearly outperforms the capabilities of individual cells, as cells in the biofilm consortia are more robust. Aim of the present study was to compare the surface proteome composition (surfaceome) of the probiotic *Lactobacillus rhamnosus* GG grown in biofilm and planktonic states and on two metabolizable carbohydrates, glucose and fructose, known to affect acid tolerance, adherence and antimicrobial activity of this probiotic. The main idea was to explore how the two carbohydrates affect the biofilm formation efficiency and antigenicity of GG and if the observed phenotypes could be explained by the specific surfaceome changes. We show that switching the carbohydrate source from glucose to fructose increased the protein-mediated biofilm formation of GG over a wider range of concentration compared to glucose. Fructose in growth medium increased the surface-antigenicity of GG, as well as the number and abundances of the surfaceome proteins during both growth modes. The surface-proteins with classical cell-wall anchors/domains (e.g., PrtP, HtrA) and adhesive moonlighting (e.g., r-proteins, tRNA synthetases, PepN/PepC aminopeptidases, sulfatases, glycolytic and stress proteins) were remarkably more produced in biofilms grown on fructose. Although the identification data suggested higher abundances for the SpaA/SpaC pilus proteins on planktonic cells, we show that biofilm cells grown on fructose were more adherent to mucus than the planktonic cells grown on glucose. To explore this further, an additional anti-SpaC antibody-mediated mucus inhibition assay and whole-cell ELISA monitoring SpaC abundances were conducted, which suggested that the adhesive moonlighting proteins, indeed, conferred increased adherence to GG cells during growth on fructose. Finally, a whole-cell enzymatic assay measuring the PepN/PepC activity was performed, which indicated the highest aminopeptidase activity with fructose-associated biofilm cells, thereby confirming the proteomic results. In conclusion, this study shows that besides the pili the non-classical protein export contributes to adherent features of this well-known probiotic strain, and that fructose could provide a selective advantage for growth of GG in the gut environment.

[P24] TARGETING AMYLOID TO COMBAT BACTERIAL BIOFILM

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Staphylococcus Aureus and *Staphylococcus Epidermis* are the main cause of bacterial infections when performing implant surgery (1) Once the patient is infected, large doses of antibiotics are needed to kill the bacteria, impart because of the bacteria's ability to form biofilms. Bacterial biofilms are formed by communities that are embedded in a self-produced matrix of extracellular polymeric substances (EPS). The EPS matrix consists primarily of eDNA, gel forming polysaccharides and amyloids. We have studied the formation of amyloids by the phenol soluble modulins β1 and β 2 from S. Aureus and S. epidermidis in vitro and analyzed them by various techniques and found a remarkable pH dependent versatility in the structure of the amyloids formed compared to other functional amyloids. Based on this work, small molecules and peptidomimetics will later be screened for efficacy in reducing biofilm strength and antibiotic resistance. A successful outcome may establish new approaches to combine biocides with matrix-targeting compounds to combat biofilms in different settings.

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[P25] IMPACT OF THE QUORUM SENSING MOLECULE JAI-1 AND THE TYPVI SECRETION SYSTEM OF JANTHINOBACTERIUM IN BIOFILM FORMATION AND FUNGAL CELL DEATH

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Janthinobacterium spp. and bacteria affiliated with the genus *Duganella* are Gram-negative, motile, and aerobic bacteria, which are commonly isolated from soil and aquatic samples. While *Janthinobacterium* as well as *Duganella* appear to be non-pathogenic to humans, animals, and plants, they are well-known for their antifungal effects. So far, little is known about the molecular aspects involved in the close bacterium-fungus interaction. To get insight into the bacterial mechanism employed for cell-cell communication and the interaction with the fungus *Fusarium graminearum* we established the genomes of 11 bacteria affiliated with the genera of *Janthinobacterium* and *Duganella*. The data indicate that all but one strain carried a single gene cluster involved in the biosynthesis of alpha-hydroxyketone-like autoinducer molecules, designated JAI-1. Additionally all strains contain genes encoding the type VI secretion system (T6SS). In coincubation assays with different janthinobacterial strains and *F. graminerarum* we observed a biofilm formation around the fungal hyphae leading to a reduced fungal growth. Genome-wide RNA-seq studies employing the background of two isolates (J. sp. HH01 and HH102) and the corresponding JAI-1 deficient strains identified a set of 45 QS-regulated genes in both isolates. Among the most strongly regulated genes were secondary metabolite and T6SS gene clusters. Interestingly the T6SS seems to be controlled by the autoinducer JAI-1 within the strain HH102, whereas the strain HH01 did not regulate the T6SS gene expression via JAI-I. To learn more about the role of the T6SS in the interaction of J. sp. HH102 with the fungal pathogen *F. graminearum* we constructed different deletion mutants in the T6SS of HH102 to analyse the involvement in bacterial fungal interactions.

[P26] THE ROLE OF CELL-CELL COMMUNICATION IN BACILLUS SUBTILIS FLOATING BIOFILMS

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Bacterial cell-cell communication coordinates gene expression in multicellular communities (biofilms) to regulate public goods production. Public goods are extracellular molecules (e.g. extracellular enzymes, biofilm matrix components) that provide a fitness benefit to the entire bacterial population. Cell-cell mediated regulation of public goods has not been extensively studied in the biofilms of *Bacillus subtilis,* a Gram-positive spore forming and industrially important model bacterium. Our aim is to understand the regulatory role of ComX, the signaling peptide of the ComQXPA system of *B. subtilis* in regulation of public goods. We use the *B. subtilis* PS-216 undomesticated strain as a model.

It is well known that ComX positively regulates development of genetic competence and the production of the lipopeptide antibiotic surfactin, but its role in exoprotease and matrix components production during pellicle formation has not been studied in depth. Our recent results confirmed that ComX is crucial for the production of exoproteases, as the activity of fluorescent reporters in the pellicle and the overall proteolytic activity of the pellicle spent media is dramatically lower in the ComX deficient mutant as compared to the wt. Furthermore, using a biosensor assay we show also that exposure of the signaling peptide to the exoproteases reduces its biological activity. Thus, the proteolysis may have important consequences for the peptide based signaling. In the absence of ComX the pellicle formation rates drastically increase compared to the wt strain, consequently speeding up the entry into the stationary phase. Finally, by measuring the overall expression and the single cell expression of operons responsible for the production of matrix components we also investigate the role of ComX in pellicle formation We find that ComX has a positive regulatory role in pellicle development, however, this is not evident at all time points of the pellicle development. We conclude that this is due to a drastically different kinetics of pellicle formation in the wt and the signal deficient mutant. Thus, ComX may be a regulatory switch that delays the entry into sporulation but promotes investment into alternative survival strategies such as production of proteases and biofilm development...

[P27] THE THREE LEVELS OF PHOSPHODIESTERASE ACTIVATION IN C-DI-GMP HYDROLYSIS

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We are interested in the molecular mechanisms controlling c-di-GMP turnover in bacteria, which in turn controls the switch between planktonic and biofilm lifestyle. We study phosphodiesterases of the EAL type, named after a conserved amino acid sequence motif, involved in biofilm dispersal. While it was known for some time that dimer formation of these enzymes is obligatory for their activity, we have demonstrated by structural studies how dimer formation influences activity through formation of substrate binding sites that are not present in monomeric structures (1). Surprisingly, all these structures can be observed in the presence of substrate c-di-GMP, suggesting that formation of the active site and binding of substrates is insufficient to attain full activity. We now demonstrate the occurrence of three metal binding sites, suggesting a further activation step (2,3). Together, dimerization, active site formation and metal binding present three activation steps that may serve as levers to tune enzymatic activity. Understanding how phosphodiesterase activity can be raised may be a tractable way to induce biofilm dispersal and clear chronic infections. Further, drugs inducing dispersal could aid in combination therapy, getting around the challenge of antibiotic tolerance in bacterial biofilms.

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[P28] DNA EXCHANGE IN B. SUBTILIS BIOFILMS

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B. subtilis is a soil dwelling microorganism capable of cooperative social behaviors as well as spiteful and antagonistic interactions. Interestingly, even *B. subtilis* strains isolated from soil micro locations are ecologically distinct and can be divided to different kinship groups based on their ability to discriminate kin and non-kin strains. During swarming and formation of biofilms on plant roots *B. subtilis* acts antagonistically towards non-kin strains by forming a death zone between two approaching non-kin swarms and outcompeting the non-kin strain on plant root, respectively. Since biofilms in natural settings are often composed of heterogeneous microbes of different kinship, antagonism and killing could promote the acquisition of foreign DNA in the vicinity of the cells resulting from the attack. In this work we explore *B. subtilis* natural transformation and DNA exchange of kin and non-kin cocultures incubated in planktonic and static (biofilm) settings. Remarkably, under static conditions *B. subtilis* develops the highest transformation frequency and transformation is the highest in exponential phase, prior the migration of cells to the liquid-air interface where they settle and become immotile. This is in line with previous findings showing that motility loss negatively affects natural competence in *B. subtilis*. In addition, we could detect that the cells expressing competence genes are forming small clusters within the pellicle biofilms and the number of cells in these clusters stayed constant during biofilm development. DNA exchange, however, seem to be decreased in structured kin-biofilms as compared to unstructured kin-liquid cultures and this implies that *B. subtilis* differs from other biofilm-forming and naturally competent species. However, this can be modulated in terms of ecological competition as DNA-exchange rate between two non-kin strains stays similar or even significantly increases in biofilms. Therefore, we need to explore interactions in biofilms composed of genetically heterogeneous populations, especially rhizospheric bacteria, whose complex interactions can significantly influence biofilm function in natural settings such as plant roots and the evolution of bacteria.

[P29] UNDERSTANDING PHOSPHODIESTERASES INVOLVED IN BIOFILM DISPERSAL

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Pseudomonas aeruginosa biofilms are involved in chronic infections, such as those affecting the lungs of Cystic Fibrosis patients. This is due to the presence of persistent cells within a biofilm which are highly tolerant to antimicrobials. We focus on comprehending the dispersal stage of pre-established *P. aeruginosa* biofilms. Phosphodiesterases (PDEs) responsible for the breakdown of the secondary messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) can induce dispersal; here we study, at the molecular level, how these enzymes are regulated.

The bi-functional enzyme RbdA (Regulation of biofilm disposal) contains a PDE for hydrolysis of c-di-GMP, but also a diguanylate cyclase domain (DGC) for c-di-GMP synthesis. We study how the activity of the PDE domain is affected by the DGC domain. With crystallographic structure determination and biochemical assays we aim to elucidate the structural mechanisms of such regulation.

We find elevated intracellular c-di-GMP levels in the knockout mutant Δ*rbdA*. Furthermore, deletion of RbdA leads to increased extracellular polymeric substances (EPS) production, reduced swarming and swimming motility, and consequently, hyper-biofilm formation compared to wild type. Biochemical assays show reduced PDE activity in the DGC-PDE tandem protein, compared to the autonomous PDE domain. This implies that the DGC domain is negatively regulating the PDE domain of RbdA. We have determined the 3D structure of the RbdA PDE domain, and compare this domain with a recently determined tandem domain structure to explain the suppressor function of the DGC domain.

[P30] HEAT ACTIVATES C-DI-GMP PRODUCTION IN BACTERIA

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Stimulus perception via signal transduction pathways is essential for all life, allowing organisms to regulate their internal processes to suit the conditions of the external environment. Environmental stimuli are often transduced into physiological responses through the production of a second messenger, which can regulate many protein targets simultaneously. The second messenger cyclic diguanylate (c-di-GMP) in the model bacterium *Pseudomonas aeruginosa* regulates biofilm formation, which may be key for survival in a host. C-di-GMP is produced by a variety of intercellular diguanylate cyclases (DGCs), and can be degraded by c-di-GMP specific phosphodiesterases. However, the stimuli and mechanisms of DGC activation are in many cases unknown. Here we identify a *thermosensing diguanylate cyclase* (*tdcA*) that enables *P. aeruginosa* to increase biofilm formation as temperature rises from 25 to 37 °C. Recombinant TdcA displays thermostatted activity: it is inactive at 25 °C, but displays linearly increasing reaction rates between 28 and 42 °C, allowing for a large change in intracellular c-di-GMP over a narrow range of temperatures. Heat-sensing is enabled via a Per-Arnt-SIM type III (PAS_3) domain, which is a previously undescribed function for this widespread family of protein domains. We propose that TdcA exemplifies a new class of heat-sensing enzymes that behave like molecular thermostats, allowing for the rapid change of cellular c-di-GMP over a narrow but physiologically relevant range of temperatures.

[P31] ANTIBIOFILMOGRAMS HIGHLIGHTED THE EARLY ADHESION INDUCTION OF P. AERUGINOSA CYSTIC FIBROSIS ISOLATES BY B-LACTAM ANTIBIOTICS

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P. aeruginosa is recognized as the most involved pathogen in the chronic lung colonization of cystic fibrosis (CF) patients. Bacteria are responsible for the decline in health of individuals and poor diagnosis. Once established, microorganisms grow in biofilm structures, highly tolerant to antimicrobial treatments. Sub-inhibitory concentrations of antibiotics were already described as having an induction effect in biofilm abilities.

According to the Antibiofilmogram® device, which allows the evaluation of sessile bacteria susceptibility to antimicrobials, we studied the effect of various concentrations of β-lactam antibiotics on clinical non-adherent strains of *P. aeruginosa*.

Results of Antibiofilmograms® showed the induction of the early steps of biofilm formation by some concentrations/antibiotics for originally non-adherent bacteria. To validate the inter-assay reproducibility, we estimated the colony forming units (CFU) on agar media of planktonic and sessile fractions of bacterial cultures supplemented or not with antimicrobials. For non-bactericidal concentrations of antibiotics, bacterial counts showed a higher proportion of adherent cells compared to the planktonic one. Conversely, for control strains growing without treatment, the fraction of free-mobile bacteria was more important than the one of sessile cells. These data correlate with previous results obtained with the Antibiofilmogram® assay and confirm the ability of antimicrobials to promote the early adhesion of microorganisms. Finally, pre-treatment of our clinical strains with tigecycline (a protein synthesis inhibitor without bactericidal effect on *P. aeruginosa*) led to the inhibition of the bacterial adhesion induction by β-lactam antibiotics. As described in literature, these results demonstrated the involvement of specific proteins in the early stages of *P. aeruginosa* attachment, whose expression can be induced by inappropriate antimicrobial treatments.

In conclusion, Antibiofilmogram®, in highlighting the inducer molecules of bacterial adhesion, appears to be a new pertinent diagnostic tool for the optimization of antimicrobials selection in CF.

[P32] TO STICK OR NOT TO STICK? PULLING PILI SHED SOME LIGHT ON BIOFILM FORMATION

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In this work, pili were produced by *Lactococcus lactis* - a widely used species in dairy industry - that has the ability to form communities so called biofilms. A pilus is an appendage emanating from the cell surface of the majority of Gram-positive bacteria. Pili biogenesis is a machinery that involves a sortase (srtC), an enzyme that assembles covalently protein subunits (pilins) and in head-to-tail series to form the backbone (PilB) in *L.lactis* IL1403 with a tip pilin (PilA) at the distal end of the pilus. This assembly is firmly anchored to the peptidoglycan wall using PilC anchor pilin. It has been demonstrated that under static conditions pili are involved in self-aggregation and modify the architecture of a growing biofilm. To provide a deeper understanding of the role played by pili in biofilm formation, this work has focused on the influence of different pilins and sortase C on (i) adhesion of cells to surfaces under dynamic conditions and (ii) nanomechanical properties of pili using single-molecule force spectroscopy. It has been reported that the presence of pili increased drastically the adhesion properties of *L.lactis* on polymeric surfaces and that sortase C was mandatory for cells to sustain the shear flow. Force spectroscopy experiments confirmed such requirement and provided data showing extreme flexibility of the pili, much more than DNA strands. Such discovery opens up new assumptions about homotypic interactions between two cells in particular and about self-assembly in general. A better understanding of the conditions that drives the bacterial colonization to biotic (e.g. intestinal tract) or abiotic surfaces (e.g. milking machine) remains a major socio-economic issue. Eventually, such type of biofilm would allow to struggle against pathogens (e.g. Listeria) but also against spoilage flora in order to propose *in fine* new solutions for food biopreservation.

[P33] A STICKY TRANSITION: THE ROLE OF EXTRACELLULAR MATERIAL IN THE TRANSITION FROM REVERSIBLE TO IRREVERSIBLE ADHESION OF STAPHYLOCOCCUS EPIDERMIDIS

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Bacteria are a major source of fouling for surfaces. Bacteria experience bond maturation phenomena that transitions the adhesion to a surface from reversible to irreversible. Recent studies have shown that the number of tethers between a bacterium and the surface is important for the transition from reversible to irreversible adhesion. The problem rests with the definition of a tether. Common perception would define tethers strictly as physical extrusions from the cell wall that can directly attach to the surface. However, extracellular material is also present on the cellular outer surface and can interact with the surface as a gel in collaboration with any physical extrusions.

Staphylococcus epidermidis ATCC 35983 was treated (buffer, DNaseI, N-acetyl-L-cysteine) prior to adhesion to a glass slide under shear stress ($10s^{-1}$). Bond maturation occurred statically or under continuous shear ($10s^{-1}$) and subsequently the vibrational movement was determined. Vibrations were monitored for 40s, and the standard deviation of the center position was converted to vibrational amplitude in nanometers. The mean-squared displacement was also monitored to describe the progression of bond maturation.

With static bond maturation, treatments containing DNaseI yielded significantly higher ($p<0.05$) vibrations while treatment with N-acetyl-L-cysteine alone yielded significantly lower vibrations against all treatments. Under continued shear stress, the vibrations were significantly reduced. The mean-squared displacement for all treatments suggests Brownian motion, with a linear increase, followed by a plateau, and ending with an exponential increase, albeit at different times and varying degrees.

Overall, the data clearly suggests that the use of DNaseI to break up eDNA on the cell surface of *S. epidermidis* promoted free movement of the bacterial cell, both during static adhesion and under shear stress. This confirms that eDNA aids the conversion from reversible to irreversible adhesion of *S. epidermidis* to a surface.

[P34] BENEFICIAL ORAL BIOFILMS AS SMART BIOACTIVE INTERFACES

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Periodontitis is a very common health problem caused by formation of pathogenic bacterial biofilm that triggers inflammation resulting in either reversible gingivitis or irreversible periodontal hard and soft tissue damages, leading to loss of teeth when left untreated. Commensal bacteria play an important role in oral health in many aspects. Mainly by colonizing oral tissues, they (i) contribute to maturation of immune response, and (ii) foreclose attachment of pathobiont and, therefore, prevent from infection. The main goal of the study was to investigate if blocking of receptors on a commensal biofilm can prevent or reduce the attachment of pathogenic strains. To do so, biofilm produced by commensal *Streptococcus sanguinis* was treated with whole cell lysate of pathobionts *Fusobacterium nucleatum* or *Porphyromonas gingivalis*, followed by incubation with respective strain(s). The study revealed significant reduction in pathobiont adhesion to lysate-treated commensal biofilm. Therefore, adhesion of pathobionts onto the lysate-blocked biofilm was hindered; however, not completely eliminated supporting the idea that such approach in the oral cavity would benefit the production of a well-balanced and healthy bioactive interface.

[P35] ATOMIC FORCE MICROCOPY TO DETAIL IN REAL-TIME THE CELL WALL REMODELING OF ADHERING STAPHYLOCOCUS AUREUS DURING BIOFILM GENESIS.

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Atomic Force Microscopy (AFM) in the approach-retract scanning mode is a powerful method to probe at the nanoscale the topography and mechanical properties of live bacteria in aqueous environment. We took benefit from this method to describe the time evolution of the surface nanotopography of the strain *S. aureus* 27217 from bacterial adhesion to the first step of biofilm genesis. Two types of adhering bacteria were observed without and with herring-bone patterned bacterial envelope decorations. These AFM observations on individual bacteria were further confirmed by Scanning Electron Microscopy (SEM) experiments on large cell population, revealing an equal ratio between the two populations. AFM revealed that the herring-bone pattern is characterized by lateral dimensions of ~70 nm and the mean depth of the trough between them is ~15nm. The corresponding root-mean-square (RMS) roughness of such cells was ~5nm (on (400nm)² image), one order of magnitude higher than that for a cell of the second population, the so-called "smooth" one. Concomitantly with these topographic investigations, AFM mechanical characterization of the two types of adhering bacteria was performed: the upper patterns (the herring-bone motif) are stiff (Young modulus of ~ 2.3 MPa) while the lower ones are much softer (~ 0.35 MPa) similar to the stiffness of the smooth population.

Another important result is that we observed, thanks to our 4-hour real-time AFM experiments, the gradual detachment of the herring-bone patterns from the bacterial surface and its progressive smoothing. Accumulation of these herring-bone tiles in the form of highly adhesive globular deposits between bacteria was observed. Furthermore we observed that these cell surface decorations were removed by centrifugation and thus loosely attached to the cell wall. Proteomic analysis of the supernatant highlighted the presence of *Staphylococcus aureus* surface proteins well-known to be implicated in adhesion and biofilm formation.

[P36] CO-AGGREGATION PROPERTIES OF TRIMERIC AUTOTRANSPORTER ADHESINS

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Trimeric autotransporter adhesins (TAAs) comprise a group of virulence-related proteins in Gram-negative bacteria. These obligate homotrimeric proteins are embedded in the outer membrane and function mainly as adhesins. Members of this family bind to extracellular matrix components such as collagen and fibronectin, but in addition to their primary adhesive activity exhibit a number of other functions, such as conferring serum resistance and autoggragation. Autoaggregation promoted by TAAs is homotypic and mediated by the sticky, globular head domains of these lollipop-like molecules. However, whether TAAs mediate heterotypic intercations (i.e. co-aggregation) has not been studied. To address this question, we investigated the co-aggregation of two model TAA groups: YadA from the enteropathogens *Yersinia enterocolitica* (YeYadA) and *Y. pseudotuberculosis* (YpYadA), and the immunoglobulin-binding Eib proteins from *Escherichia coli*: EibA, EibC and EibD. Both groups promote strong autoaggregation. To study TAA co-aggregation, we co-expressed a fluorescent label (sfGFP or mCherry) together with a particular TAA and followed the aggregative interactions using fluorescent readout. Our results show that there is co-aggregation between some population expressing different TAAs, which can be explained by relatively high sequence similarity between the interacting TAAs. In most cases, the level of co-aggregation correlated with the sequence similarity. However, in other cases the TAAs did not interact despite high sequence similarity, showing exclusion of non-self-bacteria. Based on these results, we investigated biofilm formation by a subset of co-aggregating bacteria to see whether they form mixed biofilms or segregated microdomains within the biofilm. Also in this setting, the more similar TAAs formed highly mixed biofilms, whereas non-aggregating TAAs formed separate microdomains. These data demonstrate that TAAs can mediate bacterial co-aggregation, but in some cases prevent co-aggregation of bacteria with disparate TAAs.

[P37] CANDIDA ALBICANS ADHESION ON DIELECTRIC SURFACE (SIO2) WITH TAILORED PROPERTIES AT LOW SHEAR STRESSES

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Silica (SiO_2) an inorganic solid substrate known for its optically transparent properties in the visible range of the spectrum is regularly used in microelectronics, in plasmonics as a host matrix, or in biomedicine for the development of materials useful for tissue regeneration and for drug screening systems. To understand the interactions between thin dielectric layers of SiO_2 with tailored by silver nanoparticles (AgNPs) properties and microorganisms, a primary study of adhesion on thin layer of SiO_2 only is necessary. The present study focuses on the shear-induced detachment of the yeast *Candida albicans* IP48.72 in contact with a thin silica layer at low shear stresses using a shear stress flow chamber. The experimental arrangement is designed to address large range of shear stresses, up to 80 Pa, with specific attention paid to the low shear stress domain, between 0.01 Pa and 0.05 Pa. The experiments are performed using cell suspensions adjusted to 4.10^6 CFU/mL in water for injectable preparations in order to avoid any possible interaction with extracell molecules. It was found that the duration of the *Candida albicans* cell culture (exponential growth phase vs. stationary phase) influences the percentage of microbial adhesion on the thin layer of SiO_2 in conditions of low stress ($\leq$ 0.2 Pa). Under such conditions one can observe the adhesion of new cells on the silica surface. The phenomena of rolling, circumvention or adhesion to other adherent cells have also been noted. The steps in progress are to evaluate how the AgNPs embedded in SiO_2 layer alter the observed phenomena regarding the release of bioavailable Ag^+ [1] and to address the impact of previously formed protein layer on the SiO_2 surface [2, 3] on the cell adhesion and release mechanisms.

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[P38] "FORCESCOPY" OF BIOFILMS

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In a biofilm reactor different forces act on the cells: forces perpendicular to the interface (dominate the first contact), lateral shear forces, and forces on the cell-wall by turgor pressure (viability of the cells). The interplay of these forces plays a major role in the biofilm formation.

Here, we report on the seawater bacterium *Paracoccus seriniphilus* on titanium, steel and glass. The prupose is to understand the influence of wettability, roughness, defined structures, and environmental conditions (pH, ionic strength) on the viability of the cells as well as the bacterial attachment/detachment.

The first "forcescopy" experiment (scanning force microscopy, SFM) determines the turgor pressure of the bacteria as a function of pH and salinity. *P. seriniphilus* can easily adapt to saline conditions and can survive at NaCl concentrations up to 100 gL^{-1}. Its turgor pressure and thus its elasticity and size depends on the ionic strength. *P. seriniphilus* has its optimum pH at 7, but the results at pH 4 point to an active adaptation mechanism to acidic conditions whereas at pH 11 *P. seriniphilus* is not viable.

The next "forcescopy" measures the vertical adhesion forces of a single bacterium as a function of pH, ionic strength, and substrate. The adhesion force of one single cell decreases from pH 4 to pH 11. The adhesion forces increase with increasing ionic strength with a pronounced higher adhesion force at 0.9 % NaCl. All adhesion forces on bare substrates correlate with the electrostatics as determined by zeta potential measurements. A conditioning film of growth medium strongly decreases the attachment forces which may be due to macromolecular interactions.

The last "forcescopy" experiment determines the lateral detachment forces of the bacteria. There is a clear correlation between the applied force and the number of moved bacteria, but the detachment forces vary for a individual bacterium. Overall, the surface energy of the substrate influences the detachment process while any structuring of the substrate hinders the detachment substantially. At pH 4 bacteria are harder to detach than at pH 7. This can either be due to electrostatics or changes in mebrane composition.

[P39] ENRICHING ELECTROACTIVE BIOFILMS ON ELECTRODES MIMICKING OXYGEN USING A POTENTIOSTAT

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Waste to energy transformations are gaining importance in both research and real-world applications. Direct harvesting of electricity from waste by the use of microbial fuel cells (MFC) is a promising alternative due to the low temperature environment applicability and the decentralization advantage in comparison to the more centralized infrastructures required for anaerobic digestion (AD). Nonetheless, several impediments need to be overcome before the technology can be upscaled for real-world applications. Most of the drawbacks stem from the high cost and suboptimal efficiency of the electrodes. It is hypothesized that microbial communities have an important role in improving electrode's characteristics. Consequently, this project aims to analyze the microbial communities growing at the electrode surface. Direct interspecies electron transport (DIET) and long distance electron transport (LDET) can improve waste-to-electricity transformations and electrode efficiency through microbial electrical syntrophy. For this reason, the electrode potential is posed under oxygen-mimicking conditionsby means of a potentiostat. The experiment sets triplicates of a 2 L semibatch reactor at 12.0 (± 1.0) °C with a mix of artificial seawater and synthetic wastewater as electrolyte (neutral pH; 20‰ salinity; 2.0 µm Sulfide; and COD mixture 1500 mg L^{-1} consisting on 50.0% Acetic acid and 50.0% Propionic acid). Two different mixed culture inocula are analyzed: blended AD granules and sulfide-rich sediment with focus on the presence of recently discovered cablebacteria. The community differences will be obtained by comparing the oxygen mimicking electrode with an open circuit 'dummy' electrode. The microbial community change will show the different composition between unspecific electrode attachment and exoelectrogen interacting ecosystems. The community will be characterized by a 16s rRNA composition analyses after 35 to 40 days under the stated conditions. Chronoamperometric measurements and water quality analyses will help in the comprehension of the microbial activity and waste-to-electricity transformations.

Keywords: Bioelectrochemistry, biofilm, microbial fuel cell, anaerobic digestion, waste-to-energy, microbial ecology, 16S rRNA, sustainability, electrodes, cable bacteria, sediment, biorefinery, potentiostat.

[P40] POLYMER COATINGS ON STAINLESS STEEL TO IMPROVE THE POWER OF MICROBIAL FUEL CELLS

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Microbial fuel cells (MFCs) convert chemical energy from waste water into electrical energy, however the current densities are still low and need to be improved. Because of its biocompatibility graphite is the most common material for anodes in MFCs. Stainless steel would be a great alternative material because of its lower costs and much better electric conductivity compared to graphite. However *Geobacter (G.) sulfurreducens* dominated biofilms, which are common in MFCs, do not grow well on stainless steel. To improve the growth of bacteria we test polymer coatings, which will support the bacterial colonization on the electrode. Therefore copolymers containing phosphonates or phosphates and quaternary ammonium salts have been prepared by radical copolymerization. Phosphonates and phosphates are used to chemically bind the polymer on stainless steel[1]. Furthermore it has been shown that positive charges like quaternary ammonium salts can support the growth of anodophilic bacteria like *G.sulfurreducens*[2].

Copper has an even better conductivity than steel, but it is regarded to be antimicrobial. In contrast to that Baudler et al. have shown that biofilms dominated by *G. sulfurreducens* are able to grow on copper[3]. However copper has a limited voltage range it can be used at because of its easy oxidation at low potentials. Copolymers that should bind chemically to copper and are expected not to change but to protect the good conductivity of copper have been synthesized by copolymerization of 2-Methyldisulfanylethyl-methacralate (MDEMA) and vinyl-ferrocene (VFc).

Stainless steel is coated by spin or spray coating whereas copper is coated by dip coating (formation of a self-assembled monolayer). The coatings are analyzed by ellipsometry, contact angle, Xray photoelectron spectroscopy (XPS), cyclic voltammetry (CV) and linear sweep voltammetry (LSV). The polymer coatings show layer thickness within the range of monolayers. The coated substrates are further analysed by chronoamperometry in the presence of *G. sulfurreducens*. The thickness of the biofilms obtained is subsequently analysed by confocal microscopy.

[P41] STRUCTURAL DETERMINANTS OF STAPHYLOCOCCUS EPIDERMIDIS EXTRACELLULAR MATRIX BINDING PROTEIN EMBP AND ITS INTERACTION WITH FIBRONECTIN

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Introduction
Staphylococcus epidermidis is the leading cause of foreign body associated infections. The great success of this pathogen is attributed to colonize artificial surfaces via biofilm formation. This crucially depends on interactions between *S. epidermidis* and surface immobilized fibronectin. Here, we investigated the structural basis of *S. epidermidis* – Fn interactions, focusing on the 1MDa extracellular matrix binding protein Embp. Bioinformatics analysis showed that Embp is mainly organized by stretches of ten F- and forty FG-repeats. Structural analysis showed that both repeats mainly display an a-helical secondary structure. Small angle X-ray scattering (SAXS) analysis of recombinant Embp fragments suggest that repetitive elements are organized as elongated rods rather than being globularly folded.

Material and methods
These experiments focus clinical isolate *S.epidermidis* 1585 We investigated the bacterial attachment to immobilized fibronectin in ELISA experiments. In order to map the potential site of interaction Far-Western Blot analysis and peptide library screening revealed type III repeat FN12 to be the major site of interaction.

To further elucidate the interaction of Embp and fibronectin investigation of the Embp structure was begun. Bioinformatics analysis of the Embp primary sequence revealed 170 (F-) and 126 amino acids (FG-) repeats as most presumably minimal structural units. By means of X-ray crystallography the structure of these repeats was determined. SAXS was employed to get first insights in the global Embp architecture.

Results
We identified the fibronectin type III repeat FN12 as the potential site of Embp – fibronectin interaction.

On the bioinformatics level F-repeats in the FIVAR region as well as FG-repeats in the FIVAR/GA region were identified as minimal structural units of the Embp protein. Both of these repeats mainly display a-helical secondary structure. In SAXS studies of multiple repeats Embp presumably shows an elongated rather than globular architecture.

Discussion
We showed Embp to be sufficient and of great importance for fibronectin binding.

By means of bioinformatics analysis confirmed by X-ray crystallography minimal Embp structural units, F- and FG-repeat, were identified. Results from SAXS gave the impression that Embp posses an elongated shape. Ongoing project focuses on the molecular determinants for the interaction between Embp and fibronectin.

[P42] PSEUDOMONAS DONGHUENSIS P482 - ANALYSIS OF BIOFILM FORMATION AND COLONIZATION OF PLANT TISSUE BY THE PLANT-BENEFICIAL STRAIN

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Bacteria which inhabit many ecosystems most often can be encountered interacting with various surfaces, forming complex, multicellular communities - biofilms - ensuring survival in specific environmental conditions. The abillity to colonize biotic and abiotic surfaces has been the subject of numerous research, however, mostly concerning the well-studied, model bacterial species. In some cases, a correlation between biofilm development and adhesion of bacteria to plant tissues has been observed. *Pseudomonas donghuensis* P482 strain is a little-known isolate from tomato rhizosphere, which exhibits antimicrobial activity of yet unknown background towards bacterial and fungal plant pathogens. Earlier studies demonstrated that P482 has the ability to form biofilm on abiotic surfaces and to efficiently colonize the plant rhizosphere. However, the mechanism underlying this phenomenon has not been solved up to date. The aim of the ongoing research is to identify and characterize factors (genetic and environmental) involved in the process of biofilm formation on abiotic surfaces and colonization of plant tissues by the P482 strain. To reach that goal the P482 genome was analyzed and a number of knock-out mutants in genes involved in the process of biofilm formation was constructed. Subsequently, these were investigated for the features connected with biofilm formation, *i.e.* motility on synthetic media, attachment to abiotic surfaces (polystyrene and glass) in various culture conditions (different temperatures and carbon source). For the latter purpose crystal violet staining and fluorescence confocal microscpy were utilized. Colonization of plant tissues was analyzed in an *in vitro* culture system on maize seedlings. The results obtained to date suggest possible correlation between the ability to form biofilm on abiotic surfaces and colonization of plant tissues by the P482 strain. Moreover, the strain exhibits variability of biofilm formation depending on the environmental conditions.

[P43] BACTERIAL ADHESION ON NANOSTRUCTURED SURFACES

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To prevent the inflammation of a medical implant, its material specifications are of crucial importance. An ideal surface would hinder bacterial biofilm formation and/or kill adhering pathogens without harming surrounding somatic cells. Our study focuses on the influence of surface structure of silicon on bacterial (*Staphylococcus aureus*) adhesion and viability, using AFM force spectroscopy [1], where the probe is a single bacterium, as well as flow chamber experiments. While surface chemistry and subsurface composition of the silicon surfaces are consistent, bacterial adhesion rate and viability on nano-rough silicon can be ascribed to geometry constraints, as changes in the adhesion strength due to a variation of the long-range van der Waals force can be neglected. Comparing adhesion rate and viability on hydrophobic and hydrophilic substrates of identical roughness reveals the influence of short-range, e.g. hydrophobic, forces [2]. The data obtained from our single cell force spectroscopy can be compared to our flow chamber measurement data on the same surfaces.

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[P44] BIOFILM FORMATION STUDIES IN MODIFIED PAPER-BASED ARRAYS: NOVEL PLATFORMS FOR BIOFILM-RELATED APPLICATIONS

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Earlier investigations of our research group have shown the suitability of utilizing paper-based arrays for biofilm formation studies by *Staphylococcus aureus* (1, 2). Paper is a sustainable and recyclable material, and its physicochemical properties (i.e. topography, roughness, stiffness, surface energy, polarity, porosity and pore geometry) can be modified quite conveniently by various coating materials and methods and surface treatments (3). Although paper-based arrays are excellent innovative tools for biofilm sensing purposes, they have the limitation that biofilm formation cannot be followed by microscopy evaluation techniques. In this investigation, newly developed latex-coated glass coverslips were tested for biofilm adhesion studies in representative Gram-positive and Gram-negative strains. The Static Biofilm Method was optimized to evaluate the adhesion of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 15442 at different time points ranging from 2 to 48 hours. The formed biofilms were simultaneously quantified by viable plate counts and biomass staining as well as Fluorescence Microscopy (LIVE/DEAD® BacLight™ staining). Biofilm adhesion increased over time when measured on latex-coated substrates of three different compositions. At similar times, the attachment of *P. aeruginosa* onto latex-coated coverslips seemed to be slightly higher than *S. aureus* (for instance at 24 h the attachment of *P. aeruginosa* in 40:60 substrates is 6.4 Log CFU/cm^2 compared to 5.2 Log CFU/cm^2 for *S. aureus*). As proof-of-concept, Fluorescence Microscopy images were also taken of the formed biofilms on the selected substrates. This investigation shows the feasibility of using latex-based glass coverslips as tools for orthogonal investigation approaches of bacterial adhesion.

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[P45] THE CONFORMATION OF ADSORBED FIBRONECTIN DETERMINES THE SUCCESS OF BACTERIAL ATTACHMENT

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Staphylococcus epidermidis is responsible for a large number of implant-associated infections, and its pathogenicity is linked to its ability to form biofilm. Attachment to the implant occurs through unspecific interactions or specific receptor-ligand interactions with adsorbed host proteins. One important receptor for *S. epidermidis* is the giant extracellular matrix binding protein (Embp), which binds to fibronectin (Fn). Embp, like many other bacterial adhesins, bind to host proteins that are present as soluble compound in body fluids as well as adsorbed to the surface of biomedical implants. So how can interaction with these proteins promote attachment to the surface? We hypothesized that Embp only mediates attachment to immobilized fibronectin, and that the distinction between soluble and immobilized fibronectin lies in the availability of binding domains in Fn. These domains may become exposed only when the protein adsorbs to an implant surface and undergoes conformational changes.

To investigate these hypotheses we first used fluorescently labelled Fn and confocal microscopy to show that *S. epidermidis* interacted with adsorbed but not soluble Fn. Soluble Fn is in a globular conformation, while adsorbed Fn can either remain in the globular conformation, or change conformation to form fibrils as adsorption-induced conformational changes expose Fn-Fn binding domains. To study the bacterial interaction with Fn in these two conformations, we produced surfaces coated with (poly)methyl acrylate (PMA) and (poly)ethyl acrylate (PEA), which adsorb Fn in the two different conformations. Atomic force microscopy confirmed that Fn adsorbed to PMA remained globular, while Fn adsorbed to PEA fibrillated. We then quantified Embp-mediated bacterial attachment to Fn on these two surfaces, using *Staphylococcus carnosus* expressing a recombinant fragment of Embp. Fibrillar Fn promoted bacterial attachment while globular Fn did not. This result supports our hypothesis that adsorption-induced conformational changes dictate if a host protein promotes or prevents bacterial attachment to an implant surface. Our results underline that the materials properties of implants affect biofilm formation indirectly by making host proteins available in the right or wrong conformation. This knowledge adds a new layer to the considerations made in materials design for novel implant materials that prevent biofilm infections.

[P46] ATR-FTIR OF PSEUDOMONAS FLUORESCENS FROM PLANKTONIC STATE TO EARLY BIOFILM FORMATION UNDER DIFFERENT NUTRIENT CONDITIONS

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This presentation discusses to use of Fourier transformation infrared spectroscopy combined with Attenuated total reflection accessory ATR-FTIR as a tool to investigate biofilms. FTIR spectroscopy is suitable for the identification of bacterial biofilm fingerprint allowing. The use of ATR accessory allows the observation of biofilm forming directly on the interface of an ATR crystal (Germanium or Zinc Selenide). The crystals can be coated to obtain a surface more relevant to study interfacial processes. Spectra have been acquired, non-destructively, *in situ*, and in real-time during biofilm formation. This method suitable for fundamental biofilm research, as well, as for monitoring of biofilm formation in different environment conditions (temperature, humidity, pH, medium). These different experiments demonstrate that FTIR spectroscopy is suitable for study of biofilm and biofilm/surface interaction and can be applied in many different way. In particular this presentation will show results for the three types of conditions: bacteria growing in 10% Tryptic Soy Broth (TSB) medium as a model of low nutrients environment, bacteria cultivated in 10% TSB medium and in sterile water on ATR crystal suppressed nutrient environment and bacteria cultivated in sterile water as a model of no nutrient environment.

After bacteria inoculation, the system has been monitored for 6 hours studying bacterial attachment on the surface.

Depending on condition which bacteria were inoculated, we can clearly observe the difference in the time evolution of biofilm following the changing of Amide II peak.

In particular, Amide II/PO (phospholipids) and Amide II/PS (polysaccharides) show the changes of phospholipids and polysaccharides as a function of time respect to the proteins PO/PS ratio remains at the same level. The changes of intensity peaks corresponding to protein are compared with changes occurring in the intensity of phospholipids and polysaccharides peaks.

This is an agreement in biofilm evolution onto the surface.

[P47] BACTERIAL CONTACT FORMATION AND BREAKING: REVEALING THEIR CONTACT AREA & CONTACT CHARACTERISTICS ON DIFFERENT TYPES OF SURFACES

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Pathogenic bacteria adhering to surfaces are a nuisance in a wide area of healthcare applications. We present a combined experimental and computational approach to characterize the adhesion of *Staphylococcus aureus* and other cocci. Single cell force spectroscopy (SCFS) [1] paired with Monte Carlo simulations enabled an unprecedented molecular investigation of the contact formation [2]. Our results reveal that bacteria attach to a surface over distances far beyond the range of classical surface forces via stochastic binding of thermally fluctuating cell wall proteins. Thereby, the cells are pulled into close contact with the surface as proteins of different mechanical properties attach consecutively. This mechanism, however, can be manipulated by genetically/enzymatically/chemically modifying the cell wall proteins [3]. On hydrophobic surfaces, hydrophobic interactions are dominant and many cell wall proteins contribute to the cell's stickiness. On hydrophilic surfaces, contact formation needs a longer time and only few cell wall proteins are involved in this process. By performing numerous SCFS measurements on gradually varying positions near a sharp interface between a hydrophobic and a hydrophilic surface, the contact area between staphylococcal cells and flat surfaces could be determined [4]. The contact area shows to be a cell-individual property that is quite robust against varying loading forces. Furthermore, the adhesive strength of an individual bacterial cell is not dependent on the contact area. Rather, the cell's adhesion capability is a matter of which and how many molecules of the bacterial species' cell wall form the contact.

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[P48] EFFECTS OF MILK FAT CONCENTRATION AND ABIOTIC SURFACES ON BIOFILM FORMATION OF GEOBACILLUS THERMODETRIFICANS

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The thermophilic bacillus such as *Geobacillus thermodenitrificans* is an important contaminant in the dairy industry. This bacillus is not pathogenic, its presence in dairy products is an indicator of poor hygiene. In the present study, different types of abiotic surfaces that are frequently used in dairy industry and milk products [whole (3.0 % fat content, semi-skimmed (1.5 % fat content) and skimmed (0.1 % fat content)] were evaluated for their effects on biofilm formation of *G. thermodetrificans*. The strain was cultured at both 55°C and 65°C for 48 h in standart whole milk containing different types of abiotic surfaces (polyvynil chloride, polycarbonate, polystyrene, 316 L stainless steel, glass and polypropylene; R: 14 mm). After the incubation, the biofilm cells were harvested from the surfaces and counted as logCFU/cm^2. The biofilm suspensions were also heated at 100°C for 15 min in order to eliminate vegetative cells. The remaining suspensions were also diluted and counted for the calculation of themophilic spores. Following this study, the mostly preferred two abiotic surfaces were selected and biofilm sampling was carried out in the whole, semi-skimmed and skimmed milk contents with these surfaces. The total biofilm content (vegetative forms, and spores) and only spores were also counted. While the strain preferred hydrophilic surfaces such as stainless steel and glass at lower temperatures (55°C), the hydrophobic surfaces (polyvynl chloride, polycarbonate, polystyrene and polypropylene) were preferred at higher temperatures (65°C). The whole milk (3.0 % fat content) was found to be the most problematic milk type for both biofilm formation and sporulation. It was the first report for *G. thermodenitrificans*, commonly isolated from dairy environments, in order to determine the potential risks of abiotic surfaces and milk fat concentration in terms of thermophilic biofilm formation.

Key words: Thermophilic bacilli, *Geobacillus*, biofilm formation, abiotic surfaces, milk product

[P49] BACTERIAL ATTACHMENT IS AFFECTED BY FLOW AND HYDROPHOBICITY

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Biofilms are intricate communities of microorganisms encapsulated within a self-produced matrix of extra-polymeric substances, creating complex three-dimensional structures allowing for liquid and nutrient flow through. These aggregations offer constituent microorganisms enhanced protection not only from environmental pressures like flow but are associated with higher resistance to antimicrobial compounds, providing a persistent cause of concern in relation to marine biofouling and medicinal infections. Bacterial attachment is affected by surface properties, such as hydrophobicity, as previously shown. Using an innovative microfluidic flow cell, we investigated the relationships between both shear stress and surface properties upon early biofilm formation of two biofilm forming species, *Cobetia marina* and *Pseudomonas aeruginosa*. In this study we investigated biofilm development under flow conditions and on surfaces with varying degree of hydrophobicity, namely low-density polyethylene membranes, permanox and standard glass slides. Biofilm development was measured using nucleic acid staining and end-point confocal laser scanning microscopy. Flow conditions affected biomass, maximum thickness and surface area of biofilms, with higher shear stresses (5.6 Pa) resulting in thinner, more compact biofilms than lower shear stresses (0.2 Pa). Control experiments performed under static conditions have shown that an absence of flow creates much thinner, smaller biofilms suggesting a growth response to the surrounding flow. With respect to surface properties, initial biofilm formation was also impacted as hydrophilic surfaces resulted in thinner biofilms when compared to hydrophobic ones. Alongside this, we observed the formation of biofilm streamer-like structures under laminar flow conditions within straight, micro-channels for the first time.

[P50] POLYMER SCAFFOLDS FOR BIOCATALYSIS USING BIOFILMS

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There has been growing interest in using biocatalysts in industry due to their ability to catalyse highly stereoselective transformations in a sustainable manner. A major problem however is the sensitive nature of enzymes which are easily denatured in adverse reaction conditions. A potential solution is to use enzymes expressed by bacteria in biofilms, with the robust extracellular polymeric substances protecting the enzymes from external stresses. The aim of this project is to design polymers onto which biofilm forming bacteria adhere in a controlled manner, to form protected communities that can express useful enzymes for use in stereoselective reactions.

Poly(acryloyl hydrazide) has been chosen as the polymer scaffold, due to ease of post polymerisation modification resulting in highly functional polymers. To date, the polymer has been functionalised with range of aldehydes (Imidazole-3-carboxaldehyde, 2-Amino-3-Formylpyridine, Benzaldehyde and Isovaleraldehyde) predicted to interact with bacteria. Clustering of the biofilm-forming *Escherichia coli* strain PHL644 has been analysed upon mixing with differently substituted polymers. When suspended in phosphate buffer, the polymers induce clustering of cells. However, when the same assay is performed in water or NaCl solution, clustering is significantly decreased. It is likely that in the absence of buffer, the relatively basic aldehydes become protonated whereas in the presence of buffer they remain in their native state. It is known that charge and hydrophobicity of a surface are two important factors influencing bacterial adhesion so it is possible that *E. coli* PHL644 preferentially binds to hydrophobic polymers over positively charged polymers. Further experiments have been performed to investigate crystal violet binding by the clusters, with preliminary results indicating an increase in binding by bacteria clustered with hydrophobic polymer, potentially due to the formation of adhesins formed during the early stages of biofilm formation.

[P51] QUANTIFICATION OF BACTERIAL ADHESION FORCES BY ATOMIC FORCE MICROSCOPY OF ANTIMICROBIAL-RESISTANT AND NON-RESISTANT STRAINS

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Bacterial adhesion onto surfaces of biomedical devices such as implants or catheters and the formation of biofilms are non-trivial phenomena which cause a major problem in the clinic. Opportunistic bacteria such as Staphylococci and Pseudomonas can form biofilms on medical devices introducing one of the leading factors in failures and microbial infections, which can lead to serious health concerns. Efforts to prevent or treat these microbial infections such as using antibiotics or cleaning surfaces with disinfectants, can create a potential for the evolution of antimicrobial resistance (AMR). The evolution of AMR poses a real threat in the clinic as it can hinder the effectiveness of current treatments and methods.

In order to better assess the best methods to counter antimicrobial resistance, adhesion and biofilm formation, we need to understand better the influence of surface properties on the microorganisms. Through adhesion force measurements by making use of atomic force microscopy we can gain insight into the behavior of antibiotic resistant and non-resistant bacterial strains of *Staphylococcus aureus* ATCC12600 and *Pseudomonas aeruginosa* PAO1 on surfaces with different physico-chemical properties.

We have studied the influence of treated and non-treated glass surfaces with different levels of hydrophobicity, topology and antimicrobial activity. Through adhesion studies we have found that characteristics such as hydrophobicity play a more important role on strains of *P. aeruginosa* PAO1 than on *S. aureus* ATCC12600 and that bacterial modification (antimicrobial resistance or fluorescence) can affect the adhesion of a bacterium to a given surface.

[P52] STAPHYLOCOCCUS AUREUS BIOFILM FORMATION ON VARIOUS IMPLANT MATERIALS

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Bacterial adherence and biofilm formation on the surface of biomaterials can cause intractable implant associated infections. This process is vary depending on species of microorganisms, host's immune response and characteristics of dental materials, including material surface. In this in vitro research, we evaluated biofilm production ability of the selected clinical *S. aureus* strains the main pathogen in implant-related infections isolated from patients with mucoviscidose. This study compared the susceptibility of three different implant biomaterials: titanium alloy Ti-6Al-4V (Grade 5), zirconium oxide and CoCr alloy; actually used in clinical practice with similar surface roughness with *S. aureus* biofilm formation. SEM observation demonstrate that the bacteria on Co-Cr alloy was more aggregated than for Ti-6Al-4V and zirconium oxide. After culturing for 48 hours, biofilm on zirconium oxide was more tightly colonized than for Ti-6Al-4V and Co-Cr alloy. Biofilm colonies on the surface of Ti-6Al-4V compared to the pattern for the other biomaterials tended to be scattered. This is consistent with fluorescence microscopy images with similar biological properties in terms of biofilm composition and bacterial adherence. However, the quantity of adhering microorganisms varied among the materials. The amount of *S.aureus* growth was significantly lower with statistically lower logCFU/ml values than the other materials ($P < 0.05$) on the surfaces of the zirconium oxide Our findings, evaluating CFU values, indicate that implants composed of CoCr had a statistically ($P < 0.05$) higher proclivity towards biofilm formation compared to titanium-alloy and zirconium oxide implants. In relation to the implant material types, titanium is the most commonly used material in dentistry due to its excellent physical and chemical characteristics. Overall, the conclusion derived from this in vitro investigation is that a significant correlation exists between dental biomaterials and the amount of adhering clinical *S. aureus* strains. Different implant treatment with different biomaterials including zirconium oxide *should be considered* in patients with chronic infection like mucoviscidose.

[P53] THE ROLE OF FIBRONECTIN NANOPATTERN IN LIMITING THE STAPHYLOCOCCUS AUREUS ADHESION TO BIOMATERIAL SURFACE

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Bacterial adhesion to materials surfaces is of critical importance in many application areas, in particular in controlling infection related to medical implants. A range of bacteria can adhere specifically to proteins from the extracellular matrix found in tissue enabling infection and biofilm formation. Here we study specific interactions of staphylococcus aureus with fibronectin (Fn) adhered to materials surfaces and address the following questions:

-Does the nanoscale distribution of the Fn influence the adhesion?
-How does Fn adherent layers influence bacterial adhesion to nanoscale topography?

We utilized colloidal lithography to prepare patterns of Fn (from 0.1 µm - 1 µm) in a protein rejecting background of silicon dioxide coated with a precoated layer of Fn and study bacterial adhesion under flow. The results indicate that the adhesion of S. Aureus to surface adsorbed Fn is altered at sub micrometer patterns in a size dependent manner with a threshold for between 300nm patches. Above the threshold, increased adhesion was observed for larger patterns. Moreover, there was not a significant difference between the 300 and 500 nm in number of adherent bacterial cells. However, interestingly, 800 nm patches were more influenced for a bacterial adherence. Meanwhile, no significant difference has been noticed between the former size and 1000nm on the adherence number.

[P54] PLANKTONIC AND BIOFILM PSEUDOMONAS AERUGINOSA METABOLISM COMPARED BY BOTTOM-UP PROTEOMICS

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Pseudomonas aeruginosa is a problematic opportunistic pathogen often isolated from burn wounds, pressure ulcers and cystic fibrosis patient lungs. Proteomics can help elucidate different metabolic strategies utilized by this pathogen when grown as either planktonic or biofilm cultures. The role of carbon, nitrogen and energy sources such as glucose, lactate, and amino acid nutrients are of interest as they contribute to cellular growth, maintenance, and structural support. Untargeted label free quantification proteomics was applied here to illuminate metabolic differences in a comparative analysis of planktonic cultures and sessile membrane biofilms of *P. aeruginosa*.

A clinical isolate of *P. aeruginosa* was grown in amino-acid rich (CSP) or lactate-enriched CSP media (LCSP) and sampled at various growth phases. Biofilms were grown under no-shear conditions on tissue culture insert membranes. Whole cell lysate of planktonic and biofilm cultures were treated by RIPA buffer supplemented with DTT, lysozyme, and protease inhibitors. Protein concentrations were measured using DC-Bradford assay. Liquid chromatography electrospray ionization tandem mass spectrometry was performed on a Thermo Fisher Scientific Orbitrap Velos Pro. MaxQuant software was used for protein identification and label-free quantification. Statistical analysis was performed by Perseus software while functional enrichment analyses were performed with the String database search engine.

Over ~500 proteins were identified on whole cell-lysate of *P. aeruginosa*, including membrane proteins previously found in mature biofilms and cytosolic proteins linked to key portions of cellular metabolism. Ca. 50 proteins in planktonic culture and ~55 in biofilm culture were statistically significant after 11 hours of growth. Of these, 15 proteins from the planktonic cultures and 10 from biofilms displayed significant expression differentials between LCSP and CSP. For example, the enzyme for conversion of L-lactate to pyruvate - L-lactate dehydrogenase - was expressed with large differentials in planktonic cultures and biofilms grown in LCSP. Preferential use of different substrates was observed based on protein abundance changes, suggesting shifts in metabolic strategies as a function of nutrient environment.

This proteome data will be used to refine computational models of this opportunistic pathogen. Laser ablation sample transfer is also being implemented to permit site-specific sampling of proteins from intact biofilms.

[P55] METFORMIN IS ALTERING THE COMPOSITION OF THE BIOFILM IN THE GASTROINTESTINAL TRACT AND THEREFORE AFFECTING THE VITALITY OF BROWN TROUTS

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The gut microbiome can be divided into two fraction, one allochtonous microbiome in the feces and one authochtonous, biofilm-forming microbiome in the gut mucus. Changes in the authochtonous microbiome can have a major impact on the health of the host organisms. That's why chemicals, which alter the composition of the gut flora, are coming more and more to the focus of research. For metformin, an antidiabetic drug, population-shifts in the gut microbiome of humans and mice were described, also in phyla, in which pathogenic bacteria naturally occur, for example in the phylum *Proteobacteria*. Also an increase in the expression of virulence factors was described in mice and rats during metformin-treatment. But the effect of metformin on the aquatic biocenosis und therefore on the ecosystem is largely unknown. Our research aims on the investigation of the potential impact of metformin on the gut microbiome of fishes. Such interactions might be a reason for a missing recovery of some fish species.

The experiments were executed with brown trouts (*Salmo trutta fario*) and metformin concentrations of 0 µg/L - 1000 µg/L in triplicates at 11°C and 7°C. The gastrointestinal tracts were preperated for the population analysis. For the analysis of the gut microbiome two methodic approaches were used: 1) PCR-DGGE and 2) 16S-Illumina-Amplicon sequencing. Furthermore, gene expression analysis of pathogen specific virulence factors were conducted after Reverse Transcription with subsequent qPCR. For that, bacteria cultures were exposed to different metformin concentrations. In parallel different constitutively expressed housekeeping genes were used for normailzation.

Microbial population shifts were observed in intestinal mucus as well as faecal microbiome fractions in presence of metformin. The most significant impacts was analysed for the phylum *Proteobacteria*. The expression of virulence genes in pathogens associated with the fish gut microbiomes was found to be increased in presence of metformin at distinct concentrations. These different alterations in the fish microbiome are discussed to possess a direct effect on the vitality of the fishes.

[P56] FROM DIVISION OF LABOR TO AUTONOMY DURING BIOFILM MATRIX PRODUCTION

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Microbial biofilms provide a platform for complex social interactions. Some of these interactions involve a division of labor, where phenotypically heterogeneous subpopulations, or genetically distinct lineages cooperate with each other by performing complementary tasks. We combine experimental and computational approaches to investigate benefits arising from division of labor during biofilm matrix production by root-colonizing *Bacillus subtilis*. In this species, biofilm matrix consists of two major components; EPS and TasA. We show that pellicles and root-associated biofilms can be established via phenotypic or genetic division of labor over biofilm matrix production. Specifically, we observed that clonal groups of *B. subtilis* differentiate into three subpopulations composed of matrix non-producers, EPS-producers, and generalists, which produce both EPS and TasA. We further found that this incomplete phenotypic specialization was outperformed by a genetic division of labor, where two mutants, engineered as strict specialists, complemented each other by exchanging EPS and TasA. The relative fitness of the two mutants displayed a negative frequency dependence both in vitro and on plant roots, with strain frequency reaching an evolutionary stable equilibrium at 30% TasA-producers, corresponding exactly to the population composition where group fitness is maximized. Using individual-based modelling, we reveal why asymmetry in the ratio of cooperating partners can increase productivity during cooperation. Finally, we use experimental evolution to directly challenge the evolutionary stability of genetic division of labor during matrix production. Surprisingly, long-term experiments resulted in cooperation collapse and revealed evolution of alternative strategies for biofilm formation. These were no more dependent on cooperative exchange of EPS and TasA because they allowed pellicle formation using only one type of matrix-component, either EPS or TasA. Specifically, TasA-deficient strain evolved into enhanced EPS secretion, while EPS-deficient strain evolved an unconventional substitution in TasA modulating its biochemical properties. We observed that the evolved biofilm formation strategies carried evolvability vs. exploitability trade-off. We believe that such trade-off differences may represent an additional and very unpredictable barrier to evolution of division of labour between genetically distinct microbes.

[P57] INVASION OF AN 'ALIEN' BACTERIUM IN MICROBIAL BIOFILMS RESIDING IN OLIGOTROPHIC CONDITIONS. SEEKING ALLIANCES TO OVERCOME STARVATION

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Microbial invasion concerns the establishment of an 'alien' microbial species in a resident microbial biofilm community. Understanding the process of microbial invasion is crucial as invasion is an ubiquitous process involving beneficial as well as detrimental organisms and affecting various domains with an impact on economics and health. Pollutant-degrading bacteria are such beneficial organisms for which a successful invasion of a polluted environment, also known as bioaugmentation, is aimed for. In recent years, bioaugmentation was dug up again as a technique to remove micropollutants from drinking water by applying micropollutant-degrading bacteria in existing sand filters which are part of the drinking water production process.

Challenges for micropollutant-degrading bacteria to invade the sand filter environment were explored using *Aminobacter* sp. MSH1, a strain currently investigated to remove 2,6-dichlorobenzamide (BAM) from contaminated drinking water. Strain MSH1 is able to form biofilms in the sand filter environment relying only on a very low amount of assimilable organic carbon for energy, while exposed to high shear forces. Proteomics reveal signs of starvation stress leading to a reduced gene expression and metabolisation of enzymes including BAM-catabolic enzymes. In addition, plasmids carrying the genes for BAM-mineralization become a burden due to a high maintenance cost for replication, translation and oxidative stress due to catabolic activity and are discarded.

Starvation puts MSH1 in a bad position to invade a resident sand filter biofilm community well-adapted to oligotrophic conditions in sand filters. Using a synthetic microbial community reconstituted from previously isolated sand filter bacteria, reveals, however, that introduction of an 'alien' bacterium such as MSH1, brings new opportunities for resident bacteria. Upon invasion by MSH1, a shift was observed from a community previously dominated by *Paucibacter sp*. to a community evenly dominated by *Rhodococcus* sp., *Piscinibacter* sp. and strain MSH1 itself, and corroborates with a co-localization of strain MSH1 with resident bacteria, indicating cooperative associations.

Hampered growth, starvation and shear undermines maintenance of active MSH1 cells, but adequate BAM removal from drinking water is assured largely due to MSH1's exceptionally high reactivity towards the micropollutant BAM and the resilience to cooperate with bacteria in the residing biofilm community.

[P58] CHARACTERISATION OF BIOFILM AND CELL AGGLOMERATION BY BURKHOLDERIA CEPACIA COMPLEX FROM INDUSTRIAL ENVIRONMENT

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The genus *Burkholderia* comprises a group of all-occurring, gram-negative, motile, obligatory aerobic and rod-shaped bacteria. Because of the opportunistic pathogenicity to plants, animals and humans, the members of *Burkholderia cepacia* complex (Bcc) are important among the genus of *Burkholderia*. Contamination by Bcc is found in different industrial issues. Bcc affect manufacturing process chains by contaminating the working fluids with planctonic cells and biofilms. This study aims: (i) to characterise genetic and biochemical properties of industrial Bcc isolates; (ii) to analyse planctonic growth and potential to form biofilms; (iii) to better understand the influence of antibiotics, metal ions and biocide on cell metabolism; (iv) to define the process of cell agglomeration as a precursor step of mature biofilms. For this, the wild type strain *Burkholderia cepacia* (DSM_7288) is taken into account as reference strain, belonging to the same group of Bcc. Starting with growth studies in different rich and poor culture medium, the industrial isolates showed growth even in low nutrient surroundings. Contrary to the isolates, the reference strain was able to grow in rich media. Though the Bcc showed similar metabolic properties, further investigations on the genotype were done by RAPD analysis resulting in different fingerprints for each strain. The establishment of a micro-titer-plate assay enabled the analysis of biofilm formation capabilities. Bcc isolates were strong producer of biofilm. Beside the ability to form biofilms on solids, the isolates in the planktonic form were able to agglomerate permanently by cell-cell-attachments under static and dynamic conditions. Formed agglomerations were quantified by means of optical density and visualised by microscope. Industrial isolates showed higher resistances to antibiotics compared to the wild type and were able to form biofilms in the presence of biocides. The applied biocide had a different effect on the strains: two of the isolates showed strong biofilm formation despite biocide addition although growth of the microorganisms was already prevented at lower concentrations. However, significantly higher levels of biocides were used in the industrial application. The trials with the metal ions showed that the interaction of bivalent metal ions with the biocide result in reduced biofilms.

[P59] THE ROLE OF BIOFILM FORMATION IN THE VIRULENCE OF C. ACNES

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Acne vulgaris is a common skin problem that results from abnormal inflammation of the pilosebaceous unit and causes various lesions (hyperpigmentation, papules, comedos etc.). It mainly affects the face, the torso and the upper back and arms. The proliferation of *Cutibacterium* (formerly known as *Propionibacterium*) *acnes* (*C. acnes*) is one of the factors involved in acne. *C. acnes* is a skin commensal and a Gram-positive bacillus that resides in pilo-sebaceous follicles. Nevertheless, the presence of *C. acnes* does not solely account for acne and healthy and acne prone persons can both exhibit the same level of colonization by *C. acnes*.

First, it is a well-known fact that the pathogenicity of *C. acnes* lies at least partly in its capacity to form biofilms i.e., in most cases, virulence factors are overexpressed in a biofilm state. Biofilm formation by *C. acnes* also significantly reduces its susceptibility to antibiotics (vancomycin, clindamycin, erythromycin, levofloxacin, ciprofloxacin). Secondly, at least 3 phylotypes of *C. acnes* have been described (I, II, III) and are suspected to have different implications in acne or other infections, suggesting different virulence profiles.

In order to better understand biofilm formation by *C. acnes* and its link with virulence, an *in vitro* biofilm model in 24-wells microplates was optimized. Results show that *C. acnes* is able to form biofilms of approximately 10^6 CFU/well after 72 h in a medium with a reduced concentration of nutrients, a density that is comparable to biofilms obtained in rich media like BHI (Brain Heart Infusion) or RCM (Reinforced Clostridial Medium). Interestingly, BB does not support planktonic growth, contrary to BHI or RCM.

Our results also indicate that some virulence factors (lysozyme M1, CAMP factors, GehA lipase or Dermatan Sulfate adhesin) are differentially expressed among the different phylotypes of *C. acnes*.

Current work now focuses on the influence of artificial sebum, also known to play an important role in acne, and possibly on biofilm formation by *C. acnes*.

[P60] EXOMETABOLOME ANALYSIS REVEALS UNEXPECTED METABOLIC INTERACTION BETWEEN STREPTOCOCCUS GORDONII AND PORPHYROMONAS GINGIVALIS.

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Background:

In subgingival regions, oral microbes release variety of molecules to the microenvironment and these metabolic by-products of one microbe are utilized by others as an energy or nutrient source. Very recently, we reported a metabolic cross-talk between an accessory pathogen *Streptococcus gordonii* and the periodontal pathogen *Porphyromonas gingivalis* via para-aminobenzoic acid, which promoted *P. gingivalis* accumulation on Streptococcal biofilms, whereas suppressed its virulence in mouse models. However, comprehensive metabolic interactions between these bacterial species remain unknown. In this study, we employed CE/MS-based metabolomics to examine a global metabolic interaction between *S. gordonii* and *P. gingivalis*.

Materials and methods:

S. gordonii DL1 and *P. gingivalis* ATCC33277 were individually cultured, harvested and re-suspended in a modified chemically defined medium containing neither proteins, peptides, nor amino acids. Six-transwell polystyrene cell culture plates were inoculated with *P. gingivalis* (1×10^{10} cfu) in the lower compartment and *S. gordonii* (1×10^{10} cfu) in the upper transwell insert. Both strains were co-cultured at 37 °C under anaerobic conditions. Culture supernatant was harvested at 6 hours of incubation, then ionic metabolites were extracted. CE-TOFMS was used to measure metabolite levels simultaneously.

Results:

A metabolome analysis illustrated that glyceric acid and glycerol 3-phosphate, both are intermediates between central carbon and lipid metabolic pathways, were released into the supernatant only by cooperative interaction between *S. gordonii* and *P. gingivalis*. Furthermore, accumulation of ornithine, Glu, Thr, homoserine and uracil was synergistically enhanced by the co-existence of *S. gordonii* and *P. gingivalis*, compared to those in mono-culture supernatants ($p<0.001$). His, Gln, Pro, succinate, dipeptides (Gly-Gly, Gly-Leu, Gly- Glu-Glu, His-Glu, Ser-Glu, Tyr-Glu) and some decarboxylation products, such as agmatine and GABA, were released only by *P. gingivalis*, and markedly decreased in the co-culture supernatant ($p<0.001$), indicating the consumption or inhibition by *S. gordonii*. On the other hand, Asp and carbamoyl-Asp were mainly released by *S. gordonii*, and their production was synergistically enhanced by the existence of *P. gingivalis* ($p=0.001$).

Conclusion:

These results offer new insight into the unknown metabolic flux affected by the cooperative action of *S. gordonii* and *P. gingivalis*.

[P61] COLONY MICRO-FEATURE DIVERSITY IN ENVIRONMENTAL AND CLINICAL PSEUDOMONAS AERUGINOSA ISOLATES

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Pseudomonas aeruginosa, an opportunistic pathogen and highly social microbe, is a metabolically diverse and phenotypically rich organism. Previous research has shown high levels of structural organization in assemblages driven by both physical limitations of colony growth and emergent characteristics of the population.

This study aims to survey fine-level phenotypic diversity in over 100 fully sequenced *P. aeruginosa* environmental and clinical isolates, explore techniques for characterization of observed micro-features, and automate analysis of their phenotypic diversity to look into the application of colony structure as a predictive variable of medically relevant characteristics.

Using high-resolution light microscopy, we have imaged entire centimeter scale colonies grown on 2% LB-Congo Red agar for three days, enough time to reveal major phenotypic landmarks. With this method, we can resolve colony structure on the sub-millimeter scale and uncover layers of complexity overlooked at lower magnifications.

In an effort to explain what these emergent micro-features physically are, we consider the pros and cons of various characterization techniques from white light interferometry to scanning electrochemical microscopy and imaging mass spectroscopy.

Finally, we are developing a custom deep convolutional network from the augmentation of the original image dataset in order to extract features in morphological space. We can then relate these features with 30 physiological variables related to growth, antibiotic resistance, biofilm formation and virulence factor production that we measured and explore the relationship between morphological features and these variables. If a relationship exists, it would be possible to use colony morphology, a relatively simple technique, to predict medically relevant characteristics about strains, allowing for cheaper diagnostics.

[P62] NITROGEN FIXATION IN A SYNTROPHIC COCULTURE ALTERS BIOFILM STRUCTURE AND FUNCTION

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Free-living diazotrophic bacteria and archaea are key players in global nitrogen cycling, making fixed nitrogen available in anoxic systems with significant contribution to dinitrogen flux from the atmosphere. Current research on nitrogen fixation in biofilm is dominated by examples from root-associated bacteria. In this study, a previously characterized syntrophic coculture of *Desulfovibrio vulgaris* and *Methanococcus maripaludis* was evaluated for potential to fix nitrogen under syntrophic growth conditions and in continuous culture biofilm. Extensive work has been done on the regulation of nitrogen fixation in *M. maripaludis*, while it is less studied in *D. vulgaris*, and coculture. Little attention has been directed to the role of nitrogen limitation and fixation in the SRB:methanogen syntrophy that is perched at the edge of thermodynamic constraints. The purpose of this work was to determine the feasibility of nitrogen fixation in coculture biofilm and infer the role of both members. We predicted that diazotrophic biofilm structure and composition would be distinct from ammonium-fed biofilm. Coculture growth rate was reduced in batch planktonic diazotrophic studies, and biomass yield was low, presumably due to poor transport of N_2 gas to the aqueous phase. Continuous culture in a biofilm reactor afforded constant sparging with N_2, and mixing, which resulted in biofilm formation along with a robust planktonic community. Biofilm structure was notably different under diazotrophic growth compared to ammonia conditions as visualized with electron microscopy, with a thin biofilm that appeared to be arranged to allow for gas transport. Relative abundance of *D. vulgaris* and *M. maripaludis* in the biofilm was similar to intermediate aged ammonium-grown biofilm, while the planktonic ratio was reversed with 61% *M. maripaludis* and 39% *D. vulgaris* for a comparable amount of total planktonic protein. Biofilm protein represented just under 10% of the total reactor biomass under N_2 fixation, while ammonium-grown biofilm accounted for 80% of the total reactor biomass. The total ratio of *D. vulgaris: M. maripaludis* was 1:1 compared to a ratio of 2.5:1 in ammonium-grown conditions. The results suggest that the required energy burden of nitrogen fixation lowers biofilm biomass allocation and promotes more equalized carrying-capacity for the two biofilm populations.

[P63] IMPACTS OF COLD RECOVERY ON THE MICROBIAL ECOLOGY IN DRINKING WATER DISTRIBUTION SYSTEMS: A PILOT STUDY

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The potential of Drinking Water Distribution Systems(DWDSs) to provide thermal energy in the form of cold has been recently explored, and it seems a promising resource for cooling purposes. However, due to cold recovery by means of a heat exchanger, the drinking water temperature rises instantaneously in and after the heat exchanger, and the influence of suddenly increased temperature on microbiological water quality and microbial ecology of DWDSs is still not known. This work studied DWDSs with cold recovery from the perspective of microbiological changes. Specifically, this study aimed to evaluate the impacts of cold recovery on the biofilm development in pilot distribution systems (PDSs) and the microbiological quality of drinking water. Two small scale PDSs were used in this study: a PDS with a cold recovery system, and a reference system without cold recovery. Both water and biofilm samples taken from these systems were analysed for biomass (measured by ATP, heterotrophic plate counts and cell counts(TCC) with flow cytometry) and microbial community diversity and composition (Illumina Miseq,16srRNA).After a period of 9 months, significant changes were observed in the biofilm formed after cold recovery in terms of 3 times higher TCC and ATP compared to the biofilm in the reference system. Moreover, the results of 16srRNA revealed a less diverse and significantly different (p= 0.01) biofilm community formed in the cold recovery system compared to the reference system. In the cold recovery system, bacterial groups that are related to formation of thicker and stronger biofilms are more abundant than in the reference system. The relative abundance of *Legionella spp.* was 2% higher in the cold recovery system compared to the biofilm in the reference system. In bulk water, no major changes were observed, most likely linked to the short hydraulic residence time in the system(60seconds) as compared to biofilm (38 weeks). It is concluded that temperature increase due to cold recovery inside DWDSs is more likely to impact the biofilm phase than the water phase. However, due to interaction between both phases it may contribute to changes in the water phase on the long term. Hence, cold recovery from drinking water systems combined with subsequent temperature increase requires continuous monitoring and investigation to control the drinking water quality.

[P64] HEAT AND CIP TOLERANT FILAMENTOUS YEASTS DOMINATING REVERSE OSMOSIS MEMBRANE BIOFILMS

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Reverse Osmosis (RO) membranes are increasingly being used in the food industry for treatment of product water subsequently used for different purposes within the industrial facilities in order to save water intake and discharge of waste water. Although the spiral wound RO membranes offer high filtration efficiency, their compact structure increases susceptibility to biofilm formation, which may lead to flux reduction and changes in the water quality. Even after Cleaning-In-Place (CIP) operations using strong alkaline and acidic solutions, some microorganisms may still survive in biofilms. We investigated the biofilm communities on several RO membranes used for after-treatment of ultrafiltered product water in food industry and their response to cleaning and disinfection. Light and Confocal Laser Scanning Microscopy (CLSM) were used to visualize the biofilms. The total microbial population was enumerated using Standard Plate Count (SPC) method, while 16S and 26S rRNA sequencing were used for identification of bacteria and yeast isolates. Tolerance to different time-temperature combinations and CIP solutions was tested.

A dense network of filamentous yeast covering a great area together with budding yeast and bacteria was observed before CIP, while after CIP only yeast species were detected. The dominant species were identified as the filamentous yeast genera *Saprochaete clavata* and *Magnusiomyces spicifer* and they exhibited high tolerance towards heat (15min. /75° C) and CIP compounds.

Filamentous yeast species are rarely described as part of RO membranes biofilms. We believe that although they may cover large areas on membrane surface, they can be easily overlooked due to their slow growth and low numbers relative to the many fast growing bacteria. Work is in progress to elucidate their role in microbial adhesion and survival as well as removal strategies.

[P65] A METHANOGENIC BIOFILM FOR BIOGAS UPGRADING - INVESTIGATED ON A MICROSCALE

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Hydrogen produced from periodic excess of electrical energy may be added to biogas reactors where it is converted to methane that can be utilized in the existing energy grid. It has been demonstrated that hydrogen can be readily utilized by microorganisms in anaerobic digesters. One of the major challenges with this technology is the low hydrogen solubility and gas-to-liquid mass transfer limitations when introducing hydrogen to the anaerobic digester. Gas-filled reactors containing a carrier material with a large surface area covered by a methanogenic biofilm could ensure efficient gas-liquid transfer and circumvent the problem with low hydrogen solubility.

Two different kinds of biofilm carrier material were tested; BIO-BLOK® and glass fiber filter paper. The incubations were made in slurry-filled serum bottles with 75% headspace. Controls consisted of slurry without carrier material. After five weeks of incubation the average methane production rate for the control was 0.59 (n=2) L_{CH4}/L_{slurry}/day, 0.79±0.35 (SD, n=3) L_{CH4}/L_{slurry}/day for the glass fiber filter paper and 7.43±4.84 (SD, n=3) L_{CH4}/L_{slurry}/day for the BIO-BLOK®. Hence, the methane production rate for the control and glass fiber filter paper was comparable while the BIO-BLOK® methane production rate was 13 times higher. The hydrogen flux towards the biofilm and slurry was determined with a hydrogen microsensor when a mixture of 80% H_2 and 20% CO_2 was supplied. The average hydrogen flux towards the slurry was 0.16±0.03 (SD, n=3) nmol cm^{-2} s^{-1} while the average hydrogen flux towards the glass fiber filter paper was 0.21±0.03 (SD, n=9) nmol cm^{-2} s^{-1} and 0.17±0.01 (SD, n=6) nmol cm^{-2} s^{-1} towards the BIO-BLOK®.

It was demonstrated that it is possible to grow a hydrogen consuming biofilm and enhance the methane production rate. The hydrogen fluxes towards the slurry and the different biofilm carrier materials were comparable. Hence, the enhancement of the methane production rate in the BIO-BLOK® incubation must be interpreted as a result of an increased surface area for the hydrogen to methane conversion.

[P66] BIOFILM DIVERSITY, STRUCTURE AND MATRIX SEASONALITY IN COOLING TOWER SYSTEMS

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Biofilms colonize surfaces in cooling water systems, where cause operational issues and microbiologically induced corrosion as well as may pose health problems associated with the presence of pathogens. Control strategies, mainly based on the addition of chemical additives to the water, may not be fully effective in biofilm removal resulting from the protection provided by the biofilm matrix. The scanty information on biofilm occurrence and development in industrial systems strongly limits the efficacy of the current control strategies as the development of more effective alternative procedures. Our aim was evaluate the temporal patterns in diversity and structure of seasonal biofilm samples collected from a full scale cooling tower over one year. To this end, next generation sequencing, CARD-FISH and lectin staining, combined with confocal microscopy were used. A bacterial core microbiome constituted by members of *Sphingomonadaceae*, *Comamonadaceae* and *Hyphomicrobiaceae* families was found in all samples. Marked seasonal variations were observed in the phototrophic fractions. Summer samples showed the highest species richness and were dominated by filamentous cyanobacteria immersed in a α-Mannose, α/β-Glucose rich matrix. They also formed aggregates building a scaffold for coccal cyanobacteria and bacteria, whose capsules had N-acetyl-glucosamine. Filamentous green algae were also found showing fucose on their surface. Raphid diatom species were also present, binding N-acetyl-glucosamine specific lectins on frustule surfaces. Conversely, the lowest species richness was recorded in winter biofilms, dominated by diatoms surrounded by a loose matrix containing abundant mineral particles. Autumn and spring samples had similar phototrophic composition showing a prevalence of green algae, surrounded by AAL labelled mucilaginous material.

We could suggest that a single control strategy cannot be used all over the year since as a biofilm variability has been retrieved.

[P67] ELUCIDATING PRIORITY EFFECTS AND SUCCESSION IN MULTISPECIES BIOFILM ASSEMBLY

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The arrival order of different species to a specific niche may strongly impact community assembly and succession dynamics. In this study, we used a model consortium composed of four soil isolates (*Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans* and *Paenibacillus amylolyticus*) to determine if priority effects exist and influence the assembly of a multispecies biofilm grown under different *in vitro* conditions. We tested our four species individually for exerting priority effects on later arriving species by determining the species ratios of the biofilms at different time points. The IBIDI microfluidics system was used to grow the biofilms. The procedure was to pre-inoculate the strains into separate flow-cells and grow them individually for 6h. Cultures with all four strains were subsequently inoculated in all flow-cells and incubated for a total of 48h and 96h. All biofilm biomass was collected from the flow-cells and 16S rRNA gene amplicon sequencing was performed to determine relative species ratios. Absolute bacterial numbers were estimated by flow cytometry and plate counts. Moreover, the spatial structure of fluorescently-tagged *S. rhizophila* and *X. retroflexus* in the biofilms will be assessed by confocal microscopy. Preliminary results indicate that specific species alter the niche and thus influence the quantity of biofilm produced and the composition of the community.

[P68] INCREASED INTRASPECIES DIVERSITY IN ESCHERICHIA COLI BIOFILMS PROMOTES CELLULAR GROWTH AT THE EXPENSE OF MATRIX PRODUCTION

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Intraspecies diversity in biofilm communities is associated with enhanced survival and growth of the individual biofilm populations. In here, the influence of intraspecies diversity in biofilm populations composed of up to six different *Escherichia coli* strains was evaluated. For that, standard methods were employed including crystal violet (CV) staining, cultivability in solid medium and extracellular polymeric substances (EPS) matrix quantification. In general, with the increasing number of strains in the biofilms, a slight increase in cell cultivability and a decrease in EPS matrix production were observed. It hence appears that increased genotypic diversity in a biofilm leads *E. coli* to direct energy towards the production of its offspring, in detriment of the production of public goods (i.e. matrix components). In fact, an intraspecific competition seems to take place between phylogenetically close bacteria. This behaviour is particularly noticeable during the first hours of biofilm growth, where a trend for multi strain biofilms to cluster themselves as low biomass producers is observable. Interestingly, the most phylogenetically distant isolate seems to induce an increase in biomass production when paired with the remaining strains in consortia. Multiplex PNA-Fluorescence in situ hybridization (FISH) combined with confocal laser scanning microscopy (CLSM) analysis of polymicrobial biofilms suggests a well-mixed, “carpet-like” structure of the biofilm. Adding to the ecological implications, these results can be explored in clinically-relevant biofilms, as a decrease in EPS matrix production, by introducing avirulent strains, may render these biofilms more sensitive to antimicrobial agents.

[P69] BIOFILMS OF STENOTROPHOMONAS MALTOPHILIA PROTECT LACTOBACILLUS BREVIS FOOD SPOILAGE BACTERIA AGAINST CLASSICAL CLEANING AND DISINFECTION PROTOCOLS

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The development of a biofilm can protect food spoilage microorganisms against classical cleaning and disinfection protocols used in the food industry. In this study, the tolerance of a *Stenotrophomonas maltophilia* biofilm forming strain (Sm), isolated from a food processing environment, against cleaning and disinfection agents was investigated. Its ability to form bispecies biofilms with a food spoilage organisms *Lactobacillus brevis*, isolated from the same food company, was evaluated. Mono- and bispecies biofilms of both micro-organisms were subjected to classical cleaning and disinfection protocols in a reactor designed to simulate food processing conditions. Biofilms of the Sm strain showed a high tolerance against the cleaning and disinfection agents, with on average a LOG 3.5 reduction after the total sanitation protocol and the residual biofilm completely recovering within 24 hours when supplied with new growth medium. The monospecies biofilm of *Lactobacillus brevis* was completely eradicated by cleaning and disinfection and no regrowth was observed when new growth medium was supplied. However, when co-cultured with the Sm-strain in a bispecies biofilm, the *Lactobacillus brevis* strain survived the sanitation protocols and recovered completely within 24 hours. This suggests that to reduce food spoilage, the removal of the Sm-strain from food surfaces is necessary.

The optimization of the removal strategy was done by analyzing the EPS composition of different Sm-strains, with different biofilm-forming capacities. The protein composition of the EPS determined by SDS-PAGE of the different Sm-strains was different depending on the biofilm-forming capacity of the strain. All Sm-strains show proteins in their EPS layer with a molecular weight of 54 kDa. However the EPS layer of the Sm-strains exhibiting strong biofilm forming capacities consisted of extra proteins of 18, 19 and 34 kDa.

This study shows the importance of multi-species consortia in food spoilage being the possible source of recurring contamination problems in the food industry and the need to develop a biofilm removal strategy to eliminate food spoilage organisms from the production plant.

[P70] CHARACTERIZATION AND CONTROL OF MICROBIAL PROLIFERATION ON BIO-BASED PRODUCTS FOR HEALTHY AND DURABLE BUILDINGS

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Nowadays, impacts of buildings on the environment and on the health of inhabitants are priority issues. For many reasons, the demand for building products (bricks and plasters) made of materials such as clay and bio-based materials (vegetal fibres, granulates...) is increasing. Under certain conditions, a microbial development on such surfaces can be observed, which raises many questions about their use in buildings due to potential health hazards for inhabitants.

The BIOTERRA ANR project (2014-2018) aims to identify and to characterize microbial proliferation on bio-based earthen construction products, and to propose solution(s) to reduce or inhibit these proliferations, in order to ensure healthy and durable buildings.

Samplings have been performed in several earthen dwelling, sometimes on bio-based building materials: three sampling campaigns have been carried out between 2015 and 2016, on 11 sites with an average of 4 sampled areas per site. The composition and diversity of bacterial and fungal biofilms communities on the indoor walls have been characterized and identified by high throughput sequencing (MiSeq). The main bacteria identified belonged to the *Arthrobacter*, *Bacillus*, *Actinomycetospora* and *Saccharopolyspora* genera. The *Aspergillus*, *Cladopsorium*, *Wallemia* and *Fusarium* fungal genera were the most abundant taxa on the surface of earthen building materials. The analysis of the microbial flora suggests an effect of the addition of vegetal fibres on the composition of bacterial biofilms, but not on the fungal communities. The abundance of fungal genera seems to be more associated to the sampled house than to the building material composition. Microbial communities on earthen building materials are close to those identified on other building materials and do not represent a hazard for the occupants under conventional environmental conditions.

[P71] A SIX MONTH STUDY MONITORING THE FATE OF LISTERIA INNOCUA INTRODUCED TO A MATURE BIOFILM COMMUNITY ESTABLISHED IN A MODEL DRAIN SYSTEM

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Listeria monocytogenes is a foodborne pathogen of major concern as it can establish and persist within food processing facilities. Floor drains are prime locations for buildup of *Listeria*-harboring biofilms due to constant wetting and contact with food residues. As such, the pathogen can be disseminated from these reservoirs onto foods or food contact surfaces through the production of aerosols. In this study, a five strain cocktail of *Listeria innocua* was used as a surrogate for *L. monocytogenes* to investigate the ability of *Listeria* to integrate into an established biofilm within a floor drain model, and persist following multiple exposures to benzalkonium chloride (BAC). A new cast iron floor drain was obtained, cleaned and sterilized. The drain was inoculated by the addition of ground beef slurry which was left to stand at 15°C for 24 h to allow the natural microflora associated with the meat time to colonize drain surfaces. A regimen of nutrient cycling was employed where the biofilms were subjected to alternating days of nutrient exposure (1/10 cooked meat medium) and starvation (water) over 28 weeks. The *Listeria* cocktail was introduced after biofilms were given 8 weeks to establish. At this time viable cell densities ranged from 10^7 (sides) to 10^8 (bottom) CFU/cm^2. *Listeria,* introduced at 10^5 CFU/ml was able to integrate into the biofilms and maintain levels between 10^3 and 10^5 CFU/cm^2 over a four week period prior to BAC exposures. Following initial BAC treatment (week 12) *Listeria* counts were reduced to 10^2 CFU/cm^2 but returned to near pre-treatment levels within two weeks. After a second BAC treatment (week 16) *Listeria* were reduced to undetectable levels; however over the next 4 weeks the bacterium was re-established. This pattern continued over the remainder of the study. Profiling the biofilm community through DGGE analysis and 16S rRNA gene sequencing showed that the community structure that was established early during the regimen remained relatively consistent over the entire course of the experiment; however its relative resistance to BAC appeared to increase. In this six month study the persistent nature of *Listeria* was clearly demonstrated using a model drain system.

[P72] SMALL-SCALE HETEROGENEITY IN DRINKING WATER BIOFILMS

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The majority of bacteria reside in biofilms and multiple factors give rise to heterogeneity within these. While this had already been described on large scale (e.g., through a full-scale distribution system), heterogeneity on smaller scales is poorly quantified. In this study, we assessed the heterogeneity of building plumbing biofilms on small-scale. To achieve this, indigenous biofilms were grown inside flexible shower hoses over the course of 12 months under controlled conditions; hoses (1.20 m) were horizontally aligned and exposed to twice-daily shower events (15 min) with non-chlorinated warm water (~ 42°C). For analysis, hoses were dissected into 100 cross sections, which were then halved to yield 200 pieces of approximately 1.2 cm^2. Biofilms were characterized by their thickness (µm-scale, optical coherence tomography), total cell concentration (TCC) (cm-scale, flow cytometry), and microbial community composition (cm-scale, 16S rDNA amplicon sequencing). We found considerable heterogeneity on small-scale throughout the length of a hose. Biofilm thickness along the entire hose ranged between 35.8 – 890.7 µm, with significantly higher values for the bottom (323.0 ± 91.7 µm) compared to the top part (244.1 ± 54.4 µm). On small-scale, the thickness varied up to 75% within as little as 500 µm hose length. TCC varied throughout the hose between 1.2 x 10^8 – 3.9 x 10^8 cells/cm^2, with successive 1.2 cm biofilm sections showing differences between 0.04 – 96.4 %. Interestingly, averaged TCC did not differ between bottom (2.6 ± 0.7 x 10^8 cells/cm^2) and top (2.6 ± 0.4 x 10^8 cells/cm^2) as observed for thickness. In addition, changes in the microbial community composition of the biofilm were detected throughout the hose, with a higher α-diversity for the bottom compared to the top. From these biogeographical variations, we conclude that considerable heterogeneity in biofilms does occur already on small-scale, even when biofilms were formed under controlled and consistent conditions within the same hose. These findings are particularly valuable with respect to (1) an improved understanding of biofilm ecology, (2) developing sensible biofilm sampling strategies, and (3) understanding the driving factors for biofilm formation and how small-scale development impacts the microbiology of building plumbing systems on a large-scale.

[P73] AN AUTOMATED SYSTEM FOR OCT IMAGING OF BIOFILMS UNDER DIFFERENT HYDRODYNAMIC REGIMES

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Benthic biofilms contribute substantially to the biogeochemistry of streams and provide important ecological functions. Depending on local hydrodynamics, these complex communities are constantly exposed to the shear stress of the flowing water, with consequences for mass transfer of solutes, biofilm metabolism and ultimately biofilm architecture . We developed a laboratory mesocosm setup to monitor the growth of phototrophic biofilms under a variety of hydrodynamic regimes using optical coherence tomography (OCT). OCT is a meso-scale 3-dimensional imaging technique that provides information on volume, height and roughness of biofilms. The OCT was mounted on a precision positioning device and integrated in an automated system of images collection and processing. We designed plexiglass flumes with a funnel-like shape, which generates a gradient in flow velocity within the single flume. These flumes can be utilized in a nozzle or diffuser configuration, resulting in different levels of turbulence and shear stress distribution. Here we present the capabilities of this setup to rigorously monitor biofilm growth and architectural differentiation with the aim to understand biofilm morphogenesis as a function of local hydrodynamics.

[P74] MARINE BIOFILM DIVERSITY ON ANTIFOULING COATINGS: A METAGENOMICS APPROACH

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Marine biofilms (mainly composed of bacteria, diatoms, spores/larvae of macro-organisms) constitute an important component of the overall biofouling process i.e. accumulation of marine macro-organisms (e.g. algae, barnacles, ascidians etc) on artificial surfaces. The maritime industry is largely affected by biofilms as their presence can increase roughness on ship hulls therefore impacting the ship's hydrodynamics, leading to higher CO_2 emissions and fuel consumption. Current antifouling (AF) coatings, such as Foul Release Coatings (FRCs) only inhibit colonization of macro-organisms, however biofilms remain the main issue as they tenaciously adhere on FRCs. Very little is known about biofilm species composition, especially when formed on FRCs, therefore it is crucial to gain knowledge on biofilm biodiversity. In the current work we investigated the biofilm community composition found on FRC coatings (plus controls) and exposed in two different sites, Southampton, UK and Genova, Italy for 12 days via Next Generation Sequencing targeting the 16s rRNA and 18s rRNA. Some key findings: Overall PCO analysis showed a clustering of OTUs with site and surface properties. For the 16s rRNA analysis, Cyanobacteria were the main phylum in Southampton Water while for the Mediterranean site it was Proteobacteria and Bacteriodetes. On FRCs these main taxa were significantly reduced when compared to the controls. For eukaryotes, the FRCs significantly decreased crustacean presence in both sites, while the diatom genus *Navicula* was abundant in the Southampton Water but not in the Mediterranean, however when compared to the controls, FRCs showed reduced abundance. We believe that data like the ones we present here, will aid the AF industry towards a more informed coating design.

[P75] UNVEILING BIOFILM ARCHITECTURAL DIFFERENTIATION IN SEDIMENTARY ENVIRONMENTS

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Hydrated porous systems with their high surface availability are attractive substrates for biofilm growth. A prime example of such a system is the hyporheic zone of streams hosting diverse microbial communities concealed within the sediment interstices. Within this sedimentary environment, topographic heterogeneity and fluid flow constrain biofilm growth by altering solute supply and hydrodynamic stress. The interplay between hydrodynamics and biofilm architecture and function in porous systems remain poorly understood. To better appreciate these links, we designed porous-like fluidic devices, exposed to streamwater flow containing bacterial cells. Biofilm formation and local hydrodynamics were investigated using time-lapse microscopy and micro-particle image velocimetry. We found two different architectures: a biofilm coating the grains and streamers extending into the pore space. We show that the architectural differentiation was the result of biofilm growth on the grains and of cell retention by the streamers. We also found that biofilms competed for space and substrates, and that the differentiation into streamers and coating biofilm was beneficial to the entire biofilm. Our work advances previous studies on streamer formation in porous systems and highlights the importance to work with diverse microbial communities rather than monospecies systems if the biophysical processes that shape the most successful mode of microbial life is to be understood.

Keywords: biofilm morphology, porous-media, filtration

[P76] MULTISPECIES BIOFILM FORMATION BY STREPTOCOCCUS PNEUMONIAE, HAEMOPHILUS INFLUENZAE AND MORAXELLA CATARRHALIS AND THEIR INTERACTION WITH NASOPHARYNGEAL MICROBIOTA AND PROBIOTIC SPECIES

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Streptococcus pneumoniae (*Sp*), *Haemophilus influenzae* (*Hi*) and *Moraxella catarrhalis* (*Mc*) commonly colonise the young children's nasopharynx (NP), exhibiting a ubiquitous and strong positive association. NP colonisation and subsequent biofilm formation are essential steps for the development of invasive diseases. In the present study we aimed to show the interactions between these pathogens with other members of NP microbiota and potential probiotic species in a multispecies (MS) biofilm (BF) model.

Single- (SS), dual- (DS) and MS-BF were grown *in vitro* for 72 hours, and individual species were quantified at different time points by propidium monoazide real-time polymerase chain reaction (PMA-qPCR). MS-BF were performed with *Sp-Mc-Hi* only, or in combination with *Staphylococcus aureus* (*Sa*), *Lactococcus lactis* (*Ll*) or *Lactobacillus casei* (*Lc*). *Sp-Mc-Hi* MS-BF were also qualitatively analysed by confocal laser scanning microscopy (CLSM).

Sp-Mc-Hi formed a stable MS-BF over a 72-hour period. In comparison to its SS-BF, *Hi* increases in MS, whilst *Mc* decreases by approximately 4 $\log_{10}$ units. Conversely, *Sp* BF formation was mostly unaffected by *Mc* and *Hi*, either in DS or MS-BF. In DS-BF, *Hi* shows a 3 $\log_{10}$ increase in combination with *Mc*, and *Mc* has restored its counts from SS-BF, suggesting that their association is more positive than that of *Mc* or *Hi* with *Sp*. Spatial organisation analysis of CLSM images corroborate these results. Whilst *Mc-Hi* interaction seems neutral in MS-BF, their interaction with *Sp* is negative.

The presence of *Sa* or *Ll* reduced *Sp-Mc-Hi* MS-BF, although an increase of *Mc* on BF containing *Ll* was noted. Conversely, when in combination with a bacterial species not commonly found on NP, *Sp-Mc-Hi* either increased or maintained similar numbers, even in the presence of high *Lc* counts.

In conclusion, the results show that the interplay amongst *Sp-Mc-Hi* seems to be the balance of cooperative and competitive interactions. In addition, *Ll* and *Sa* (a known NP competitor) have affected but not eradicated *Sp-Mc-Hi* on MS-BF, which might reflect some of the interactions occurring in the NP. Additionally, these results can provide a basis for future studies of *Ll* as a probiotic for the respiratory tract.

[P77] PHOTOTROPHIC MICROBES FORM ENDOLITHIC BIOFILMS IN IKAITE TUFA COLUMNS (SW GREENLAND)

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The Ikka Fjord (SW Greenland) harbors a unique microbial habitat in the form of several hundred submarine tufa columns composed of ikaite, a special hexahydrate form of calcium carbonate that precipitates when alkaline phosphate- and carbonate-enriched spring water seeping out of the sea floor meets cold seawater. *In situ* underwater microsensor measurements in the porous ikaite crystal matrix, revealed an extreme microenvironment characterized by low temperatures, strong light attenuation, and gradients of pH changing from pH 9 at the outer column surface to >pH 10 over the first 1–2 cm into the ikaite. This outer layer of freshly deposited ikaite matrix contained densely pigmented yellow and green zones harboring diverse phototrophic biofilms dominated by diatoms and cyanobacteria, as revealed by molecular analyses and pigment analysis. *In situ* measurements of O_2 and variable chlorophyll fluorescence measurements demonstrated high levels of oxygenic photosynthesis with strong irradiance-driven O_2 dynamics ranging from anoxia to hyperoxic conditions in the ikaite matrix, which exhibited strong attenuation of visible light (400–700 nm) down to 1% of incident irradiance at 20 mm depth into the ikaite. Variable chlorophyll fluorescence imaging showed active photosynthesis with high-light acclimation in the outer diatom layer, and low-light acclimation in the underlying cyanobacterial part. Bright field, CLSM and electron microscopy showed that the microbial phototrophs were embedded in exopolymers forming endolithic biofilms in close association to the ikaite crystal matrix. These biofilms may interact with mineral formation and cementing of ikaite crystals and thus might play a significant role in ikaite column formation.

[P78] MICROPLASTICS GET COLONIZED BY NON-NATIVE BACTERIAL COMMUNITIES INCLUDING POTENTIAL CORAL PATHOGENS

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Microplastic particles (MPs) represent a new type of surface in marine ecosystems, which can efficiently be colonized by specific bacterial communities forming dense surface covering biofilms. Due to the specific surface structure, it is expected that MP colonizing bacterial communities significantly differ from those of natural particles in marine ecosystems. It is furthermore hypothesized that MPs can act as vectors for the transmission of pathogens to eukaryotic marine organisms as e.g. corals.

We studied the colonization of MPs and sediment particles 12 weeks after those were added surface-sterilized to marine microcosms containing corals. As shown by Reichert *et al.* (2017) the uptake of the here studied MPs by corals negatively impaired coral health. Using scanning electron microscopy, we could show particle-specific colonization by complex eukaryotic and prokaryotic communities. Bacterial community fingerprinting by 16S rRNA gene sequence based DGGE analysis and 16S rRNA gene amplicon based Illumina MiSeq sequencing revealed the formation of stable and exclusive bacterial communities on MPs, which clearly differed from bacterial communities developed on sediments. In contrast to MPs, communities developed on sediments were similar to those present on detritus and particle-associated bacterial communities of the microcosm water (>5 µm water fraction). A high relative abundance of *Jejudonia*, *Roseivivax*, and *Marinobacter* was determined specifically on MP. Beside the cultivation-independent approach abundant surface colonizing bacteria were cultured from the different particle types and identified by partial 16S rRNA gene sequencing and differentiated at the strain level by genomic fingerprinting. Several abundant cultured bacteria represented abundant taxa found also in the cultivation-independent molecular approach. Most of the isolates identified as *Vibrio* spp. (potential coral pathogens) from MP were genetically different compared to *Vibrio* spp. isolates from other samples. Our study confirmed the development of specific bacterial communities on MP. The strain collection of isolates is now the starting point to study the biofilm formation of the specific isolates on MPs and the potential pathogenicity of MP colonizers to corals.

Reichert J, Schellenberg J, Schubert P, Wilke T. (2017). Responses of reef building corals to microplastic exposure. Environ Pollut. pii: S0269-7491(17)32953-6.

Keywords: microplastic particles, bacterial communities, plastisphere, genomic fingerprint.

[P79] THE ROLE OF ADAPTIVE DIVERSIFICATION IN BURKHOLDERIA CENOCEPACIA BIOFILM COMMUNITY

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Bacterial biofilms evolve strategies that reduce intercellular conflicts and develop cooperative interactions that enhance population-level fitness. The evolutionary process is characterized by the diversification of genotypes shaping the ecological structure and productivity within.

In long-term evolved populations of *Burkholderia cenocepacia* propagated in a biofilm model, the lineages selected not only underwent ecological differentiation into an early attaching wrinkled ecotype, a densely clustering ruffled ecotype, and an exterior colonizing studded ecotype but also increased the overall biofilm productivity. The main factor driving this adaptation is the coordination of quorum sensing (QS) and regulation of intracellular cyclic-di-GMP (cdG) levels. Here, we distinguish these effects genetically to determine how different mutations influence both the genotype and other ecotypes in the evolved community.

We observe distinct mutations in *rpfR* that cause increased levels of cdG and alter the binding of Burkholderia Diffusible Signal Factor (BDSF). This causes higher biofilm attachment and shunts BDSF mediated biofilm dispersion. One such genotype associated with the studded lineage- Y355D- stimulates the activity of diguanylate cyclase domain that produces cdG; as a result, the biofilms formed exhibit uniform surface coverage. This genotype is dominant in co-cultures and outcompetes most other genotypes except A106P. A106P mutation selected in the ruffled lineage produces large aggregates in biofilms and coexists when grown in co-cultures with A106P. Uniquely, we also describe it as a facilitator as it enables the growth of other partner genotypes in clusters increasing their fitness in co-culture communities.

Other nonsynonymous mutations in the membrane- bound WspA or terminal kinase WspE observed in wrinkled lineage cause constitutive activation of phosphorelay regulator, upregulating the biofilm matrix production. These *wsp* genotypes along with the facilitator ecotype synergistically increase the total biofilm biomass.

Overall evolved biofilm ecology demonstrates both competitive and cooperative assembling of genotypes. It is remarkable how enhanced biofilm formation is the feature observed among most evolved populations in different bacterial species. In the ongoing study, we plan to ask if the ecological significance of these ecotypes plays role in multispecies bacterial interactions.

[P80] KIN DISCRIMINATION IN BACILLUS SUBTILIS BIOFILMS

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In their natural habitats microorganisms are surrounded by other strains and species with whom they engage in competitive, cooperative or neutral interactions. Understanding decision making that drives competition and cooperation and the consequences of this interactions is of fundamental importance. We have recently shown that Bacillus *subtilis*, a gram positive bacteria, engage in cooperative swarming only with highly related (kin) strains, but not with less related (non-kin) strains of the same species. We here address whether this differential behavior is also evident during other types of multicellular existence, namely in floating biofilms (pellicles) where *B. subtilis* invades liquid – air interphase or in colony biofilms that form on nutrient agar. Cell distribution of two strains in mixed biofilms was monitored by fluorescent technology and confocal microscopy. In addition we also quantified viscoelastic properties of biofilms and fitness of strains in biofilms initiated by two self, kin or non-kin strains. These were labeled with different antibiotic resistance and fluorescence markers. We observed a spatially close intermixing of cells in kin biofilms. In contrast, segregation into larger and well visible patches of cells was evident in non-kin biofilms. Mixed biofilms also showed changed viscoelastic properties but this change did not correlate with the kinship of the two strains. Fitness of kin biofilms was comparable to fitness of the monoculture biofilms. However, in non-kin combinations one strain of the two always experienced a decreased fitness. The observed fitness dynamics indicates a more intense ecological competition or even interference competition between non-kin, which underscores the importance of kin discrimination for social outcomes in different modes of bacterial multicellular existence.

[P81] COMMUNITY STRUCTURE AND IDENTIFICATION OF BIOFOULERS IN A SEAWATER REVERSE OSMOSIS DESALINATION PLANT.

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The acceleration of climate change and the increasing population growth has placed additional stress on an already vulnerable global water supply. Seawater reverse osmosis (SWRO) is considered the simplest and most cost-effective method of freshwater production to overcome this current problem. However, even after seawater pre-treatment and cross flowing within the system, SWRO is often hindered by biofouling. The conditioning of the membrane and adhesion of microorganisms allow for the rapid growth of biofilms on the reverse osmosis (RO) membrane. This study is novel in that SWRO intake water and pre-treated feed water were assessed as a biofouling indicator. Communities from the intake water and autopsied spiral bound RO membranes following a lead and lag 2 – 4-year operational life-span were characterized by 16S and 18S gene metabarcoding. Proteobacteral prevalence within microbiomes of the intake water as well as the fouled membranes reflecting natural seasonal variation within seawater and also the natural aging process of biofilms. However, the communities differed from those found in the intake water and on the RO membranes indicating selection pressures with the desalination plant. On fouled membranes, Prokaryotes, Actinobacteria, Alphaproteobacter and Thermoleophila and eukaryotes, Chromodorea, Cristidiscoidea, Trebouxiophyceae and Sordariomycetes dominated the biofilms. Whereas, the prokaryotic communities of the intake water were found to be dominated by Cyanobacteria, Acidobacter, Fibrobacteres, Marinimicrobia, which suggests that a sole survey of seawater cannot predict the biofouling potential of the community. This study also highlights the role that eukaryotic organisms such algae, fungi and protists have on the formation of biofilms with desalination plants.

[P82] ACTIVITY PARTITIONING IN AN ARCHAEAL-BACTERIAL CO-CULTURE BIOFILM

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Symbiosis is widespread throughout the biosphere with well-studied examples in and across all three domains of life. In communities of bacteria and archaea, mutualism is typically referred to as syntrophy ("eating together") where by-products of one metabolism serve as substrates for another metabolism. The syntrophy between sulfate-reducing bacteria (SRB) and methanogenic archaea is of interest because these guilds both play crucial roles in many different anaerobic environments. In the absence of sulfate as an electron acceptor and the addition of the hydrogenotrophic methanogen, *Methanococcus maripaludis,* the two cell types are interdependent via previously proposed product inhibition syntrophy, and cross-feeding of by-products allows a cooperative syntrophic relationship to be established. In monocultures, only *D. vulgaris* Hildenborough readily forms biofilm, and the topography of the biofilm is relatively flat and thin. However, the co-culture biofilm is evenly interspersed with *M. maripaludis*, and is thicker and filled with topographical features such as ridges, spires, and valleys. In order to better understand the interactions between *M. maripaludis* and *D. vulgaris* Hildenborough, deuterium-labeled proteomics and BONCAT microscopy was used delineate activity states of the two biofilm populations. Deuterium-labeled proteins were observed in both populations, and the *D. vulgaris* labeled proteins were enriched in proteins involved in carbon oxidation and electron transfer while the *M. maripaludis* proteins were enriched in carbon dioxide processing and methane generation. Interestingly, BONCAT labeling was observed for both organisms grown as monocultures under sulfate-reducing or hydrogen-utilizing conditions, respectively. However, under co-culture biofilm conditions, only *D. vulgaris* was detected to be BONCAT active, yet methane was being actively produced. In addition, when an active methanogenic co-culture biofilm was perturbed with a constant sulfate influx, the methanogen population not only persisted but increased and remained viable. The data suggest that a mutualistic co-culture biofilm partitions activity to optimize carbon processing and energy conservation.

[P83] AGENT-BASED MODELLING OF THE FACTORS AFFECTING BIOFILM SURFACE ROUGHNESS.

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Biofilm surface roughness, or variation in surface height, can play a crucial role in biofilm function. Surface roughness controls diverse characteristics such the extent of pathogen adhesion, genetic mixing and hence potential for cooperation, antibiotic penetration and the chances of fixation of antibiotic resistant mutants. These characteristics, in turn, can feed back on the roughness of the biofilm. We have used the iDynoMiCs agent-based biofilm modelling software to establish how the roughness of model *Pseudomonas aeruginosa* biofilms in a flow cell set up changes with time, nutrient availability and bacteria maximal growth rate. We analyse our results firstly in terms of the Kardar–Parisi–Zhang equation for interface growth, which is well known in statistical physics, and secondly in terms of the variations in the thickness of the layer of actively growing cells at the top of the biofilm, or 'active layer'. We investigate in detail the phase transition from a low roughness regime, characterised by a smooth biofilm, to a high roughness regime, characterised by the presence of biofilm "fingers". This transition happens when the active layer becomes discontinuous and the troughs of the biofilm fingers become stationary, or 'pinned'. We also present preliminary results on how biofilm roughness relates to genetic diversity, which has implications for the evolution of resistance to antimicrobials in biofilms.

[P84] THE DEVELOPMENT OF AN IN-VITRO DYNAMIC FLOW MODEL TO MIMIC A DENTAL UNIT WATER SYSTEM

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Dental unit water systems (DUWS) are used to provide water to irrigate the operative site and cool instrumentation during different dental treatments. This elaborate network, of tubing and connectors, forms an ideal niche for bacteria to form biofilms. Infection prevention guidelines in The Netherlands dictate that water delivered from these systems has to meet European drinking water standards and recent studies have shown that 65% of the sampled units do not comply. To gather more insight in the biofilm dynamics within these systems, our aim was to develop an *in-vitro* dynamic flow model mimicking DUWS, which would be (i) reproducible, (ii) able to differentiate between multiple inoculation sources and (iii) able to recover from an intervention.

A model was built using polyurethane tubes (n=16), connectors and computer programmed pumps. The model was inoculated with either non-potable or potable water and incubated under static conditions for 24h at room temperature. Each workday, filter-sterile water was fed to the tubes during 30 cycles (30s, 30ml/min, 9.5 min static). The model was run for 30 days, followed by a 5 min. 1000 ppm hypochlorite treatment to simulate a biofilm disinfection protocol, and ran for an additional 14 days to monitor regrowth. Effluent and biofilm samples were taken at multiple time points for heterotrophic plate counts (HPC) and microbiome analysis.

HPC showed that, for both non-potable and potable water, effluent samples reached an equilibrium around day 16 (10^5-10^6 CFU/ml) and for biofilms between day 23 and 30 (10^7-10^8 CFU/cm^2). In effluent samples, the hypochlorite induced a LOG 5 reduction in HPC, but regrowth in both effluent and biofilm samples occurred within 14 days.

Microbiome analysis revealed that both non-potable and potable water, but also biofilm and effluents, differed significantly in composition. The use of hypochlorite resulted in a selection of organisms.

To conclude, this model (i) can produce replicable biofilms, (ii) differentiate between different inocula and (iii) can recover from an intervention. Further studies to test the efficacy of common dental unit disinfectants are being performed to answer more fundamental questions on how disinfectants act on biofilm structure and development.

[P85] SEQUENCING-BASED MICROBIOME ANALYSES FOR PREDICTIVE BIOFILM MODELS

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Laboratory biofilm *in vitro* models are only a rough approximate for the complex situation in reality. Modelling bacterial biofilm formation in clinical and environmental settings requires mimicking the situation in reality as close as possible. For the efficacy assessment of antimicrobial materials or antibiotics, mostly laboratory model strains or clinical and environmental isolates are being used, but the interplay of pathogens with associated commensal bacteria is mostly neglected.

We have performed a 16S microbiome profiling study to explore bacterial communities on clinical ureteral stent samples, thin plastic tubes inserted into the urinary tract between kidney and bladder in case of blockage by kidney stones or malignancy. Beyond sequencing, we additionally characterized the biofilms by X-ray diffraction analysis of crystals, cultivation of bacteria, and microscopy. While microbiome profiling indicated a vast variety of bacteria, complementary methods indicated low numbers of bacteria in most of the investigated samples. Only few samples contained high numbers of bacteria, with limited bacterial diversity.

In summary, we suggest that for biomaterial assessment bacterial communities including both pathogens and commensals to simulate the relevant (clinical/environmental) situations should be considered. Our research directs towards the closer investigation of the members of the identified bacterial communities regarding their function in their ecosystem and possible roles in health and disease.

[P86] STRUCTURE OF A 1.5-MDA BACTERIAL ADHESIN REVEALS ITS ROLE IN THE MIXED-SPECIES BIOFILM WITH DIATOMS ON ICE

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Bacterial adhesins are modular cell-surface proteins that mediate adherence to other cells, surfaces and ligands. The Antarctic bacterium *Marinomonas primoryensis* uses a 1.5-MDa adhesin comprising over 130 domains to position it on ice at the top of the water column for better access to oxygen and nutrients. We have reconstructed this 0.6 µm-long adhesin using a 'dissect and build' structural biology approach and have established complementary roles for its five distinct Regions. Domains in Region I (RI) tether the adhesin to the Type I secretion machinery in the periplasm of the bacterium and pass it through the outer membrane. RII comprises ~120 identical Ig-like β-sandwich domains that rigidify on binding Ca^{2+} to project the adhesion regions RIII and RIV into the medium. RIII contains ligand-binding domains that join diatoms and bacteria together in a mixed species community on the underside of sea ice where incident light is maximal. RIV is the ice-binding domain; and the terminal RV domain contains several 'Repeats-in-Toxin' motifs and a non-cleavable signal sequence that target proteins for export via the Type I secretion system. Similar structural architecture is present in the adhesins of many pathogenic bacteria and provides a guide to finding and blocking binding domains to weaken infectivity

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[P87] ECTOPIC SUPPLY OF IL-1BETA PROMOTES BIOFILM GROWTH OF STAPHYLOCCOCUS AUREUS ON PRE-COLONIZED IMPLANTS IN VIVO

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Biofilms are communities of microorganisms attached to hydrated surfaces and enclosed in a self-produced extracellular polysaccharide matrix. By that, the bacteria become resistant to exogenous assaults like antibiotics or the conventional immune defense mechanisms of the host. Biofilm infections represent a serious health burden in the clinics since the microorganisms are able to colonize biological surfaces or surfaces of indwelling medical devises. Presently, too little knowledge exists on biofilm physiology to clear such infections efficiently, if at all. We here studied the biofilm forming, Gram positive bacterium *Staphylococcus aureus* Xen29 in the mouse model of a pre-colonized subcutaneously implanted osmotic pump. Biofilm formation on this device was confirmed by Light-sheet microscopy and Scanning Electron Microscopy. The model permits the ectopic provision of different innate immune recruiting and activating agents like cytokines or chemokines to test their role during biofilm development *in vivo*. When applied ectopically, the pro-inflammatory cytokine IL-1b had a very strong effect on biofilm development compared to all other agents tested. Its presence resulted in a significant increased magnitude of infection and biofilm development. This was concluded from noninvasive imaging of the bioluminescent signal emitted by the lux expressing bacteria and the quantification of bacterial load in blood and other tissues and confirmed by the microscopic techniques. In addition, increased numbers of neutrophils were observed under these conditions. The correlation between these two findings will be studied now.

[P88] CORRELATION BETWEEN BIOFILM REMOVAL AND BACTERIAL KILLING IN A MODEL MIMICKING DISINFECTION OF CONTAMINATED ENDOSCOPES

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Background: Growth of biofilms inside endoscope channels can be the cause of device-related nosocomial outbreaks. Therefore, it is essential to clean and disinfect them effectively between patients to avoid this risk. The objective of this study was to examine the impact of biofilm removal by detergents in bacterial eradication achieved by high-level disinfection (HLD) in a biofilm model mimicking the accumulation of bacteria within endoscopes.

Methods: Two clinically relevant species *P.aeruginosa* and *K.pneumoniae* were used to grow biofilm in the Buildup Biofilm model (BBF) that mimics the endoscope environment (1). Briefly biofilm was developed in MBEC 96-well plates (Innovotech, Canada). Bacteria were suspended in Artificial Test Soil (ATS) to achieve 10^8 CFU/ml. Biofilm was formed for 8 days at room temperature in ATS, with rocking action. Four rounds of HLD using 2.6% glutaraldehyde were included. Biofilms were then treated with four detergents: EnziQure, Revital-Ox , Prozyme Active , Endoclean at their recommended dosage (1 hour at 40°C) in the absence of friction. Optionally, after the treatment with cleaners, biofilms were exposed to peracetic acid (PAA) at a concentration of 900 ppm (3 min at 40°C). Remaining biofilm biomass was assessed using crystal violet assay (2) and residual bacterial viability was quantified by CFU counting (1).

Results: Regression analysis revealed that the cleaner plays a predominant role in biomass and CFU elimination. The Figure shows the effect of the treatment with the 4 cleaners included, either with or without PAA, on biofilm biomass removal and CFU/cm^2. EnziQure showed biofilm biomass and CFU removal activity superior to the three other cleaners both with and without PAA. Prozyme Active, Revital-Ox and Endoclean displayed very similar performance and showed biofilm biomass and CFU removal significantly higher than the control after PAA treatment. Correlation was found between biofilm biomass and CFU reduction after PAA treatment but not when the biofilms were treated only with the cleaners (no PAA).

Conclusions: Strong correlation between biofilm removal and CFU reduction was observed suggesting that treatment with a potent biofilm-disruptive cleaner before disinfection is necessary to achieve successful decontamination of biofilm-colonized endoscopes.

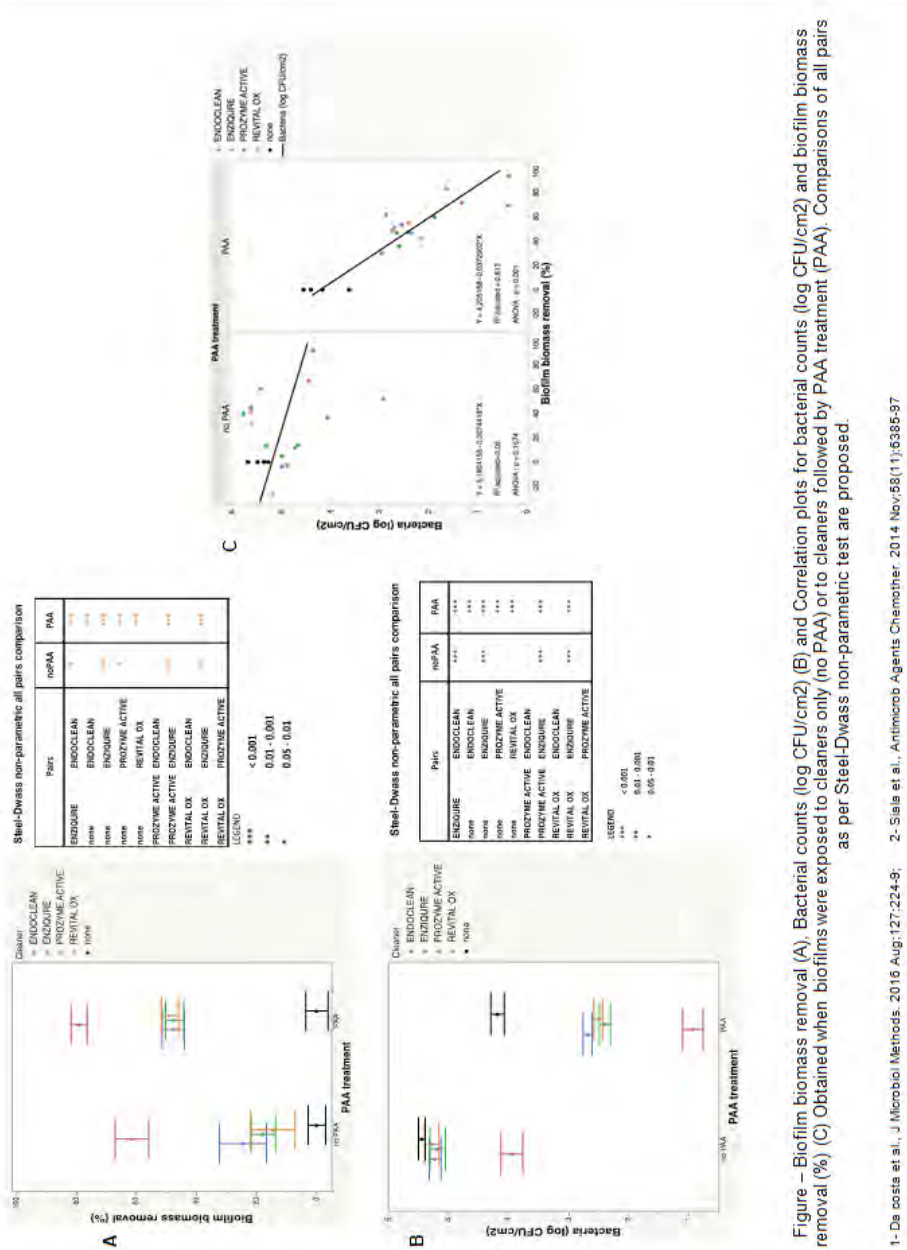

Figure – Biofilm biomass removal (A), Bacterial counts (log CFU/cm2) (B) and Correlation plots for bacterial counts (log CFU/cm2) and biofilm biomass removal (%) (C) Obtained when biofilms were exposed to cleaners only (no PAA) or to cleaners followed by PAA treatment (PAA). Comparisons of all pairs as per Steel-Dwass non-parametric test are proposed.

1- Da costa et al., J Microbiol Methods. 2016 Aug;127:224-9; 2- Sisla et al., Antimicrob Agents Chemother. 2014 Nov;58(11):6385-97

[P89] COLONIZATION AND BIOFILM FORMATION BY STAPHYLOCOCCUS AUREUS ON ENDOTHELIAL CELL LAYERS UNDER FLOW

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Staphylococcus aureus is a major human pathogen and known for causing vascular infections such as sepsis and infective endocarditis. It has previously been proposed that *S. aureus* succeed in colonization of the endothelial wall by specific surface attachment likely followed by biofilm formation. Furthermore, *S. aureus* is known to invade human cells, which has been proposed to promote persistence through immune and antibiotic evasion. In the current study, we sought to investigate endothelial colonization, invasion, and biofilm formation by *S. aureus* using a newly developed *in vitro* flow chamber model. We show that under physiological shear rates, *S. aureus* utilizes cellular invasion to enable the following surface colonization and biofilm formation. These observations might help explain the success of *S. aureus* as a bloodstream pathogen and guide further studies in *S. aureus* pathogenesis and treatment of *S. aureus* biofilms.

[P90] PHENOTYPIC GROWTH IN EXPANDING BACILLUS SUBTILIS BIOFIILMS

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We develop an optical imaging technique for spatially and temporally tracking biofilm growth and the distribution of the main phenotypes of a Bacillus subtilis strain with a triple-fluorescent reporter for motility, matrix production, and sporulation. We develop a calibration procedure for determining the biofilm thickness from the transmission images, which is based on Beer-Lambert's law and involves cross-sectioning of biofilms. To obtain the phenotype distribution, we assume a linear relationship between the number of cells and their fluorescence and determine the best combination of calibration coefficients that matches the total number of cells for all three phenotypes and with the total number of cells from the transmission images. Based on this analysis, we resolve the composition of the biofilm in terms of motile, matrix-producing, sporulating cells and low-fluorescent materials which includes matrix and cells that are dead or have low fluorescent gene expression. We take advantage of the circular growth to make kymograph plots of all three phenotypes and the dominant phenotype in terms of radial distance and time. To visualize the nonlocal character of biofilm growth, we also make kymographs using the local colonization time. Our technique is suitable for real-time, noninvasive, quantitative studies of the growth and phenotype distribution of biofilms which are either exposed to different conditions such as biocides, nutrient depletion, dehydration, or waste accumulation.

[P91] FROM SPORES TO BIOFILMS - GROWTH UNDER SIMULATED MICROGRAVITY

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During the Apollo 16 space mission in 1972, *Bacillus subtilis*, an endospore- and biofilm-forming microorganism, became one of the first microbial model-systems used in space experiments. Since then, space facilities (i.e. MIR-space station or ISS) have become burdened with microbial growth, which has generated an emerging interest in the highly resistant endospores and biofilms problematic for both crew and spacecraft machinery. Indeed, the resultant clogging or contamination from biofilm growth on life-support systems, such as air or water leading pipes, endangers the security of space missions.

Due to various constraints (financial, time, and sample amount) associated with space missions, we simulate space conditions (vacuum, radiation, microgravity) in the German Aerospace Center facilities and other research institutes in order to study the biological response and adaptation to spaceflight-relevant conditions. We use the biofilm-forming *B. subtilis* strain NCIB 3610 to study the impact of microgravity and additional space related parameters on young biofilms (Fuchs et al., 2017). Our major research goal is to monitor and visualize the overall process, starting with spores differentiating into biofilms in simulated microgravity (sim-µg), using a fast-rotating 2D clinostat, and terrestrial gravity (1g) conditions. White light profilometry, scanning (SEM) and transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) are then used to analyse spores and biofilms regarding their topology and structure.

During early phases of spore germination and outgrowth, no structural differences were observed between spores germinating at 1g or sim-µg. While, young biofilms formed at sim-µg and 1g showed qualitative architectural differences in cross-sections, no significant qualitative variations in biofilm surface topography were shown. When comparing the CFU (colony forming units), spore levels, and sporulation efficiency in biofilms formed at 1g or sim-µg, no differences were observed. However, spores isolated from sim-µg biofilms showed spontaneous germination in water, indicating a potential instability. We also found that biofilms formed at sim-µg exhibited similar resistance to space-conditions, such as vacuum or ionizing and galactic cosmic radiation, compared to biofilms formed at 1g.

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[P92] GRADIENTS OF ANTIMICROBIALS CAN INFLUENCE THE DEVELOPMENT OF ANTIBIOTIC RESISTANCE

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Antimicrobial resistance (AMR) is becoming an increasingly problematic healthcare issue as it has been predicted that by the year 2050 more deaths will be associated with AMR than with cancer (AMR review, 2016). To combat AMR it is essential to understand its development and the underlying evolutionary mechanisms. AMR development has been studied by conducting planktonic evolution experiments which provided deeper insights into AMR development. However, planktonic bacterial cultures do not reflect reality sufficiently as in nature bacteria are mainly found in biofilms. An important characteristic of biofilm growth is the presence of antimicrobial gradients. These could influence AMR development differently than conventional homogeneous treatments used in planktonic studies. The research hypothesis is that gradients (of different steepnesses) could influence the resulting resistance levels and resistance conferring mutations.

To test this hypothesis, a planktonic gradient evolution experiment was designed to mimic biofilm gradients, because manipulating spatial antibiotic gradients in biofilms is technically very challenging. In this experiment, 30 Salmonella Typhimurium ATCC 14028 populations were evolved in increasing antibiotic concentrations, subjecting the populations to temporal antibiotic gradients of cefotaxime (CTX). Furthermore, gradient steepness was varied by five fixed factors, called steepness factors which represent the daily concentration increase. For example, in a strong selection treatment the antibiotic concentration increased with 1xMIC per day (steepness factor 1). Other treatments had decreasing selection strengths as the steepness factor decreased to 1/2, 1/4, 1/8 and 1/16. When populations reached a predetermined maximum concentration their MIC was determined. Resistance levels of populations evolved under steep gradients (1, 1/2) were lower than those of populations evolved under less steep gradients (1/4, 1/8, 1/16). Furthermore, all populations were sequenced which revealed mutations in resistance associated genes including envZ, multidrug efflux pumps, and in the CTX target penicillin-binding-protein3 (PBP3). However, PBP3 mutations only occurred in populations grown under less steep gradients in contrast to the other genes which were common in all populations.

These results imply that gradients might influence AMR development. Moreover, it seems that populations grown under slowly increasing gradients are capable of reaching higher resistance levels than those grown under faster increasing concentrations.

[P93] EXPERIMENTAL EVOLUTION OF PSEUDOMONAS AERUGINOSA IN TOBRAMYCIN REVEALS ANTIMICROBIAL RESISTANCE PATHWAYS

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Opportunistic pathogens like *Pseudomonas aeruginosa* may evolve resistance to antibiotics by mutations in pathways outside the direct drug target. This poses a challenge in identifying the causes of clinical resistance. Evolution experiments of clinically relevant bacteria in antibiotics can be used to screen for the spectrum of possible pathways by which pathogens can evolve antimicrobial resistance. *P. aeruginosa* causes chronic respiratory infections in cystic fibrosis patients that are associated with growth in a biofilm, and the biofilm mode of growth has been shown to confer antimicrobial resistance. Therefore, biofilm associated growth may confer different mechanisms of antimicrobial resistance compared to planktonic populations typically studied *in vitro*. In this study, *P. aeruginosa* strain PA14 was experimentally evolved in varying concentrations of tobramycin, an aminoglycoside antibiotic commonly administered to treat infections in cystic fibrosis, for twelve days. This experiment was performed in a planktonic environment as well as in a biofilm environment modeled through a daily bead transfer method. Lineages were inoculated with an ancestral clone and evolved in either no tobramycin or increasing concentrations of tobramycin that doubled every three days. Periodic sampling of the populations was combined with whole genome sequencing to determine the mutations conferring antimicrobial resistance in the different growth conditions. All populations evolved in a biofilm environment with antibiotic pressure acquired mutations in the *fusA1* gene, which encodes elongation factor G, reaching a cumulative frequency of 100% by day twelve. Biofilm populations evolved without antibiotic pressure did not acquire these mutations, indicating that mutations in *fusA1* conferred resistance to tobramycin. Populations evolved in a planktonic environment with tobramycin acquired mutations in the *fusA1* gene, but only one of the three sequenced populations evolved mutations in *fusA1* that reached a cumulative frequency of 100%. Instead, these populations were found to acquire a four-nucleotide insertion in the 23S rRNA. Mutations in *fusA1* at the positions identified through this experiment have also been identified in clinical samples from cystic fibrosis patients, indicating that evolution experiments of *P. aeruginosa* in a biofilm environment with antibiotic pressure can identify clinically relevant pathways to antimicrobial resistance.

[P94] DETERMINING FITNESS OF CLINICAL P. AERUGINOSA ISOLATES ASSOCIATED WITH PERSISTENT LUNG INFECTIONS USING A PHARMACO-DYNAMIC BIOFILM MODEL SYSTEM

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Pseudomonas aeruginosa is a dominating airway colonizer in patients with cystic fibrosis (CF). Longitudinal investigations of bacterial isolates from CF patients have shown that persistent infection by *P. aeruginosa* is associated with adaptive evolution of the bacteria, suggesting a gradual increase in fitness properties of the infecting bacteria. Few investigations, however, have addressed bacterial fitness development, since it cannot be performed in the specific environment (the patient airways), from which the bacteria are derived. As an alternative we apply an *in vitro* model system mimicking the airway environment with emphasis on the antibiotic selection pressure and the structural architecture of the lung environment.

The pharmaco-dynamic/pharmaco-kinetic (PD/PK) system used is based on bacterial biofilms grown under continuous culture conditions, simulating the changing antibiotic concentrations in CF patients during intravenous dosing. The antibiotics will decay in a way similar to what takes place in CF patients during treatment. Using differentially tagged sub-lineages isolated from the patients, competition experiments are performed in this system including confocal microscopy imaging after live dead staining in combination with quantification using COMSTAT.

In an investigation of *P. aeruginosa* variants derived from CF patients, isolates with increased persister levels after treatment with high doses of ciprofloxacin (Hip mutants) were tagged with Cfp and co-cultured with Yfp tagged wild type persister bacteria from the same patient (and with the same MIC) in our PD/PK dbiofilm system treated with one bolus ciprofloxacin. Using dead staining (red) it was found that mainly the wild type persister bacteria were killed.

In a second application of the PD/PK biofilm system, the fitness properties of frequent *mexZ* mutations observed in isolates from CF lungs were investigated. A *mexZ* deletion mutant tagged with Cfp was constructed in the reference *P. aeruginosa* strain, PAO1, and competed against Yfp tagged wild type bacteria using one bolus ciprofloxacin added to the biofilm system. The Cfp tagged *mexZ* deficient bacteria were found to survive treatment significantly better than the Yfp tagged wild-type biofilm cells.

We suggest that the PD/PK biofilm system can be applied as an alternative to animal models for investigations of antibiotic treatment of biofilm associated infections.

[P95] INDUCTION OF VIABLE BUT NON-CULTURABLE P. AERUGINOSA IN IN VITRO BIOFILMS. ROLE OF SUB-INHIBITORY ANTIBIOTIC CONCENTRATIONS

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Pseudomonas aeruginosa pulmonary infections are the main cause of morbidity and mortality in Cystic Fibrosis (CF) patients and result characterized by biofilm formation and recurrence. The latter feature is likely due to the presence, within biofilms, of persistent bacterial forms, including those known as Viable But Non-Culturable (VBNC), usually undetected by routine cultural diagnosis. According to the hormesis hypothesis, the antibiotic treatment, specifically subinhibitory antibiotic concentrations, could be responsible of the VBNC state induction, modulating the bacterial gene expression. In this study, we evaluated the ability of antibiotics used to contrast *P. aeruginosa* infections (ciprofloxacin and tobramycin) to induce *P. aeruginosa* VBNC forms in *in vitro* biofilms. Bacterial biofilms were developed in microtiter plates and exposed to starvation (growth in Non Nutrient broth) and starvation supplemented with subinhibitory (1/4xMIC) antibiotic concentrations for 170 days. *P. aeruginosa* culturability and viability were monitored by CFU counts, performed after 24 and 72 h of incubation at 37°C, *ecfX*-targeting qPCR and epifluorescence microscopy. Although biofilms did not lose their culturability, a slow-down of *P. aeruginosa* growth was observed in tobramycin-exposed biofilms, since, starting from 40 days of antibiotic exposure, CFU counts were available only after 72 h incubation. Biofilms exposed to starvation alone and starvation plus ciprofloxacin did not show any particular effect. Moreover, the presence of a stable *P. aeruginosa* VBNC population was detected, starting from 80 days of antibiotic exposure, in tobramycin-exposed biofilms by qPCR counts, that resulted about 1-2 log higher than CFU counts. The same difference was not evidenced in starved biofilms, while subinhibitory ciprofloxacin concentrations induced only a transient VBNC population, between 120 and 140 days, most likely a pre-mortem state. The molecular data were confirmed by epifluorescence microscopy experiments. In conclusion, our study demonstrated the tobramycin inference in inducing a stable VBNC population in *P. aeruginosa* biofilms, probably responsible for the CF infection recurrence. These data, despite preliminary, give new insights about the bacterial dynamics occurring during the *P. aeruginosa* CF lung infections and allow to design novel therapeutic treatments, affecting even VBNC forms, in order to improve the patients' life expectancy.

[P96] PROSTHETICS AND BIOCOMPATIBILITY: ASYNTHETIC BIOLOGY APPROACH FOR BETTER INTEGRATION AND FUNCTIONALITY

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The International Genetically Engineered Machine competition, also known as iGEM, is a competition that focuses on synthetic biology. Every year, hundreds of teams coming from all around the world meet in Boston in an event organized by the MIT to try and solve problems from our day-to-day lives. This year at Institut Pasteur, we created a team composed of students working in different engineering fields such as biology, physics, chemistry, industrial design, and intellectual property law allowing us to develop a project from A to Z. The project we will be working on is at the interface of prosthetics and molecular/bacterial interfaces. Indeed, bacterial biofilms often develop on prosthetics or other implantable devices. These biofilms can cause infections, and major health risks to patients. In the USA, it is estimated that 5% of people who are implanted with an orthopedic prosthesis require re-intervention on their devices [1]. Some of the possible remedies include the improving of the interface at the nerve / prosthetics junction, and secondly preventing this interface form getting infected by other pathogenic bacteria. We will be discussing these possibilities at the Biofilms 8 conference to meet with researchers and professionals.

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[P97] MECHANISMS OF ANTIMICROBIAL PEPTIDE ACTION AGAINST CELL MEMBRANES

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The prevalence of antibiotic-resistant infections has led to a need to develop alternative treatments, among which antimicrobial peptides (AMPs) are most promising. AMPs are short, cationic proteins with broad-spectrum activity attributed to biophysical interactions with bacterial membranes. AMPs have potential to be used against bacterial in biofilms, as they are effective against Gram-negative and Gram-positive bacteria, and have even show efficacy against multidrug resistant bacteria. Our work has centered on a few AMPs, including alimethicin, a 20- amino acid, alpha helical peptide, and LL37, which is a human derived AMP. We are developing a detailed understanding of the mechanism of interaction between AMPs and cell membranes, using supported lipid bilayers as models for either bacterial cell membranes or mammalian cell membranes, depending on the specific lipids chosen. Most AMPs initially adsorb onto cell membranes, but there is controversy over whether the next steps involve detergent-like action, transient pores, and/or whether toroidal pores form as mechanisms of toxicity. We measured AMP interactions in real-time with model lipid bilayers using quartz crystal microbalance with dissipation (QCM-D). The lipids studied included phosphatidylcholine (PC), or 4:1 POPC:POPG mixtures (4:1 molar ratio of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol). Using QCM-D, we related interfacial changes on the bilayer to peptide adsorption and aggregation kinetics and bilayer integrity. We used the Voigt-Kelvin model to calculate membrane thickness changes at various LL37 concentrations, allowing us to propose a model for its real-time interaction mechanism at different peptide-to-lipid (P/L) ratios. The relationship between P/L ratio and the mechanism of AMP action against the two different types of membranes was described. These data provide a real-time mechanistic insight into AMP interactions with mammalian cell membranes, which will enable rational design of less toxic AMP therapeutics for clinical applications.

[P98] NANOPARTICLES TO OVERCOME THE OBSTACLES OF BIOFILMS UNDER STATIC AND DYNAMIC FLUIDIC CONDITIONS

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Cystic fibrosis (CF) is a genetic disorder that affects mostly the lungs, associated with chronic bacterial infections and subsequent inflammation. Infections with pathogenic bacteria, like *Pseudomonas aeruginosa* and *Burkholderia cepacia complex* (*Bcc*), cause high mortality due to the recurrent formation of antibiotic-resistant biofilms in the abnormal CF mucus, and both the biofilm matrix and mucus hinder efficient treatment with inhaled antibiotics. In this study, tobramycin (Tb) was encapsulated in nanoparticles based on poly(D,L-lactide-co-glycolide) (PLGA) and poly(ethylene glycol)-co-poly(D,L-lactide-co-glycolide) diblock (PEG-PLGA) to overcome these barriers with particle types of 225 - 231 nm (nanoparticles, NPs) and 896 - 902 nm (microparticles, MPs) and negative zeta potentials. The effectiveness against biofilms of both pathogens *P. aeruginosa* and *Bcc* was strongly enhanced by the encapsulation under static and fluidic experimental condition as well as in artificial mucus (AM). The biofilm-embedded bacteria were killed by less than 0.77 mg/l encapsulated Tb, whereas 1000 mg/l of free Tb was ineffective. Moreover, encapsulated Tb was even effective against biofilms of the intrinsically aminoglycoside-resistant *Bcc*, indicating the synergistic effect of PEG and PLGA with Tb. No cytotoxicity was detected *in vitro* in human lung epithelial cells with any formulation.

[P99] SELENIUM NANOPARTICLES INHIBIT PSEUDOMONAS PUTIDA GROWTH AND BIOFILM FORMATION

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Selenium nanoparticles (SeNPs) have been suggested as antibacterial and anti-biofilm agents for biomedical devices and instruments. Increase in widespread use also leads to increase in the environmental concentration of nanoparticles. Targeted for pathogenic microorganisms, nanoparticles can also kill microorganisms helpful for the environment. Present study evaluates the effect of SeNPs on bacterial growth, change in concentration of extracellular polymeric substances (EPS), their ability to inhibit biofilm formation and disrupt grown biofilms.

SeNPs were synthesized using *Shewanella putrefaciens* CN-32 under anaerobic conditions. EPS (loose & tight bound) was extracted from *Pseudomonas putida* (ATCC 23483) grown for 48 hours under shaking in presence of varying concentration of SeNPs. Proteins, polysaccharide, and total organic content were measured along with 3D EEM fluorescence spectra of EPS. Nanoparticles coated plastic surface was used to test the ability to inhibit bacterial adherence on the surface. Fluorescence microscopy was utilized to determine the effect of increasing concentration of nanoparticles on the growth of bacterial biofilm.

Biochemical tests of EPS show the decrease in protein, polysaccharide, and TOC values with increase in NPs for LB-EPS while values for TB-EPS initially increased and then decreased more with increasing concentration of nanoparticles. 3D EEM indicated the presence of aromatic and tryptophan type of proteins in EPS. An increasing concentration of SeNPs coating on polystyrene surface decreased adherence of bacteria and biofilm formation. Bacterial viability staining show inhibition of biofilm growth at the high concentration of NPs with further confirmation of cell death inside the biofilm using confocal laser scanning microscope.

Experiments demonstrated that elevated concentration of SeNPs is capable of disturbing the metabolism of *P putida*. High concentration can not only inhibit bacterial adherence to a surface further leading to inhibition of biofilm formation, however, it is also capable of causing cell death in already established biofilms. Therefore, increasing use of nanoparticles has shown potential to affect environmentally important microorganisms, and capability to alter the ecological balance.

[P100] ELECTROCHEMICALLY DEPOSITED SURFACES BASED ON COPPER AND SILVER WITH BIOCIDAL EFFECT AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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Introduction: Inert surfaces can be a reservoir for pathogenic agents and play an important role in the acquisition and spread of healthcare infections. Therefore, surface treatments that aim to provide the surfaces with antibacterial activity are receiving increasing attention and scientific interest. Copper can inactivate a multitude of bacteria, fungi and viruses and copper or copper alloys have been suggested as alternative to stainless steel to help reduce the occurrence of hospital-acquired infections. Silver also has antibacterial activity and it has been suggested to combine these for enhanced, potentially synergistic, antibacterial action.

Aim: The purpose of the present study was to investigate the antibacterial efficacy of a novel electroplated copper-silver alloy coating against methicillin resistant *S. aureus* (MRSA) with the aim of developing antibacterial surfaces for the medical and health care sector. We investigated if the alloy could prevent adhesion and biofilm formation.

Methods: The EPA Test Method for Efficacy of Copper Alloy Surface as a Sanitizer was carried out on Cu/Ag coating and stainless steel against MRSA. In a static biofilm model, four different surfaces were evaluated in parallel (Cu/Ag, Cu, Ag coatings and stainless steel) to estimate MRSA biofilm formation.

Results: Under dry conditions, the Cu/Ag coating reduced in numbers of MRSA on the surface with more than 99.9% after 2 hours of exposure as compared to numbers on stainless steel. When testing for MRSA biofilm formation, no difference was observed between silver and stainless steel coupons. However, compared with stainless steel, the most significant bacterial number reduction was found for the copper surface (close to 100 fold) followed by the Cu/Ag electroplated surfaces (10 fold) ($P<0.001$).

Conclusions: Pure copper-coated and copper-silver alloy surfaces were effective in killing bacteria and preventing MRSA biofilm formation *in vitro*. Further research is planned to determine the efficacy against other clinically relevant pathogens and to do *in vivo* test for biocidal and antibiofilm efficacy in healthcare settings.

[P101] ANTI-BIOFILM EFFICACY OF TRICLOSAN-AMPHOTERICINB COMBINATION AGAINST FILAMENTOUS FUNGUS, ASPERGILLUS FUMIGATUS

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Triclosan (TRC), an antimicrobial agent, has been reported to be safe for topical and surface-coating applications. It possesses a broad-spectrum of antimicrobial activity. The combination of TRC and DispersinB (DspB, a biofilm disruptor) displayed synergistic efficacy against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli,* and *Candida albicans*. There was a significant difference in the adherence of each of these microorganisms to TRC+DspB-coated silicone catheters compared with uncoated control catheter. Therefore, TRC+DspB has antibiofilm effect against both gram positive and gram negative, as well as yeast strains. Furthermore, for the first time, TRC effect against *Aspergillus fumigatus* biofilm formation on a glass surface was investigated alone and in combination with amphotericinB (AMB). AMB is effective against fungal infections. Viability was measured by determining colony forming units (c.f.u.) using 6-mm paper disks impregnated with TRC (0.5 to 32 mg/l) and AMB (0.125 to 16 mg/l). The diameters of the growth inhibition zone on agar plates were measured after incubation at 37° for 24 hrs. Determination of metabolic activity of hyphae was assessed using viability staining with FUN-1. Double-strength RPMI–2% glucose medium+MOPS containing 10^6 conidia/ml was incubated at 37° for 24 hrs. Subsequently, TRC and AMB at Minimum Inhibitory Concentration (MIC) doses were added and incubated at 37° for more 24 hrs. As control, *A. fumigatus* hyphae were incubated in the absence of TRC and AMB in the medium. Microscopic visualization and image acquisition of biofilms were conducted using a confocal laser scanning microscope (CLSM). Based on the optical microscopy and CLSM images, the number of hyphae structures as well as extracellular polymeric substances (EPS) formation were reduced in TRC and AMB/MICs treated samples in comparison with the non- treated control groups. Also, 3D surface plots showed the least biofilm depth in TRC/MIC treated sample in comparison with AMB/MIC treated, and control groups. Finally, Synergy Checkerboard Assay revealed that there is a synergistic activity when *A. fumigatus* was treated with TRC following by AMB.

[102] RESISTANCE SELECTION IN BACTERIAL BIOFILMS GROWING ON ANTIMICROBIAL SURFACES IN A MULTIDRUG ENVIRONMENT

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Background Bacterial biofilms are regarded as the most common cause of chronic infections and are often associated with medical devices, such as implants and catheters. Therapeutic strategies against biofilm infections in clinical settings commonly involve the application of multiple antimicrobials: biocidal coatings on the implanted biomaterials in combination with systemically administered antibiotics. Due to their inherent tolerance towards toxic compounds, bacteria growing in biofilms can survive high concentrations of antimicrobials long enough to acquire antimicrobial resistance. The frequent practice of combination therapy harbors the risk of the development of cross-resistance via shared resistance mechanisms between antimicrobials used in material coatings and administered antibiotics.

Aim Our goal is to determine how bacteria adapt to antimicrobials during biofilm formation on surfaces coated with antimicrobials and how population dynamics within biofilms affect the transmission of resistance mutations. Specifically, we want to identify antimicrobial-antibiotic-combinations that select for and against antibiotic resistance in biofilms by following the population dynamics of resistant and susceptible strains in competition assays on a single cell level.

Methodology To study the effect of antimicrobial-antibiotic exposure on resistance development and population dynamics on bacterial biofilms in a multidrug environment, we will grow *Pseudomonas aeruginosa* on glass surfaces with and without antimicrobial coatings and expose them to antibiotics. First, we will screen *in vitro* for combinations of antibiotics and antimicrobials that select for and against antibiotic resistance. Second, effective combinations will be chosen for in-depth investigations during bacterial adhesion and of mature biofilms of resistant and susceptible genotypes. Third, based on the outcome of the screen and the obtained mechanistic understanding we will choose a clinical example in which we study the relevance of our findings in biofilms grown *in vivo*.

Relevance Studying the bio interfacial interactions between bacterial biofilms and medical devices in terms of population dynamics as well as on single cell level during multidrug selection will help us understand how drug resistance develops and spreads in persistent biofilm infections. Based on our findings we aim to provide clinical recommendations for improved administration of antibiotics/antimicrobials in combination with medical device materials in order to mitigate against biofilm associated antimicrobial resistance.

[P103] SELECTING RAW GOAT MILK LACTOCOCCUS LACTIS FOR SUSTAINABLE BIOPRESERVATION?

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Biofilms forming in dairy installations can dramatically affect the quality of dairy products and cause technological and health issues. Colonizing milking machines (MM) with lactic acid bacteria (LAB) such as lactococci to form positive biofilms and compete with negative flora would be an interesting solution. The MM is known to be colonized by both lactococci and negative flora such as *Pseudomonas sp*. which cause defects on cheese. In order to prevent the presence of spoilage microorganisms and pathogens in the MM, this project aims to select indigenous strains of lactococci isolated from goat raw milk, candidates for the establishment of a positive biofilm in the MM.

In this study, 37 strains of *Lactococcus lactis* ssp *lactis* (*L. lactis*) were isolated from raw goat milk. These strains exhibited a wide genetic variety and various physicochemical surface properties. Among them, 9 representative strains were tested in a shear-stress flow chamber to evaluate their adhesion to stainless steel (the main component of MM). The biofilm-forming ability was also checked. All strains were able to form biofilms in our laboratory conditions. The two most adherent strains on stainless steel were then selected and used to form biofilms. Dual-species biofilms were performed to test the aptitude of *L. lactis* to control *Pseudomonas fluorescens* CIP6913^T as a model for negative flora. No difference was observed in bacterial density when *L. lactis* and *P. fluorescens* were inoculated simultaneously, whichever was the material used as substratum (polystyrene or stainless steel). Moreover when *L. lactis* was inoculated before *Pseudomonas* (24 to 48 h), the same bacterial density was obtained after 24h to 72 h of incubation. These results were confirmed with two environmental isolates of *Pseudomonas* (*P. fluorescens* and *P. korensiis*). Finally, Confocal Laser Scanning Microscopy revealed that both species coexisted together on surfaces.

This study demonstrates that the tested *L. lactis* strains are not good candidates to control *Pseudomonas* in MM in spite of their biofilm forming capacities. Other *L. lactis* or other species of bacteria of interest or a dairy microbial ecosystem should thus be tested to address this issue.

[P104] CONFOCAL LASER SCANNING MICROSCOPY AND ATOMIC FORCE MICROSCOPY OF PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS BIOFILMS TREATED WITH SILVER NANOSPHERES AND NANOSTARS

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Bacterial resistance to commonly used drugs is one of the greatest threats to global health. Biofilm formation may be deemed one of the main determinants accounting for treatment failure of infections. Nanotechnology is offering new options for treatment, including for biofilm-associated infections. Spherical silver nanoparticles (AgNPs), also called nanospheres, are well known potent antimicrobial agents.

This study aims to explore the inhibitory and antibiofilm activities of AgNPs and star-shaped silver nanoparticles, the nanostars (AgNSs), against susceptible and multidrug-resistant Gram-positive and Gram-negative bacteria, as the starting point for further development of silver-based nanomaterials specifically targeting biofilms unique features, such as the biofilm matrix. Therefore, the initial assays reported herein consisted in determining i) minimum inhibitory concentrations (MICs) of AgNPs and AgNSs against several reference strains and multidrug-resistant isolates and ii) the antibiofilm activity of AgNPs and AgNSs against preformed biofilms (so far only the biofilms of *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were studied). For the antibiofilm activity evaluation, 24-h biofilms were treated with AgNPs and AgNSs (at concentrations equal to 0.125 nM, which correspond to the MIC for AgNSs and to a sub-MIC for AgNPs) for further 24 h, after which biofilms were visualized microscopically by both confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM). Tryptic soy broth (TSB) was the medium used in all assays.

MIC values of AgNPs and AgNSs ranged from 0.25 to 0.50 nM and from 0.125 to 0.25 nM, respectively. The generally lower MIC values of AgNSs may indicate an influence of the shape in the antibacterial activity. Regarding the microscopic analysis of AgNSs-treated biofilms, CLSM images showed a bactericidal effect on some of the bacterial cells composing the biofilm both of *P. aeruginosa* and of *S. aureus*, in comparison to the controls. By AFM, damaged bacterial cells could be observed in the treated biofilms. AgNPs-treated biofilms were practical identical to the controls (non-treated).

These preliminary results need to be further confirmed by repeating the antibiofilm experiments in more strains and by treating the biofilms with concentrations higher to the MIC (2× MIC, 4× MIC or even higher).

[P105] NATURALLY OCCURRING SMALL MOLECULES INHIBIT FIBRILLATION OF BACTERIAL AMYLOID FAPC FROM PSEUDOMONAS SP. UK4

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Biofilms result from microbial attachment to surfaces and are known to occur in many environments, leading to significant economic and health-related problems. Key to biofilm formation is the extracellular matrix (ECM), which consists of different types of biopolymers such as exopolysaccharides, extracellular DNA, and proteins, the latter sometimes in the form of amyloids. We previously showed that functional bacterial amyloids in opportunistic pathogen *Pseudomonas* strains are important for biofilm architecture and stability. Here we show that the plant-derived compounds epigallocatechin gallate (EGCG), Penta-O-galloyl-β-D-glucose (PGG), Baicalein, Oleuropein and Procyanidin B2 inhibit the amyloid formation of FapC protein from *Pseudomonas sp. UK4* with various degrees of effectiveness. A combination of ThT-based plate reader assays, Transmission Electron Microscopy (TEM), SDS-PAGE, circular dichroism and size exclusion chromatography showed that EGCG and PGG as the best inhibitors in our study and redirect the aggregation of FapC monomers into high molecular oligomeric FapC species. We speculate that the inhibitory effect arises because these oligomers are unable to form amyloid. To gain further insight into the inhibitor effect, we studied deletion mutants of FapC lacking one or more of the three imperfect repeats found within the sequence. The small molecules reduce amyloid formation in all deletion mutants of FapC, but PGG and EGCG have the greatest effect on FapC constructs containing the R1 repeat, indicating that the inhibitory effect of these small molecules is particularly directed towards the N-terminal part of the protein.

[P106] ORGANIC BIOELECTRONICS FOR CONTROL OF BIOFILM FORMATION

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Bacterial metabolism and is governed by an intricate electrochemical balance and biofilm formation has been directly linked to the periplasmic redox status in *Salmonella* Typhimurium as periplasmic oxidoreductases participate in regulation of biofilm formation. It is therefore likely, that control of redox microenvironments will allow for control of biofilm formation. To achieve electrochemical control of the redox status in the vicinity of an active device surface, we prepared a range of polyelectrolyte composites based on the conducting polymer poly(3,4-ethylenedioxythiophene (PEDOT), known for its superior biocompatibility and electrochemical stability. The redox potential of PEDOT is very low and application of a small potential leads to electrochemically oxidized $PEDOT^{+}$ or reduced $PEDOT^{0}$ while charges are equilibrated by ions in the electrolyte. We used a two-electrode device with PEDOT electrodes covering the wall of a culturing plate. Applying a continuous electrical potential over the PEDOT electrodes led to opposing redox states on each electrode while *Salmonella* Typhimurium was cultured and allowed to form biofilm at the air-liquid interface. Removing the electrodes and analyzing biofilm formation using crystal violet and a new stratogram technique, we found that biofilm formation is promoted on oxidized surfaces and minimized on reduced surfaces. This implies that electron acceptors provided by various biotic and abiotic materials can favor biofilm formation, while electron saturation of bacterial growth substrates might be a means to prevent biofilm formation.

As biofilm was reduced, but not abolished on reduced PEDOT electrodes, we explored functionalization of a PEDOT derivative with silver nanoparticles (AgNPs). We used a two-electrode device with AgNP-functionalized PEDOT electrodes covering the bottom of a culturing plate. Application of a transient electrical potential over the two PEDOT electrodes led to a continued current flow. While *Staphylococcus aureus* was cultured in the device, near prevention of biofilm formation was obtained through the synergistic effect of AgNPs and current flow. The work performed in these studies shows the potential of conducting polymers to control biofilm formation. We suggest that these polymers provide an excellent toolset to study the influence of redox environments on biofilm growth and architecture.

[P107] QUATERNARY AMMONIUM CHITOSAN DERIVATIVES FOR CONTROLLING STAPHYLOCOCCUS AUREUS BIOFILMS

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Due to the increased emergence of antibiotic resistant biofilms, several strategies for combating bacterial biofilms have recently been developed such as using amphiphilic small molecules, nanoparticles, biomaterials and plant extracts. One such new approach uses the biodegradable biopolymer chitosan and its derivatives as antimicrobials for the treatment and prevention of bacterial biofilms. Chitosan and its derivatives are widely used for controlling the growth of planktonic bacteria. However, due to poor aqueous solubility, the application of this polymer is limited to few areas. In this study, we aimed at synthesizing chitosan derivatives that would improve the aqueous solubility of the polymer under neutral pH. At the same time, we also intended to enhance the antimicrobial efficacy of chitosan, particularly towards mature biofilms. Hence, we synthesized as series of water soluble chitosan derivatives containing quaternary ammoniumyl groups and investigated their efficacy towards *Staphylococcus aureus* biofilms. For this purpose, we utilised TBDMS-protected chitosan to selectively introduce different moieties at varying ratios at the 2-amino position of chitosan. Selective modification enabled us to have cationic groups, such as trimethyl amine (linked directly to chitosan or via an acetyl spacer) and hydrophobic alkyl chains. Simultaneously, we also synthesized a chitosan derivative containing multiple functional groups. The average molecular weight of the polymers after size exclusion chromatography was 8-21 kDa, and dispersity was ~1.

We then investigated how the combination of different functional groups influenced chitosan's efficacy against planktonic bacteria and biofilm. Minimum Biocidal Concentrations (MBC) against planktonic bacteria was 16 µg/mL for all compounds, while the Minimum Biofilm Eradication Concentration (MBEC) was more variable. The antimicrobial efficacy of cationic chitosan derivatives towards biofilms was greatly enhanced in presence of hydrophobic groups (alkyl chains), and the extent of their effect was determined by the ratio and length of the alkyl chains. Living and dead cells were visualized by fluorescence staining, and three-dimensional imaging of biofilms confirmed the accessibility and antimicrobial effect of chitosan derivatives with alkyl chains in the full depth of the biofilms.

[P108] DETECTION OF P. POLYMYXA BIOFILM AND DAIRY FOULING BY A PORTABLE NIR DEVICE

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A variety of processing conditions in food manufacturing can cause unwanted biofilm and fouling formation. Biofilms and fouling in dairy processing equipment can lead to transmission of diseases, food spoilage, metal corrosion, and constitutes economic losses. Psychrotolerant sporeformers, specifically *Paenibacillus spp*., are important spoilage bacteria for pasteurized milk due to the bacterial adhesion in production lines. Moreover, *Paenibacillus polymyxa,* which is isolated from processing plant environments, produces extracellular enzymes causing spoilage of pasteurized milk. Lecithinase activity of *P. polymyxa* is responsible for "bitty cream" defect in milk because of the floating clumps of *fat*. Due to the current lack of early warning systems, the presence of biofilms is often presumed when poor process performance and product quality is observed. Therefore, there is a need of an on-line monitoring system.

The present work investigates a NIR portable device to detect *P. polymyxa* biofilm and dairy fouling in the wavelength range from 900 to 1630 nm. The NIR device was tested in static and dynamic conditions using 6-multiwell culture plates and a polycarbonate flow cell produced by 3-D printing, respectively. The spectra of specifically produced *P. polymyxa* biofilms, dairy fouling films and their references (sterile nutrient medium and milk, respectively) were collected after 24 h of incubation. All spectra were smoothened using Savatzky-Golay method and a principle component analysis (PCA) was performed. In addition, ATP-bioluminescence test was carried out to verify microbial growth and fouling formation after incubation.

In static experiments, the most promising results with good reproducibility were obtained for dairy fouling samples. A clear difference between dairy fouling samples and the reference (milk) is evident. The difference between *P. polymyxa* biofilm and corresponding reference, however, was less pronounced. In dynamic experiments, higher reflectance at 900 to 1130 nm was observed during biofilm formation compared to the reference (sterile nutrient medium). Different flow rates showed no effect on NIR results. Therefore, after further improvements, NIR devices can be potentially implemented in dairy production lines to detect the formation of biofilms and fouling.

[P109] SPATIAL ARCHITECTURE OF E. FAECALIS IMPACTS AND IS SHAPED BY RESPONSE TO ANTIBIOTICS

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We investigate the impact of antibiotic expose on the formation and spatial architecture of *E. faecalis* biofilms at single cell resolution. At sub-Minimum Inhibitory Concentrations (MIC), we find antibiotics inhibiting cell wall synthesis promote biofilm formation. Using a range of in-vitro assays combined with confocal microscopy and live-dead cell staining, we show that the counterintuitive increased biofilm mass reflects a true increase in the number of viable biofilm cells and is also associated with an increase in both cell lysis and extracellular DNA (eDNA). Furthermore, non-antibiotic chemical agents that inhibit cell lysis shift the peak in total biofilm mass to higher drug concentrations, while agents that promote cell lysis exhibit a similar increase in biofilm mass at low doses, even in the absence of antibiotics. We similarly tested other antibiotics outside the cell wall inhibitor class, but found no such increase in biofilm formation. Our results, along with a simple mathematical model of biofilm formation, suggest that environmental perturbations leading to small increases in cell lysis may actually promote biofilm formation by increasing eDNA concentrations.

As drug concentration increases beyond the Sub-MIC regime, biofilm formation eventually plummets in homologous populations of drug-sensitive cells. However, the size, composition and architecture of the biofilms can change dramatically in mixed populations of drug-sensitive strains and drug-resistant strains expressing β-lactamase, an enzyme responsible for degradation of β-lactam antibiotics. Here we use confocal microscopy and mathematical modeling to show that β-lactamase producing cells dramatically impact biofilm architecture at single-cell resolution. Surprisingly, drug-sensitive cells constitute a significant fraction of mixed-strain biofilms, even at drug concentrations that completely eliminate sensitive-only biofilms. Furthermore, we show these mixed-strain biofilms are characterized by islands of sensitive cells spatially arranged in local neighborhoods enriched for protective resistant cells. Finally, we combine these experiments with mathematical model to predict the impact of different time-dependent drug dosing protocols on biofilm formation and eradication. Our results highlight the important roles of cooperation and spatial heterogeneity in shaping spatially structured microbial communities and modulating their responses to antibiotics in the sub- and super-MIC regimes.

[P110] A TROJAN HORSE STRATEGY FOR ANTIMICROBIAL BIOLOGICALS

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Background: Glycoside hydrolases have emerged as potent, novel therapeutics that can disrupt biofilms, thereby increasing the susceptibility of the residing bacteria to co-administered antibiotics [1]. The broader clinical use of glycoside hydrolases such as alginate lyase (AL) is limited due to challenges in maintaining enzyme stability, adequate delivery and release of the enzyme at the site of infection. Herein, we present a Trojan Horse carrier for AL using environment-sensing lyotropic liquid crystalline gels (LLC) [2].

Aim: To design a LLC-gel carrier based on the lipid glycerol monooleate to protect, deliver and release AL in combination with the antibiotic gentamicin (GENT) as a novel anti-biofilm strategy.

Methods: The effect of *Pseudomonas* lipase on the release of AL/GENT from LLC-gels was evaluated and the efficacy of the gel was determined over 1 week *in vitro* against biofilms formed by alginate producing *P. aeruginosa* (clinical isolate) and compared to an unformulated simple drug solution. Finally, the stability of AL after fabrication of the LLC gel was assessed.

Results: GENT and AL were released at different rates and extent from the LLC-gels (10% AL over 9 days; 60% GENT over 2 days, respectively). Addition of *Pseudomonas* lipase increased AL release >2-fold (20-30% within 2 days). The LLC-gel demonstrated similar anti-biofilm activity (2.5 log reduction in CFU) compared to unformulated solution, confirming preservation of AL activity in the LLC-gels. Interestingly the antimicrobial effect could not be sustained over extended period (>2 days) which was attributed to a gradual loss of AL activity from prolonged exposure to 37°C during the assay, rather than short exposure to higher temperatures (60°C) during LLC-gel fabrication.

Summary: LLC gels present a promising Trojan Horse strategy to conceal and protect biologicals such as glycoside hydrolase. The ability of infection-triggered release provides potential as a future site-specific anti-biofilm therapeutic system.

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[P111]

Abstract withdrawn

[P112] ANTIBIOFILM ACTIVITY OF SALINE BASED NASAL SPRAYS EVALUATED WITH A NOVEL NASAL BIOFILM METHOD

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Introduction and Aim Nasal colonization with *Staphylococcus aureus* is common and is a major source of health care associated infections. A complicating factor is that these pathogens form biofilms that drugs like antibiotics have difficulties to eliminate. Moreover, with the increasing rise of resistance to antibiotics, current drugs have limited success. Thus, alternative approaches must be developed. Nasal sprays, are commonly used to clear the nose. Also, to relieve sinusitis. The aim with this study was to evaluate, *in vitro*, the antibiofilm activity of saline based nasal sprays with a novel biofilm assay mimicking the nasal surface including mucin, to which *S. aureus* adheres to and thus critical for its colonization.

Method *S. aureus* was mixed with mucin, a glycoprotein covering the nasal surface. This mixture was inoculated onto membranes that were transferred to agar plates and incubated for 24 hours. The formed biofilm on the membrane were then moved to a cell strainer placed in a 6-well plate before treatment. Treatments, once or daily for 4 days, were done for 1 second and the biofilm load analyzed with plate count. In addition, the biofilm on the membranes was assessed qualitatively by staining with Filmtracer LiveDead and analyzed with fluorescence microscopy. Also, Crystal Violet staining was performed on 24 hours biofilm, established in a 96 well plate, to evaluate if the nasal sprays had a biochemical activity.

Results All nasal sprays reduced the biofilm load after 1 treatment with more than 93% and after daily treatments for 4 days with more than 99%. Fluorescence imaging of live and dead cells showed a substantial decrease of cells after treatment and that most cells stained red indicating dead cells. Crystal Violet staining showed that mature biofilm was reduced by nasal sprays.

Conclusion In conclusion, the saline based nasal sprays reduced *S. aureus* biofilm which was shown with the novel nasal biofilm method, LiveDead and Crystal Violet staining assays. This study suggests that the reduction of the biofilm load was due to that saline based nasal sprays irrigate, i.e. removes the biofilm by physical force and by biochemical activity on the extracellular matrix.

[P113] INDUCING BIOFILM FORMATION WITH POLY(ACRYLOYL HYDRAZIDE) POLYMERS

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About 99% of the world's population of bacteria are found in the form of a biofilm at various stages of growth. Biofilm could be described as a consortium of bacteria attached to a surface and embedded in a matrix of extracellular polymeric substance. In this stage, the bacterial community behaves more like a supra-cellular organism rather than a unicellular one, showing off traits that individuals lack.

Our aim is to synthetize polymers that induce biofilm formation in *Escherichia coli*. We chose to synthetize Poly(acryloyl-hydrazide), every monomer unit is easily modifiable with functional groups that carry ketones or aldehydes, these groups will be positively charged, or able to interact with membrane receptors. We used the RAFT polymerization technique, this allow control over the average length of the polymer chain.

We were able to successfully synthetize the backbone and eight different modifications. Polymers were tested against *E. coli* PHL 644 and its effect measured via: spectrophotometry, flow cytometry, optic microscopy, and metabolic assays.

Three polymers were able to induce aggregation on E. coli PHL644: protonated Poly(acryloyl hydrazide), Poly(acryloyl-hydrazide-glucopyranose), Poly (acryloylhydrazide-mannopyranose). Clusters appear between the first 3 hours and aggregates can be seen with the naked eye, this aggregation is stable after applying dissociative forces. As clusters turn bigger and denser we measure a drop in optic density, as bacterial aggregates fall to the bottom of the culture. Optic microscopy pictures of bacterial samples inoculated with these polymers show clusters of bacteria embedded into a extracellular mucus like substance.

We suspect that the protonated backbone induces clustering due to charge interaction with the negatively charged bacteria, and that the sugar functionalized polymers are recognized by FimH adhesins inducing biofilm formation.

[P114] BIOFILM FORMATION IN SPACE (BFS): DESIGNING A SPACEFLIGHT EXPERIMENT

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Fungal growth has been detected aboard the Russian Space Station (Mir) and the International Space Station (ISS), promoting biodegradation of the spacecraft materials and compromising life-support systems [1-2]. Defining filamentous fungi biofilms is currently in debate among the scientific community. Nevertheless these are indeed associated with higher tolerance and resistance to adverse conditions [3-5]. To better monitor and control fungal contamination during long duration spaceflight missions, the NASA-funded project "Biofilm Formation in Space (BFS)" comprises a spaceflight experiment aboard the ISS, planned to be launched late 2018/early 2019 on a SpaceX flight. It will study growth and biofilm formation in microgravity also testing coupons of different materials (such as quartz, aluminum silicone, and polycarbonate developed by the Saarland University), in the search for antimicrobial surfaces.

To mature the experimental design to be spaceflight ready, several pre-flight tests need to be performed. For this, one of the tasks of the German Aerospace Center (Institute of Aerospace Medicine, Cologne) is to help define and optimize the culturing conditions for the fungus *Penicillium rubens* on the space hardware: the 12-well BioCell (developed by BioServe Space Technologies). Because growth in the BioCell will inevitably differ from common laboratory containers (such as flasks or multi-well plates), it is important to assess: i) growth and biofilm formation in the BioCell; ii) growth and adherence to coupons compared with planktonic growth iii) needed adaptations for the space-proven culturing system. Two different material coupons were tested – cellulose membrane and aluminum.

Results revealed fungal growth on all the 12-wells of the BioCell culturing system, within 48h and 96h of incubation. Biomass measurements showed more adhered biomass in cellulose membrane coupons (average 100% increase) than in aluminum coupons (average 3% increase). Additionally, fluorescence microscopy of coupon-attached biomass disclosed their hyphal structure and surrounding matrix. The 12-well BioCell was established as an adequate culturing system for growth of *P. rubens* in the upcoming spaceflight experiment aboard the ISS. This marks an important step in the development of novel methodologies to study filamentous fungi biofilms, leading to new ways of controlling them both on Earth and in space.

[P115] PHOTODYNAMIC INACTIVATION OF ORAL BIOFILMS WITH INDOCYANINE GREEN AS PHOTOSENSITIZER

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Due to the increasing antibiotic resistance, the development of alternative therapies such as antimicrobial photodynamic therapy (aPDT) is required. The aim of this study was to investigate the effect of visible light and water-filtered infrared A (VIS + wIRA) in combination with indocyanine green on the initial and mature oral biofilm grown *in situ*.

In situ biofilm samples were cultivated on disinfected bovine enamel slabs (BES) which were fixed on individual splint systems of three volunteers. The irradiation of the biofilm samples with VIS + wIRA was conducted for 5 minutes in combination with different concentrations of indocyanine green. Biofilms treated with 0.2% chlorhexidine (CHX) served as positive control and the untreated biofilms served as negative control. The colony forming units (CFU) were quantified. Additionally, the bacterial vitality was determined using live/dead staining and ApoTome microscope or confocal laser scanning microscopy (CLSM).

The applied aPDT reduced the CFU of initial biofilm up to 4.3 $\log_{10}$ whereas the killing effect of CHX was lower (3 $\log_{10}$). The CFU of mature oral biofilm were killed at a range of 2.5 $\log_{10}$ which was also higher than the CFU reduction caused by CHX (1.5-2 $\log_{10}$). A high bactericidal effect of aPDT against the initial (92%) and mature oral biofilm (74%) was revealed by the live/dead staining. CHX reduced the vitality of the initial and mature biofilm at a range of 96% and 55%, respectively.

The aPDT using VIS + wIRA and indocyanine green is a promising adjunctive therapy to treat periodontitis and peri-implantitis.

[P116] EFFECTS OF QUORUM SENSING AND PROPHAGE INDUCTION ON BIOFILM FORMATION AND PHAGE SENSITIVITY IN ISOGENIC POPULATIONS OF VIBRIO ANGUILLARUM.

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Biofilms established by fish pathogenic strains of *Vibrio* causes large problems in aquaculture by increasing fish mortality. Application of bacteriophages (phages) has been suggested to reduce biofilm formation, however little is known about phages and biofilm formation by *Vibrios* and how it is influenced by Quorum-Sensing (QS).

The main causative agent of Vibriosis is *V. anguillarum*, whose QS system resembles the phosphorelay arrangement in *V. harveyi*. There are three parallel systems transmitting signals via phosphorylation of a single regulatory protein, LuxO, activating or repressing the translation of the master regulator LuxR, depending on the level of autoinducer. We have constructed Δ*vanO* and Δ*vanT* mutants, whose protein products are homologous to LuxO and LuxR, which are locked in states of high- and low cell density, respectively.

Within *Vibrios* there are examples of both QS-activated and QS-repressed biofilm formation. With our mutants we aim to analyze how QS affects biofilm production, structure and dispersal in *V. anguillarum* and whether this affects protection against phages. Prophage induction has been linked to QS and we speculate this could stimulate biofilms through the release of different components, such as eDNA, and influence biofilm structures, potentially resulting in increased protection from phage infections. We use a *V. anguillarum* strain, which contains an H20-like prophage and we are constructing prophage-free strains harbouring the QS-mutations mentioned above. We compare non-prophage and prophage harbouring strains exposed to phages, in order to test our hypothesis.

Previous studies have shown that QS regulates the expression of the phage receptor, OmpK, in *V. anguillarum* PF430-3 resulting in reduced phage sensitivity in mutants mimicking high cell density [1]. Our mutants enable us to test if QS-mediated anti-phage defence is a unique or general phenomenon in *Vibrio anguillarum*.

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[P117] DEFERIPRONE AND GALLIUM-PROTOPORPHYRIN HAVE THE CAPACITY TO POTENTIATE ANTIBIOTIC ACTIVITY AGAINST RESISTANT S. AUREUS BIOFILMS

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Background: Staphylococcus aureus has the capacity to form biofilms and small colony variants (SCVs), which are slow-growing subpopulations with distinctive phenotypic and pathogenic traits, such as an atypical metabolism, altered production of virulence factors and intracellular lifestyle. S. aureus biofilms and SCVs are linked to antibiotic tolerance and resistance, are challenging to eradicate and have significant clinical implications. Despite aggressive antimicrobial therapies and surgery, infections often recur after weeks, months and years due to persistent biofilms and SCVs.

Aim: In the context of infectious diseases involving S. aureus biofilms and SCVs, a therapy targeting bacterial iron metabolism was evaluated.

Methods: The combination of the iron-chelator deferiprone (Def) and the heme-analog gallium-protoporphyrin (GaPP), in solution and incorporated in a surgical wound gel, was tested for antibiofilm and anti-SCV activity. The Def-GaPP activity was determined using multidrug-resistant S. aureus SCVs in an intracellular infection model. Moreover, the antibiofilm activity was assessed in vitro in the colony biofilm model and an artificial wound model, as well as in an in vivo infection model in nematodes (Caenorhabditis elegans).

Results: While Def alone failed to show substantial antibacterial activity, GaPP and the combination of Def-GaPP demonstrated concentration- and strain-dependent antibacterial properties. Specifically, the Def-GaPP combination significantly reduced the bacterial load in an artificial wound model (1.4 log10 reduction) and increased the survival of S. aureus SCV infected nematodes (86% survival of infected, treated worms vs. 25% survival of infected, untreated worms over 3 days). When Def-GaPP were combined with ciprofloxacin (Cip) or gentamicin (Gent), the triple combinations exceeded the antibiofilm activity of the individual compounds in the colony biofilm model against Cip- and Gent-resistant strains (5.4 log10 reduction for Def-GaPP-Cip and 3.4 log10 reduction for Def-GaPP-Gent). Moreover, Def-GaPP-Gent eradicated intracellular SCVs in human bronchial epithelial cells.

Summary: In targeting bacterial iron metabolism, Def-GaPP showed significant activity against S. aureus biofilms and SCVs. Def-GaPP could potentiate the activity of Cip and Gent against resistant strains. Delivered in a wound healing gel, Def-GaPP has potential to be used as a biofilm control strategy for the topical treatment of S. aureus infections.

[P118] BACTERIAL COMMUNITIES FORMING BIOFILMS IN WASHING MACHINES

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Biofilm formation is a widely known phenomenon in both natural and technical systems. In technical systems such as water pipes they are also known to cause problems due to the pathogenicity of some organisms inhabiting the biofilms. Also washing machines are subject to biofilm formation, including the risk for pathogenic bacteria to accumulate. Nowadays washing habits changed to washing at lower temperatures in order to save energy. This trend leads to a better survival of biofilm bacteria during laundry processes. Biofilm growth within washing machines can have varying effects ranging from unpleasant odors or biocorrosion to the risk of infection with pathogenic organisms.

Eliminating this problem has been subject to ongoing research. Respected work examined the effects of washing temperature and detergent composition on biofilms in the washing machine. Our approach to deal with the problem of biofilms is to identify the organisms inhabiting washing machines via Next Generation Sequencing (NGS) for a more customized approach to eliminating biofilms. To determine the best way to tackle this problem, samples from the inner and outer tub were taken to get insight into the composition of the bacterial communities inhabiting biofilms in washing machines.

[P119] TOPOGRAPHICAL CHANGES INDUCED BY ETHANOL TREATMENT RENDER BIOFILMS WETTABLE AND INCREASE BIOFILM EROSION

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Biofilm control strategies rely on the inactivation and/or removal of the bacterial material via chemical and/or physical processes. In both cases, it is crucial to sufficiently wet the biofilm surface with antibacterial or eroding agents; however, certain biofilms efficiently resist wetting, and the origin of this behaviour is to date still not fully understood.

Here, we show that depending on the nutrient media biofilms are grown on, three different modes of wetting can be observed: hydrophilic, lotus-like hydrophobic and rose petal-like hydrophobic. Using light profilometry, a variant of confocal reflectance microscopy, we demonstrate that the topography of the biofilm surfaces and their wetting behaviour are related. In particular, the degree of microscopic surface roughness present on those biofilms determines whether lotus-like or rose-like hydrophobicity occurs.

Although certain biofilms efficiently resist wetting of ethanol up to 70% concentrations, this solvent is still used as disinfectant in both industrial and medical settings. We here show that biofilm exposure to ethanol solutions with concentrations above 70% can induce a smoothening of the biofilm surface – even for ethanol exposure times as short as 10 min. As a consequence of this smoothening effect, the wetting resistance of the biofilm surface is reduced and the erosion sensitivity of the biofilm is increased.

Converting the surface polarity of a biofilm, as demonstrated here, could facilitate biofilm removal in industrial or medical settings by increasing the biofilm wettability.

[P120] TIME-KILL ANALYSIS TO SCREEN AN ANTIMICROBIAL WOUND IRRIGANT AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) BIOFILM CULTURED IN 3D ACELLULAR SOFT TISSUE

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Aim: To assess the biofilm killing activity of antimicrobial solution against Methicillin resistant *Staphylococcus aureus* (MRSA) in a novel 3D collagen biofilm model at four different timepoints, i.e. 5, 10, 60 minutes and 24 hours.

Method: Acellular Synthetic Soft Tissue (ASST) was prepared containing collagen and serum proteins. To each well of a 96-well plate, ASST was applied and cured. To form the biofilm, 1-3 x 10^5 CFU/well of methicillin resistant *Staphylococcus aureus* (MRSA) was inoculated and incubated for 24 hours before application of antimicrobials. After treatment, for different time periods, samples underwent neutralization and the biofilm matrix was digested. Kill was assessed by taking drops of digested ASST and applying them onto TSA-plates with a multichannel pipette. The TSA plates were then incubated until colonies of the growth controls were clearly visible.

Results: A MRSA biofilm was formed within the ASST and reached a biofilm load of 1-3 x 10^8 CFU/well after 24 hours incubation. Microbial inhibition could be detected but was obscured for substances that precipitated in the ASST. Kill was detected as absence of viable colonies on TSA plates.

Conclusion: It was shown that MRSA could grow a biofilm in the *in vivo* like 3D skin tissue (ASST). This study also demonstrated that the inhibitory effect of antimicrobials could be assessed directly by visual inspection of the ASST and the killing effect by assessing growth of treated ASST applied as drops on agar plates. In conclusion, we successfully developed a time-kill method assessing MRSA biofilm, grown in a clinical relevant soft tissue adopted to a microplate assay format, that is very rapid and screens both inhibitory and killing activity of antimicrobials.

[P121] DO BLOCKSTRUCTURES IMPROVE THE COPOLYMER'S PROPERTIES COMPARED TO THEIR STATISTICAL ANALOGUES?

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Worldwide, more than one million in dental implants are placed per year [1] whereof up to 4.2% of the patients suffer from postoperative infections [2]. The mouth's humidity, temperature and food remnants favor an increased bacteria growth that often results in a biofilm formation. The biofilm causes infection of the bone with severe consequences up to failure of the implant. One current solution approach is the synthesis of copolymeric coatings containing an antimicrobial moiety like a quaternary ammonium group [2] and a phosphonate group to attach the implant [3].

Statistical copolymers (Poly-BMADUA-*co*-DMMEP) containing the monomers *N*-benzyl-11-(methacryloyloxy)-*N*,*N*-dimethylundecane-1-ammoniumbromide (BMADUA) and dimethyl(2-methacryloxyethyl)-phosphonate (DMMEP) were prepared via free radical polymerization in tetrahydrofurane (THF) by Wassmann [3]. The analogous diblockcopolymers Poly(BMADUA-*b*-DMMEP) were synthesised via sequential reversible addition-fragmentation chain transfer (RAFT) in *N*,*N*-dimethylformamide (DMF). All polymers were synthesised with different BMADUA/DMMEP ratios, then *spin-coated* onto titanium plates and characterised by ellipsometry as well as water contact angle measurements. Antimicrobial tests are existent for the statistical copolymers by now; they show good results against (*S. aureus*) [3]. In general, the ellipsometry and contact angle measurements of the diblockcopolymers had smaller values than Poly(BMADUA-*co*-DMMEP). Comparing similar BMADUA percentage contents, the wettability increases by approx. 10° and the layer thickness increases by approx. 10 nm for statistical copolymers. It appears as though different polymer architectures have an influence in physicochemical properties for the described copolymer system.

Derived from these results the antimicrobial properties might change positively for blockcopolymers as well. Necessary measurements will be performed in the near future.

[P122] DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW QUORUM SENSING INHIBITORS FOR MULTI-SPECIES WASTEWATER BIOFILMS

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Keywords: Multi-species biofilms, *Quorum sensing*, inhibitors, wastewater, auto-inducers

Microbial biofilms colonize water systems and are the major cause of membrane biofouling leading to reduced efficiency of water treatment processes. Biofouling starts with the formation of a biofilm followed by growth and colonization across the overall surface. Bacteria living within biofilms are markedly more tolerant to disinfectants. Therefore, understanding and controlling bacterial biofilms is crucial for a better water systems management.

One of the mechanisms behind biofilm formation is *Quorum Sensing* (QS), a communication system where bacterial cells produce, detect and respond to small diffusible signal molecules, also called auto-inducers, such as AHLs in Gram negative bacteria [1], or PQS, specially involved in *P. aeruginosa* QS [2]. Some QS signals can also be recognized by many species, thereby allowing inter-species communication [3].

Since the signal molecules are the key factor in the bacterial communication processes that lead to biofilms, their inhibition is a promising target to control biofilms. However, very few studies on targeting Quorum Sensing have been applied to *multi*-species biofilms for so far. In view of this, our group is focused on developing an anti-biofilm strategy based on synthetic molecules as inhibitors of the natural AHLs and the PQS [4].

Here, we have synthesized and evaluated various structural analogs of the PQS. We have also developed a 96-well high throughput assay to evaluate their ability to inhibit wastewater-related biofilms.

Several compounds showed significant anti-biofilm activity, with a significant decrease of the total adherent biomass, making them promising leads for further development in the prevention of *multi-species* biofilms.

The protocol optimization, inhibition results and structure-activity relationships will be presented.

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[P123] OPTIMIZATION OF A HIGH-THROUGHPUT SCREENING PLATFORM IN 384-WELL PLATES FOR EXPLORATION OF ANTI-BIOFILM ACTIVITY

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Bacteria biofilms are responsible for 60-80% of human infections. They can be over 1000-times more tolerant to antibiotics compared to single floating i.e., planktonic cells, therefore frequently leading to chronic infections and complicating the treatment using the current prophylactic regiments. Thus, there is an urgent need for next-generation drugs specifically developed for treating biofilm-related infections. One way to achieve this goal is to screen a large set of different compounds to pinpoint potential leads. In the present study, we have optimized the in-house screening platform based on the sequential staining with resazurin and crystal violet, giving confirmatory and complementary information on biofilm formation and formed biofilms in term of viability and total biomass. Here, the two staining assays were optimized for high-throughput drug screening in a 384-well format using clinical *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 15442 as the gram-positive and -negative biofilm models, respectively. Both pre- and post-exposure modes were applied for investigating the drug-induced effects to gain a better insight into the mechanistical action of the most promising leads. For maximising the statistical reliability of this screening assay, several parameters (growth characteristics, sample volumes etc.) were first examined. With the optimised assays we have now screened over 2700 compounds from libraries of repurposed drugs, both for their activity in preventing biofilm formation and in eliminating mature biofilms. The advantage of screening FDA approved and investigational drugs is that information on their structure and toxicity is already available, which reduces the cost and time before reaching the next step in drug development (*in vivo* analyses). The most active compounds will be selected for further mechanistic studies and structural optimization with the ultimate goal for preventing and treating biofilm and/or device-related biofilm infections.

[P124] PERTURBATION OF EFFLUX BY AN AMPHIPHILIC CARGO-LOADED NANOMATERIAL: POTENTIAL SYNERGY FOR MITIGATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS BIOFILM

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Staphylococcus aureus is the one of the major organism causing nosocomial infection in the clinical set up. The development of resistance in *S. aureus* is creating obstacle to treat infection with the normal antibiotics. Fluoroquinalone resistance in *S. aureus* is one of the major resistance mechanism conferred by this organism and treating such pathogen would require elevated concentration of the antibiotic. The combination therapy is an alternative strategy to minimize the therapeutic concentration of an antibiotic to treat such infection. In this regard, a pyridinium based cationic amphiphile-loaded poly (lactic-co-glycolic acid) (PLGA) nanocarrier (C1-PNC) was developed as a potential adjuvant to eliminate clinically isolated methicillin-resistant *S. aureus* (MRSA) biofilm in combination with ciprofloxacin. Interestingly, C1-PNC was found to render 6-fold reduction of minimum biofilm elimination concentration ($MBEC_{90}$) of ciprofloxacin against a clinical MRSA strain in combination therapy. Further, C1-PNC found to inhibit efflux pump activity in MRSA planktonic cells which can be correlated with the superior intra-cellular accumulation of ciprofloxacin in presence of C1-PNC. The molecular modelling of the Nor A protein and consecutive docking of the C1 with Nor A model revealed its potential in comparison with the standard efflux pump inhibitor. Interestingly, the combinatorial dosing regimen of C1-PNC and the ciprofloxacin was nontoxic to cultured Human embryonic kidney (HEK293) cells. This nontoxic amphiphile-loaded nanomaterial holds considerable promise as an adjuvant for antibiotic-mediated elimination of MRSA biofilms.

[P125] ACTIVITY OF COMBINATIONS OF AN ENZYMATIC COCKTAIL (ENZ) WITH TOBRAMYCIN (TOB), AMIKACIN, (AMK), CIPROFLOXACIN (CIP), MOXIFLOXACIN (MXF) , VANCOMYCIN (VAN) OR LINEZOLID (LDZ) AGAINST BIOFILMS OF CLINICAL ISOLATES FROM ESKAPE PATHOGENS

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Background: The capacity of bacteria to form biofilms is a major cause for nosocomial infections. *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are ESKAPE pathogens that are frequently associated with biofilm infections. Antibiotics and antiseptics are usually considered as weakly active against bacteria growing in biofilms as they have only a poor access to bacteria. We developed an enzymatic cocktail containing DNase and polysaccharidases (ENZ) which allows the dispersion of bacterial biofilms of species mentioned above. We examined whether ENZ could restore activity of antibiotics against these biofilm, taking TOB, AMK, CIP, MXF, VAN and LDZ as examples.

Materials/methods: We used 6 clinical isolates of *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (coming from infections on medical devices). Biofilms were grown for 24 h in 96-wells plates and then exposed for 24 h to Cmax of CIP, TOB, AMK, MXF, VAN and LDZ, alone or in combination with ENZ. Bacterial viability in biofilms was quantified using the redox indicator resazurin as previously described (1). Cytotoxicity induced by ENZ (5X concentrated) was tested towards 4 human cells lines (THP-1; HA-60, U937 and T24) by measuring the release of the cytosolic enzyme lactate deshydrogenase (LDH) into the culture supernatant (cytotoxicity detection kit PLUS; Roche, Basel, Switzerland).

Results: See Table. At human C_{max}, CIP, TOB, AMK, MXF, VAN and LDZ alone reduced bacterial viability of 20-47%, 0-17%, 0-13%, 10-48%, 0% and 47-57% respectively. Combining ENZ with each of these antibiotics improved efficacy, with reduction in viability reaching 69-89 %, 65-94%, 68-96%, 66-99%, 69-92% and 79-89% when CIP, TOB, AMK, MXF, VAN and LDZ were used at their C_{max}, respectively.

No cytotoxicity of the enzymatic cocktail was observed when incubated with four human cells lines.

Conclusions: Combining ENZ with 6 antibiotics belonging to 4 classes proves highly synergistic against biofilms of 6 clinical isolates. This opens perspectives for testing these enzymes as adjuvant for the treatment of biofilm infections.

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	% reduction in viability[a] within biofilms for antibiotics alone or combined with ENZ at their C_{max}[b]											
Strains	TOB[c]		AMK		CIP		MXF		VAN		LZD	
	alone	+ ENZ	alone	+ ENZ	alone	+ ENZ	alone	+ ENZ	alone	+ ENZ	alone	+ ENZ
PA20 (*P.aeruginosa*)	47	89.20	13	78.84	13	78.84	48	77.47	na	na	na	na
PA500 (*P.aeruginosa*)	0.66	79.44	11	96.90	11	96.90	0	99.40	na	na	na	na
2003/1083 (*S.aureus*)	30.6	89.41	na	na	na	na	10	89.56	0	92.37	47	79.86
2009/SO25 (*S.aureus*)	50.34	77.23	na	na	na	na	15	88.36	0	69.96	57.97	89.29
010 (*K. pneumoniae*)	20.34	69.56	3.66	69.20	3.66	69.20	17.36	66.36	na	na	na	na
826 (*K.pneumoniae*)	34.66	75.13	0	68.28	0	68.28	12	77.36	na	na	na	na

[a] reduction in viability compared to untreated control
[b] CIP C_{max}: 3.2 mg/L; TOB C_{max}: 5 mg/L; AMK C_{max}:24 mg/L; MXF C_{max}: 3.6 mg/L; VAN C_{max}: 21 mg/L LZD C_{max}: 17 mg/L
[c] na: not applicable.

[P126] EVALUATION OF THE ACTIVITY OF A NOVEL ANTIMICROBIAL PEPTIDE AGAINST STAPHYLOCOCCUS AUREUS BIOFILMS

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Bacterial antimicrobial resistance is of great concern to global health. Biofilm formation is usually involved in increasing bacterial resistance. With regard to this, the development of new antimicrobial and anti-biofilm molecules, such as antimicrobial peptides (AMPs), is of pivotal importance.

AMPs are effector molecules of the innate immune system of Mammalia that present biocidal activity. Because of their properties (e.g., low propensity for developing bacterial resistance, broad antimicrobial spectrum activity, synergy with common antimicrobials) are emerging tools to contrast antimicrobial resistance and to control microbial biofilms.

Staphylococcus aureus is an ubiquitous worldwide-distributed pathogen responsible for both nosocomial infections and food poisoning.

The aim of this study was to evaluate the antimicrobial action of HCAT, a novel AMP obtained by modifying the cathelicidin-derived peptide IDR-1018, against biofilms of *S. aureus*.

S. aureus ATCC 35556 (a well-known strong biofilm producer) was used in this study. The antimicrobial activity of HCAT (80 µmol/L) was measured with Minimum Biofilm Eradication Concentration (MBEC) assay at different treatment time-points (24, 48, and 72 H) against 24 hours-old biofilms, obtained using two different initial inoculum concentrations (10^5 and 10^3 cfu/ml).

Results showed anti-biofilm activity of HCAT against *S. aureus*. In details, 6.14±0.17 Log_{10} cfu/peg, and 5.57±0.23 Log_{10} cfu/peg reductions were observed following treatment at different time-points against staphylococcal biofilms grown starting from 10^5 and 10^3 cfu/ml initial inoculum concentration, respectively. Interestingly, the characterization of HCAT showed a remarkable stability under a wide range of pH and temperature conditions.

Although further studies are necessary to confirm these initial findings, our results suggest that HCAT might represent a useful alternative to commonly used antimicrobials to contrast biofilms. Furthermore, because of its properties, HCAT might be involved in a wide range of applications in both food and healthcare industries.

[P127] MULTIPLATFORM APPROACH TO PROBE PENETRATION, ACCUMULATION AND EFFLUX OF ANTIMICROBIAL AGENTS INTO BIOFILMS OF PSEUDOMONAS AERUGINOSA

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The structure of *Pseudomonas aeruginosa* biofilms is not uniform and this heterogeneity influences the effectiveness of antimicrobials by altering their penetration and accumulation. This heterogeneity combined with mechanisms such as porin overexpression and development of persister cells contributes to the innate resistance of Pseudomonas in biofilms to antibiotics, creating a significant issue in the treatment of infections and resulting in elevated levels of mortality and morbidity. Whilst the mechanisms through which innate resistance arises have been elucidated, the dynamics of these processes are significantly less understood.

Here, we use a microfluidic system coupled with time-lapse microscopy to investigate the rate of cell death within the biofilm following treatment with a variety of antimicrobial agents. Using fluorescent labels we examine the effect antimicrobial agents have on distribution of exopolysaccharide (EPS) and extracellular DNA (eDNA) within the biofilm. Further, working with partners in the European Association of National Metrology Institutes (EURAMET) we are developing cross-platform methods to study the dynamics of the penetration, accumulation and efflux of antimicrobial agents within the biofilms using a variety of techniques including 3D OrbiSIMS (secondary ion mass spectrometry) and X-ray photoelectron spectroscopy (XPS) amongst others. Here we describe initial findings and demonstrate their potential in label-free imaging of bacterial biofilms.

[P128] A TARGETING AGENT FOR DRUG DELIVERY TO STAPHYLOCCUS AUREUS BIOFILM INFECTIONS

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Targeted drug delivery is a promising new strategy to combat biofilm infections because it ensures delivery of antibiotics in close proximity to the bacterial cells and thereby maximizes exposure. However, a key challenge to this strategy is to find a suitable targeting agent that provides specific interaction with bacteria in biofilms.

Nucleic acid aptamers are uniquely suited as targeting agents for drug delivery due to the ease of their synthesis, and their high target affinity and specificity.

In this study, we investigate the use of DNA aptamers as a targeting agent to deliver encapsulated antibiotics to *Staphylococcus aureus* biofilms. We first compared the ability of different *S. aureus* specific aptamers to target *S. aureus* biofilms, and then investigated if the aptamer could be used to target and accumulate drug-loaded liposomes in *S. aureus* biofilms. Lastly, we investigated if the retained targeted drug-loaded liposomes could eradicate *S. aureus* biofilms.

The *S. aureus* specific aptamer or a non-specific scrambled version of the aptamer was conjugated to fluorescent liposomes by click chemistry. To mimic *in vivo* conditions, *S. aureus* biofilms were grown in brain heart infusion broth with 5% human plasma in microwell plates preconditioned with 50% human plasma. The functionalized liposomes interacted with 24-hour-old biofilms for one hour. Upon removal of unbound material, fluorescent signal from the retained liposomes confirmed that liposomes accumulated in the biofilm if they were functionalized with *S. aureus* specific aptamers, while the non-specific aptamers did not promote binding. Confocal laser scanning microscopy showed that liposomes functionalized with the *S. aureus* specific aptamers penetrated the entire depth of the biofilm and bound to the surface of bacterial cells. Finally, preliminary studies have shown that the retained drug-loaded and targeted liposomes can mediate full kill of *S. aureus* biofilms upon 24 hours of treatment.

In conclusion, the aptamer candidate investigated is a suitable targeting agent for drug delivery to *S. aureus* biofilms.

[P129] STUDYING ANCIENT MECHANISM TO SYNTHESIZE NOVEL ANTI-BIOFILM DRUGS

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Bacteriophages are the natural enemies of bacteria. They have co-evolved with bacteria from the beginning of life and presumably have evolved evolutionary optimized mechanisms to inhibit biofilm formation. Bacteriophages are able to diffuse biofilm, infect and kill cells, even persisters can be infected. Some phages even contain enzymes that enable them to extract extracellular matrix before they reach to their prey; others can encode biofilm degrading enzymes once they have taken over hosts transcriptional machinery and release them after lysis. The latter can be coded from host genome or from phage itself (as reviewed by Abadeon, 2015 and Harper et al., 2014). Phage genes that inhibit biofilm formation are not been described to date. Here we present four non-toxic biofilm-inhibiting phage proteins from different lytic *Pseudomonas aeruginosa* phages that were identified out of 170 tested proteins. Their anti biofilm effect on laboratory, clinical and synthetic strains was evaluated using Calgary device and flow cell system. The phage proteins inhibit biofilm formation significantly, up to 85% biomass reduction in 48 hour flow-cell biofilm. These phage proteins can be used to synthesize target-based small molecules, that will mimic the anti biofilm effect (Wagemans & Lavigne, 2012).

[P130] PREVENTING MEMBRANE BIOFOULING WITH A MICROPARTICLE REACTOR

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Introduction: Biofouling is undoubtedly one of the major problems affecting the performance of membrane water treatment processes. In fact, membrane flux decline results from a set of combined mechanisms where biofouling is considered to be responsible for 45% of the problems. Some rather recent approaches to water treatment processes for membrane biofouling prevention include the development of new antimicrobial compounds to kill microorganisms before membrane filtration.

Aim: The main purpose of this work was the development of a bed reactor with biocide loaded microparticles for water pre-treatment in order to prevent membrane biofouling.

Materials and Methods: A synthetic crystalline aluminosilicate also designed zeolite (3-5 µm) and hydroxyapatite (5 µm), were selected as core materials for functionalization. The particles were prepared using the layer-by-layer (LBL) self-assembly technique and benzyldimethyldodecyl ammonium chloride (BDMDAC) was selected as biocide. Microparticles were used in the reactor to determine the log-reduction of *Escherichia coli* in 30 min. In order to determine if BDMDAC was released from the particles, the biocide concentration was determined after six washing steps. Microparticles were after tested to evaluate their antimicrobial efficiency. These microparticles were further tested to prevent biofouling formation on filtration membranes.

Results: The zeolite particles functionalized by LBL with BDMDAC at 200 and 500 mg/L were able to totally reduce the presence of viable microorganisms in 30 min. Regarding antimicrobial hydroxyapatite functionalized particles, a concentration of BDMDAC of 100 mg/L was able to completely kill *E. coli*. After six washing steps, particles kept their antimicrobial load.

Conclusion: This work showed that zeolite and hydroxyapatite microparticles are efficient cores as biocide transporters since these particles can target bacteria without releasing biocide. This is of utmost important since chlorine (which damages membranes) can now be replaced for a safer biocide, without releasing harmful chemicals to the water. This is also a proof of concept on the development of a new microparticle reactor as water pre-treatment strategy to impair biofouling development on filtration membranes.

[P131] INHIBITION OF BIOFILM FORMATION ON URINARY CATHETERS BY VANILLIN: A STUDY USING ESCHERICHIA COLI ISOLATED FROM URINARY TRACT INFECTIONS

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Urinary Tract Infection (UTI) is a primary bacterial infection affecting around 150 Million people in the world every year. UTI can even lead to death in children, older men, and women. Catheterisation was involved in 70-80% of complicated UTI. More than 50% women are affected with UTI at least once in a lifetime. It is recurrent in 40% cases. Most common bacterial pathogen associated with UTI is Escherichia coli. Uropathogens commonly form biofilms in the bladder which helps these organisms to inhabit the host. Use of antimicrobial agents can lead to the emergence of antibacterial resistance. In the present study, Escherichia coli from hundred patients of both the sex and diverse age group suffering from UTI were isolated and analysed for their sensitivity against a panel of 10 different fluoroquinolone antibiotics. This analysis revealed that nearly 80% of the isolates are resistant to fluoroquinolone antibiotics The resistant organisms were further checked for their ability to produce biofilms and to produce haemolysis in blood agar. The ten high biofilm producers showing significant antibiotic resistance were chosen for further studies. Biofilm production of selected UPEC strains was quantified using microtiter plate assays. Vanillin, a naturally occurring phenolic aldehyde is found to inhibit the biofilm formation in the microtiter plate assays. A microtiter plate assay was performed using vanillin against selected isolates of E.coli to quantify the inhibitory effect of vanillin on biofilm formation. Further, these isolates were tested on their haemolytic potential in the presence of sub MIC concertation of vanillin. The biofilm forming ability of selected strains was evaluated on urinary catheters. Vanillin was found to efficiently reducing the formation of biofilms on urinary catheters and prevent the haemolysis in some cultures. Thus incorporation of vanillin, a non-toxic natural product, which is the primary component of the extract of vanilla bean is revealed to be a practical approach to prevent biofilm formation and expression of virulence factors on urinary catheters.

[P132] ANTIFOULING PROPERTIES OF DNA COATINGS

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Fouling is a major concern for surfaces subjected to the liquid environment in applications ranging from medical devices to food contact surfaces, industrial heat exchangers, and marine equipment. One of the approaches to fouling control is the use of hydrophilic polymer coatings. Coatings made from poly-anions and poly-cations with the layer by layer (LBL) method are straightforward to produce, inexpensive, and provide a high degree of control over coating thickness compared to thin film deposition methods. DNA is a poly-anion and therefore suited for LBL coatings. We hypothesized that the poly-anionic properties and the poly-phosphate backbone of DNA would provide DNA coatings anti-biofouling and anti-scaling properties. To test this hypothesis, we characterized the physicochemical properties of DNA/poly(ethyleneimine) LBL coatings and evaluated their resistance against microbial fouling and scaling ($CaCO_3$ precipitation). The coatings reduced scaling from tap water when incubated statically or under flow and temperature conditions that mimicking surfaces in heat exchangers. The DNA coating also impaired microbial fouling from tap water, and resulted in lower adhesion force and less colonization by the human pathogens *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The antifouling properties of DNA presented in this study demonstrates a proof of concept that LBL coatings with poly-anions harboring phosphate groups can address fouling in several applications.

Keywords: Biofouling, DNA, antifouling, biofilms, heat exchangers, calcium carbonate, scaling, calcite.

[P133] REAL-TIME OPTOTRACING OF CURLI AND CELLULOSE IN LIVE SALMONELLA BIOFILM USING LUMINESCENT OLIGOTHIOPHENES

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Extracellular matrix (ECM) is the protein- and polysaccharide-rich backbone of bacterial biofilms that provides a defensive barrier in clinical, environmental and industrial settings. Understanding the dynamics of biofilm formation in native environments has been hindered by a lack of research tools. We have developed a method for simultaneous, real-time, *in situ* detection and differentiation of the *Salmonella* ECM components curli and cellulose, using non-toxic, luminescent conjugated oligothiophenes (LCOs). These flexible conjugated polymers emit a conformation-dependent fluorescence spectrum, which we use to kinetically define extracellular appearance of curli fibers and cellulose polysaccharides during bacterial growth. The scope of this technique is demonstrated by defining biofilm morphotypes of *Salmonella enterica* serovars Enteritidis and Typhimurium, and their isogenic mutants in liquid culture and on solid media, and by visualizing the ECM components in native biofilms. Our reported use of LCOs across a number of platforms, including intracellular cellulose production in eukaryotic cells and in infected tissues, demonstrates the versatility of this opto-tracing technology, and it's ability to redefine biofilm research.

[P134] FAST, A NEW QUANTITATIVE REPORTER FOR BIOFILM REAL-TIME MONITORING

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In nature, biofilms are composed of a multitude of micro-organisms that can be bacteria, fungi or micro-algae, all embedded in an extracellular matrix. Thus, these communities of microbes form a highly concentrated and heterogeneous material displaying cell sub-populations, multiple gradients of nutrients, metabolites and physico-chemical parameters. Even the single-species laboratory models of biofilm demonstrate considerable microscale heterogeneity. In this context, optical microscopy imaging strategies have widely spread to grab local information and to capture the spatial complexity of these adherent communities.

In our laboratory, we are studying biofilms in millifluidic channels under a constant flow of nutrient medium using video microscopy to monitor real-time development processes. Our approach requires reliable specific and quantitative labeling strategies, which prompted us to investigate more closely the relevance of the fluorescent reporters to draw the spatio-temporal distribution of a gene expression in the biofilm. We examined GFP and mCherry fluorescence signal along the formation of a model *Escherichia coli* biofilm in parallel with the microscopic optical density signal which reports biomass formation. We found a strongly non-linear response of these fluorescent reporters that we could relate to the environmental oxygen shortage occurring during biofilm growth. To overcome this problem, we propose an alternative strategy using a novel protein tag so called Fluorescence-Activating and Absorption-Shifting Tag:FAST. This protein has the ability to bind to a small synthetic molecule which activates the fluorescence of the complex with a non-oxygen-dependent mechanism. We will show here the advantages of using this protein in our *E. coli* model biofilm and show the first results we obtained in the more complex background of a multi-species community, monitoring *Bacillus thuringiensis* among three other species.

We conclude to the efficient and accurate properties of this new family of genetic reporters for biofilm imaging and point out the hazardous interpretation of the fluorescence signal of the popular GFP and its derived analogs in the context of the bacterial biofilms.

[P135] MAPPING THE CHEMICAL AND STRUCTURAL LANDSCAPE OF MICROBIAL INTERACTIONS IN A 4-MEMBER MODEL CONSORTIUM OF SOIL BACTERIA

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Soil bacteria live in complex communities that host a plethora of different species. Knowledge of community composition, growth and function is essential for understanding and potentially modulating microbial life e.g. in the rhizosphere for plant growth promotion or in treatment of pathogens. Microbial community ecology is in part determined by inter-species interactions, but the underlying mechanisms driving such interactions are often unknown and difficult to identify due to community complexity. Inter-species interactions are therefore often identified and studied in simpler model communities. By applying microsensor measurements of N_2O, O_2 and pH, we identified key functional mechanisms governing community synergy and development from a previously assembled bacterial model consortium with four co-isolated soil isolates: *Microbacterium oxydans*, *Xanthomonas retroflexus*, *Stenotrophomonas rhizophila* and *Paenibacillus amylolyticus*. We show that *in vitro* acid and alkali production from individual community members leads to an overall pH stabilisation of the local environment over time, which in turn leads to enhanced community growth. As *in vitro* observations were made with standard laboratory culture media, we continued by investigating whether metabolic activity of these isolates would be able to affect pH in bulk soil and observed pH changes within the natural habitat. Identifying metabolic interactions between bacteria is essential to understand their effects on the local environment and to harvest the full potential of bacterial consortia. The combination of sensor-technology and microbiological culturing methods appears to be ideal to do so.

NOVEL METHODS FOR
BIOFILM CHARACTERIZATION

[P136] NISIN PRODUCTION IN LACTOCOCCUS LACTIS BIOFILMS

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Biofilms can be considered complex agglomerates of microorganisms, which grow associated to a surface. This naturally predominant way of bacterial growth has already been used for decades in wastewater treatment, but their application in biotechnological production processes is still limited. To assess their applicability, the model organism *Lactococcuslactis* is used. This lactic acid bacterium (LAB) is commonly applied in the large-scale production of dairy products and is known to be capable of biofilm formation. As a secondary metabolite, *L.lactis* produces the antimicrobial peptide Nisin which contains several uncommon amino acids and is a member of the class of lantibiotics. This peptide is effective against many Gram-positive organisms and is already used as a food preservative (E234). The focus of this project is the cultivation of *L. lactis* as a self-immobilized biofilm in flow-through bioreactors (A) on different metallic and structured surfaces (Fig. 1B). After an initial batch-phase of 24 h with a flow rate of 5 mL min^{-1} that allows for sufficient biofilm formation, the setup is switched to a continuous process for Nisin production at 0,376 mL min^{-1} (dilution rate D = 2 h^{-1}). Due to its inhomogeneous nature and structures, the productivity supposedly varies not only over time, but throughout the biofilm itself. To assess these differences on a molecular level, an assay for gene expression analysis using quantitative real-time polymerase chain reaction (qRT-PCR) will be used. This technique allows the analysis of the regulation of the Nisin gene cluster with comparably small sample volumes and thus an increased spatiotemporal resolution.

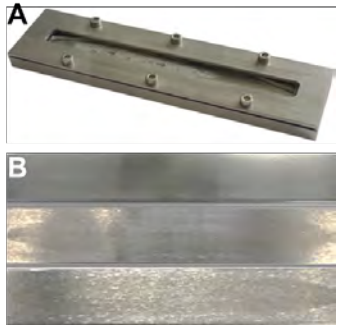

Fig. 1: A: Assembled flow-through bioreactor for biofilm cultivation; B: Titanium substrata with different surface roughnesses R_a

[P137] PROCESS BEHAVIOR OF PRODUCTIVE BIOFILMS

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Heterogeneities are a common occurrence in biofilms. A biofilms is never absolutely uniform, but rather composed of several layers and areas that differ in e.g. gene expression levels, what genes are expressed, the amount of environmental stress it is exposed to, or metabolic stress.

In order to elucidate the processes in a biofilm and gain a better understanding of them, this work focuses on establishing a reaction-diffusion model for nutrient and product transport in *Lactococcuslactis* biofilms. *L.lactis* is a lactic acid bacterium used in production of cheese and yoghurt, but it also produces the antimicrobial compound Nisin, which is used as a food additive. To study the production behavior of Nisin in biofilms of *L.lactis* confocal microscopy (CLSM) and different FRAP (Fluorescence Recovery After Photobleaching) techniques are employed to on-line assess diffusion constants of glucose and Nisin in the biofilm with a focus on how diffusion may differ in different strata and growth phases of the young biofilm. Different sensor systems are integrated in a continuous reactor system with micro structured surfaces for online monitoring of e. g. pH changes. The whole reactor set-up is integrated into the CSLM system (Fig. 1).

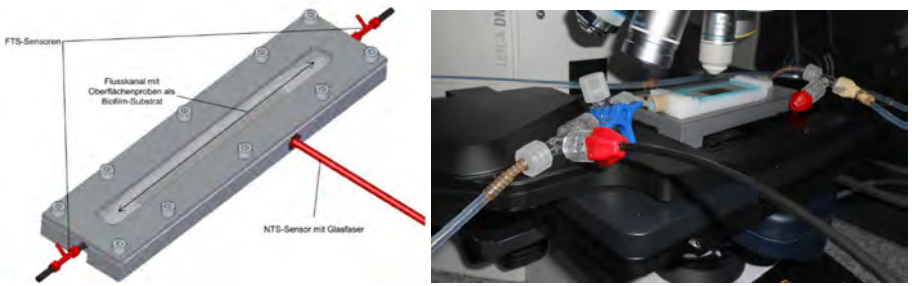

Fig. 1: Set-up for online biofilm monitoring in a CLSM system

Different reaction rates are assessed via HPLC and LC-MS-MS. Based on these data the reaction model is iteratively developed by simulation and fitting. The project is funded by the DFG (UL 170/14-1) and the collaborative research center (SFB) 926 on “microscale morphology of component surfaces” (MICOS).

[P138] STUDYING THE ANTIBIOFILM PROPERTIES OF THE PEPTIDE PEPR USING A BIOPHYSICAL APPROACH

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Infections caused by bacterial biofilms are a major healthcare problem given their reduced susceptibility to conventional antibiotics. Thus, there is a demand for new antibacterial molecules that are active against bacterial biofilms. Antimicrobial peptides (AMPs) have been considered potential alternatives as antibiofilm agents. However, more work is still needed specially to understand their mode of action at the molecular level, which will contribute to their optimization towards drug development. In this work, we studied the antibiofilm activity and mode of action of pepR, a previously developed cationic peptide derived from the dengue virus capsid protein, against Staphylococcus aureus (*S. aureus*) biofilms. The activity of pepR against *S. aureus* in the planktonic form was evaluated using standard microdilution procedures to determine the minimum inhibitory concentration and the minimum bactericidal concentration. Different assays were used to study the activity of the peptide on *S. aureus* biofilm formation and preformed biofilms: the total biomass was quantified using crystal violet staining, and the bacterial cells viability within the biofilm was tested using a resazurin reduction assay. The mechanism of action of pepR against planktonic and biofilms forms of S. aureus was investigated through a combination of different assays including colony count, flow cytometry and confocal microscopy. To assess locally the peptide antibiofilm mechanism of action we used time-lapse confocal scanning laser microscopy. The technique allowed for the simultaneous imaging biofilm structure and followed disruption of cellular membrane integrity along the biofilm. Our results showed that pepR was able to prevent biofilm formation and act on preformed biofilms. The peptide exhibits a fast bactericidal activity against planktonic bacteria, which is related to its ability to disrupt the bacterial membrane integrity as demonstrated by flow cytometry data, and is also involved in its capacity to prevent biofilm formation. Our confocal microscopy data showed that the peptide is able to diffuse through a preformed biofilm and significantly reduce bacterial cells viability in a dose-dependent manner by direct killing of embedded bacteria.

Overall, our studies demonstrate the potential of pepR against staphylococcal biofilms and suggest this peptide as a promising lead towards the development of new antibiofilm agents.

[P139] COMPARISON OF THE PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES OF ATTACHED OR CLUSTERED ALGAE WITH PLANKTONIC ALGAE

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Photosynthetic electron transports of attached or clustered algae were compared with those in a freely living state by the pulse amplitude modulation fluorometry.

Methacrylic fluorescence cuvettes were placed in *Chlorella saccharophila* liquid culture to obtain the formation of algal biofilms on the surface of the cuvettes. Every two weeks, the cuvettes were taken out and the alga attached to the outer surface of the cuvettes was wiped off and photosynthetic electron transport of the attached algae in the inner surface was measured by pulse amplitude modulation fluorometer. Among the parameters indicating the status of photosynthetic activity, ΦII, which is indicative of photosynthetic activity under light condition, of attached alga was low compared to those of planktonic life form. However, ΦII of the attached alga increased as the biofilm developed towards maturation.

After one month of liquid culture, *Chlorella vulgaris* cells sediments in the bottom of the bottle forming cell clusters. The cell cluster samples were carefully transferred to the methacrylic fluorescence cuvettes and the cells were dispersed to the medium. Photosynthetic parameters of the samples were measured daily thereafter. Instead of reconstructing the cell clusters in the cuvettes, the viscosity of the cell samples decreased in time indicating the increase of free living alga. During the experiment, the II increased over time and significant increase was observed after five days from the dispersion. Dispersion may have initiated growth of the planktonic cells. Free-living algal cell may indicate an increased photosynthetic activity than the sedimented cluster of cells. The non-photochemical quenching, a heat dissipation, parameter differed greatly among samples just after the dispersion, but they approached to a similar value in a few days.

From these results, the development of algal biofilms may enhance phototrophic activity of the cells composing the biofilms. However, a mere clustering of the cell did not promote photosynthesis compared to the planktonic state. Hence, the formation of the biofilms may mean more than a concentration of the population.

[P140] RESAZURIN ASSAY FOR HIGH THROUGHPUT ANALYSIS OF ANTIMICROBIAL PROPERTIES OF NANOFIBRE MEMBRANES

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Adhesion of microorganisms and organic matter to membrane surfaces triggers the development of biofilms that can decrease transmembrane flux and affect filtration efficiency, therefore, biofouling control is challenging. High throughput method using resazurin dye (known also as alamarBlue™) as metabolic indicator, was used to assess an antimicrobial (antifouling, anti-biofilm) efficiency of electrospun nanofibre filtration membranes functionalized by quarternary ammonium salts and nanosilver particles. Membrane cuts were placed in 12-wells microplate, inoculated by *Escherichia coli* suspension and incubated at 37 °C for 4 and 24 hours for resazurin assay and cultivation, respectively. Then, resazurin was added, incubated for another 30 minutes. Membranes were removed, and the fluorescence was measured each 30 minutes for 6 hours. To express amount of bacteria in colony forming units per milliliter (CFU/mL), relative fluorescence units were converted through the calibration curve that formulates relation between growth rate as OD_{600} and respective fluorescence of metabolized resazurin. Based on the calibration curve, limit of detection was set to 4.5 logCFU/mL. Cultivation method was also used to relate the results with resazurin assay. The results showed good relation between resazurin and cultivation methods. Membranes with nanosilver resulted in >5 logCFU/mL removal compared to control membranes. Membranes with quarternary ammonium salts were far less efficient with bacterial removal only about 1 logCFU/mL. Compared to standard cultivation method, resazurin assay does not require sonication or vortexing even when working with porous and complex matrices, and results are delivered in shorter time period. Moreover, microplate design is much less laborious compared to work with tubes and agar plates.

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[P141] RAMAN SPECTROSCOPIC DISTINCTION OF SMALL COLONY VARIANT FROM WILD TYPE PHENOTYPE AS NON-DESTRUCTIVE METHOD TO ANALYZE STAPHYLOCOCCUS AUREUS BIOFILMS

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S. aureus is frequently found in biofilm-associated infections on implants (1). Bacteria in biofilms can develop small-colony variants (SCVs) characterized by smaller colonies on solid media due to reduced metabolism. It is assumed that this adaptation mechanism allows the bacteria to persist in the biofilm for a long time. Additionally, SCVs from *S. aureus* are also increasingly formed as intracellular adaptation during long-time infection of specific host cell types (2). To identify SCVs the bacteria are plated on solid media and cultivated for at least two days. Since this method is time-consuming and destructive, it would be advantageous to have a tool to distinguish the SCV from wild type (WT) phenotype directly in the biofilm sample.

Raman-spectroscopy is a non-invasive and label-free method that is based on inelastic scattering of light through different vibrational modes of molecules. The recorded spectra thus represent important biochemical information from the sample. Furthermore, images can be obtained by point-by-point scanning. They can for example be used to detect and analyze *S. aureus* bacteria for their growth state intracellularly in host cells (3).

Here, we would like to present Raman spectroscopy as a tool to differentiate SCVs from WT bacteria in a rapid and non-destructive way. We first selected stable SCV strains that do not revert back to WT from *S. aureus* and their original WT strains for measurement. The obtained Raman spectra were used to build a classification model for discrimination of both phenotypes. We used independent stable SCV and WT strains for testing the model. As result our model was able to readily discriminate both phenotypes with high accuracy and sensitivity.

The SCV-WT discrimination model transferred on biofilm samples by using Raman spectroscopic imaging will provide helpful information about the complexity of the biofilm composition and spatial arrangement.

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[P142] PIXEL RESOLVED MICRORHEOLOGY OF BIOFILMS USING OPTICAL COHERENCE TOMOGRAPHY

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Particle tracking techniques are used to assess the rheological properties and structural heterogeneities of complex fluids. The motion of the particles is captured and used to extract the mean-square displacements (MSD); this gives information about the environment in which the particles reside. Optical Coherence Tomography (OCT) is a useful microscopy tool for biofilm studies, it allows for depth resolved structural imaging with micron level resolutions. With the addition of nanoparticles to the fluid (<1µm), depth resolved particle dynamics can be measured in and around biofilms. In a previous study we investigated the use of OCT to measure the flow around a biofilm. This allowed the estimation of local shear forces over the surface of a biofilm growing in a microfluidic channel [1]. The velocities of the particles were found to be sensitive to small changes on the channel walls. Looking at the flow around biofilms can help detect their locations before any significant growth could be seen with regular OCT imaging; specifically when the biofilm has a refractive index similar to water. To measure the flow around the biofilms it is important to minimize particle-biofilm interactions, otherwise they will "stick" to the biofilm. This was achieved by using coated particles with polyethylene glycol. Currently we are investigating the dynamics of particles trapped in the biofilm. This allows for the estimation of the rheological properties of biofilms growing under different shear stress condition. The dynamic information of the particles is obtained from the autocorrelation function (ACF) at the each pixel of the OCT detector. The ACF contains both diffusive and convective dynamics. The ability of distinguishing between the two depends on their ratio defined as the Péclet number (Pe = convective motion/diffusive motion). For Pe > 1 convection dominates the signal and only velocities can be measured, at Pe < 0.1 diffusive motion dominates. When Pe≈1, both the diffusive and convective signals are present and care should be taken to correct for both.

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[P143] DETACHMENT OF BACTERIAL AND FUNGAL BIOFILMS FOR SPECIES SPECIFIC CELL COUNT USING DITHIOTHREITOL

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Biofilms are communities of one or multiple species adherent to a surface. To estimate species specific cell count in multispecies biofilms, biofilm detachment and subsequent determination of colony forming units (CFU) on selective agar is an appropriate method. Commonly used methods to detach biofilms are sonication as well as scraping. The biofilm detaching capabilities of dithiothreitol (DTT; Sputasol®), which is able to reduce disulfide bonds of proteins, are investigated in this study, revealing the most efficient method for the detachment of fungal and bacterial biofilm.

Five different methods for biofilm detachment were tested on biofilms of the bacteria *P. aeruginosa*, *S. maltophilia*, *S. aureus* and the fungus *E. dermatitidis*: Incubation with 1 g/L DTT for 15/30 minutes under slight agitation, sonication for 10 minutes at 60 Hz with DTT or phosphate buffered saline (PBS) and scraping. Therefore, biofilms were formed over a period of 24 (bacteria) to 48 hours (fungi) on the surface of a polystyrene 24 well-plate. An inoculum was set to 1 x 10^7 and 1 x 10^6 cells/mL, respectively. The biofilm was washed thrice in sterile PBS. A dilution row was prepared after biofilm detachment and two appropriate diluted suspensions were plated in a volume of 100 µL on suitable selective agar plates. After incubation, colonies were counted and CFUs were calculated. Every strain was examined in double determination and every experiment was repeated three times. The means of the double determination were compared to each other using the statistical program GraphPad Prism 6.

Each method applied showed biofilm detachment of viable cells in a method dependent-manner. The least effective and accurate method of *S. maltophilia* and *S. aureus* biofilm detachment was scraping. For *P. aeruginosa* and *E. dermatitidis*, the sonication showed weakest effect. The incubation with DTT was highly effective for the four tested species. The extension of the incubation period from 15 to 30 minutes did not show a significant effect.

On basis of these results, 15 minutes of incubation with 1 g/L DTT was chosen as most appropriate for biofilm removal and subsequent CFU determination.

[P144] OPTIMIZING METHODS FOR BIOFILM ASSESSMENT USING PATHOGENIC BACTERIA FROM CLINICAL SAMPLES ISOLATED IN POLAND

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Bacterial biofilms are structures consisting of extracellular polymeric substances and typically multiple species of bacteria, which form clusters and adhere to surfaces. Compared to non-adhered bacteria, biofilm show a nearly 1,000-fold increase antibiotic resistance, thus being the cause of 80% reported nosocomial infections. Methods to characterize biofilms are most often are carried out on microtiter plates. Crystal Violet (CV) staining for biofilm quantification remains the most frequently used as well as the fluorometric-ones to assess cell viability (e.g. the resazurin assay). By assessing these methods and others, and optimizing the parameters, the aim of this study was to retrieve multiple data points from one sampling well by using combinations of different assays, and to find other simple tools that can provide information about bacterial biofilm. The combination of resazurin and CV assays within the same well has been previously reported, but for low biofilm formers, e.g. *Enterococcus faecium* strains, it does not work well, resulting in loss of biofilm mass after incubation with resazurin reagent followed by its removal. CV assay was used as our method of choice to determine total biofilm mass formed by *Staphylococcus aureus*, *Enterococcus faecalis* and *E. faecium*. As a result, those strains were classified in strong, intermediate and low biofilm formers, and compared to results obtained with the other assays. An innovative tool was used for biofilm mass quantification: scanning of biofilm wells, followed by image analysis using volume tool of Quantity One software. Results obtained showed a similar pattern compared to CV assay. The resazurin assay was modified in order to be performed on resuspended biofilm (versus the conventional method in which the reagent is applied on biofilm adhered to the bottom of wells) so determination of viable cell numbers could also be done from the same sample. In this study we demonstrated that biofilm measurement can be done using volume tool present in standard image analysis software. We also showed that multiple data points e.g. biofilm mass (by volume measurement), metabolic cell rate (by resazurin) and enumeration of viable cells (by colony forming unit determination) can be collected from one sampling well.

[P145] DIRECT IDENTIFICATION OF FUNCTIONAL AMYLOID BY QUANTITATIVE MASS SPECTROMETRY

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Amyloids are highly ordered fibrillar protein polymers used by organisms from all domains of life due to their exceptional properties. We have previously shown that amyloids are widespread among microorganisms in biofilms from various habitats. They are therefore believed to play important roles in biofilm ecology. Despite their common appearance in biofilms, few amyloids have been characterized from biofilm-associated bacteria. However, the few amyloids that have been studied so far have already provided an astonishing demonstration of how the amyloids can be exploited with roles ranging structural components of biofilms, cell envelopes and spore coats to cytotoxins and as reservoirs for quorum-sensing signaling molecules.

The identification of novel functional amyloids is key to understand the many roles of amyloid in biofilms. Isolation of amyloids is unfortunately not a straightforward task. The insolubility and extreme stability of most functional amyloids exclude them from traditional protein analyses. Many functional amyloids are also highly adhesive and therefore bind to pipette tips and other consumables. Pure cultures, large sample volumes and high productivity of amyloids are therefore required for successful purification. We here present a quantitative proteomics technique that allow direct identification of functional amyloid candidates in complex samples based on their structural stability in the presence of increasing concentrations of formic acid.

[P146] VIBRATIONAL SFG SPECTROSCOPY SHEDS NEW LIGHT ON BACTERIAL ADHESION ON CONDITIONED SURFACES WITH ANTI-MICROBIAL PEPTIDES

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These last years, specific surface coatings able to prevent bacterial adhesion are a very active topic of research which may concern many applications (food industries, health, environment, etc.). In this context, bioinspired strategies, such as the use of antimicrobial peptides (AMP), are recognized as promising. These compounds have a broad spectrum of antibacterial, antiviral, antifungal and antiparasitic activity. They are usually active at low concentrations and can act in synergy with antibiotics. In this study, the AMP Nisin Z was covalently tethering on acid-terminated self-assembled monolayers (SAMs). The acid-terminated SAM (MUA) was either concentrated, or four time diluted with hydroxylated thiol (MUA25). As expected, both conditioned surfaces were efficient against *Staphylococcus aureus* contamination (less bacterial adhesion and increased bacterial mortality), but surprisingly higher antibacterial activity was measured for MUA25-Nin Z coated surface (90% inhibition of bacterial adhesion). This raises the interest in a better understanding of the interaction between the bacteria and the surface at the molecular scale. For this purpose, the vibrational Sum Frequency Generation (SFG) spectroscopy, a second order non-linear optical method, appears highly appropriate for probing ordered surfaces and interfaces in *in situ* conditions (e.g., in aqueous environment in order to preserve biomolecules structure, functionality and viability). It consists in overlapping in time and in space onto the sample a visible beam (800 nm) and an infrared beam corresponding to the wavelength of molecular vibrational bands of interest, and to detect the spectrum of the emerging beam at the sum-frequency wavelength. We have used the wavelength tunability capabilities of our device to probe C-H bonds of MUA as well as amid I and II bands of the AMP Nisin Z. Results will be presented, showing the sensitivity of SFG to the peptide film. This approach was a mandatory step to improve peptide-tethering strategy and thus to obtain their maximum effectiveness.

[P147] NOVEL METHOD FOR TRANSCRIPTION FACTOR PURIFICATION AND CHARACTERISATION PROVIDES THE FIRST INSIGHT INTO THE TRANSCRIPTIONAL REGULATION OF THE FAP OPERON RESPONSIBLE FOR AMYLOID BIOGENESIS IN PSEUDOMONAS

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The functional amyloid *of Pseudomonas* operon *(*fapA*-F)* encodes the molecular machinery for amyloid fibril biogenesis, and it is found in multiple *Pseudomonas* species. Expression of the Fap system leads to highly aggregative phenotypes that produce very stable biofilms, which may have consequences, e.g. for the establishment of chronic infections. There is currently no information about how transcription of the Fap system is regulated. To gain in-depth knowledge regarding the regulation a new method for isolation and identification of transcription factors was developed. Biotinylated double-stranded DNA fragments, corresponding to overlapping regions of the *fap* promoter, were used as probes for affinity purification of transcription factors, which were then analysed with label-free quantitative MS/MS. The analysis of samples purified in the presence of various concentrations of competitor DNA and eluted with increasing concentrations of NaCl allowed not only identification of the transcription factors but also characterisation of their specificity and binding strength. Using the method, we identified multiple transcription factors that bound specifically to the *fap* operon, and due to the sensitivity of the MS/MS analysis, we were even able to characterise the composition of the transcription machinery. The method is universal and can be used to identify DNA-binding proteins for other systems as well.

[P148] A META-PROTEOMICS APPROACH TO IDENTIFY INTER-SPECIES INTERACTIONS DURING BIOFILM DEVELOPMENT IN SIMPLE MICROBIAL MODEL COMMUNITIES

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Microbial biofilms are omnipresent in nature and relevant to a broad spectrum of industries ranging from bioremediation and food production to biomedical applications. To date little is understood about how multi-species biofilm communities develop and function on a molecular level, due to the complexity of these biological systems. Here we apply a meta-proteomics approach to investigate the mechanisms influencing biofilm formation in a model consortium of four bacterial co-isolated soil isolates, which has previously been shown to interact synergistically; *Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydans* and *Paenibacillus amylolyticus*. Biofilms of mono- and four-species cultures were cultivated in drip flow reactors to allow biofilm formation under low-shear stress and in the air-liquid interphase. To obtain full taxonomic resolution on proteomic profiles between closely related species in the biofilm and to enhance protein quantification, we developed a pipeline for generating reduced reference proteomes for spectral database searches. By performing comparisons of proteomic profiles from mono- and four-species biofilms, we identified occurring inter-species interactions and the resulting changes in active metabolic pathways. By example, meta-proteomics profiling indicated that community development was influenced by cooperative interactions between community members facilitating e.g. cross-feeding on specific amino acids. However, opposite regulation patterns of fermentation and nitrogen pathways in *Paenibacillus amylolyticus* and *Xanthomonas retroflexus*, also indicated that competition for limited resources and/or niche separation influenced community development. By sampling developing four-species biofilms over time and comparing different time points we were able to show that these inter-species interactions developed over time during biofilm growth.

[P149] ADVANCED SINGLE MOLECULE OPTICAL MICROSCOPY FOR THE INVESTIGATION OF COAGULASES IN STAPHYLOCOCCUS AUREUS BIOFILMS

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Staphylococcus aureus is a leading cause of implant associated infections, which are notoriously difficult to treat using antibiotics. The ability of *S. aureus* to form biofilms is a major cause of its pathogenicity, so it is important to understand the fundamental molecular mechanisms behind the extracellular matrix formation. A bespoke fluorescence microscope capable of imaging single molecules inside biofilms is under development, which will be used to address questions regarding the production, localization, and roles of extracellular matrix proteins during biofilm formation.

Single molecules inside biofilms are challenging to image using fluorescence microscopy: samples have a large thickness, and the optically heterogeneous environment strongly scatters light and lowers the signal to noise ratio. The new microscope features transverse illumination, which reduces background noise from out of focus fluorescence. Samples will be excited using a single non-diffractive, self-reconstructing Bessel beam instead of a traditional Gaussian profile laser beam, giving an improved depth of imaging in a strongly scattering sample, and increasing the signal to noise ratio to enable single molecule detection on a millisecond timescale.

The microscope will be used to investigate the formation of fibrin, which is the key matrix component in *S. aureus* biofilms *in vivo*. *S. aureus* produces two coagulases to trigger fibrin formation: Coagulase (Coa) and von Willebrand factor binding protein (vWbp). Fluorescent fusion proteins of Coa-mCherry and vWbp-GFP have been developed, and they will be visualized in real-time with single-molecule resolution to study their production and localization during biofilm development, and to determine the different roles that these two coagulases play in biofilm matrix formation.

[P150] INVESTIGATION OF THE INFLUENCE OF FLOW ON THE PH MICROENVIRONMENT IN UREOLYTIC BIOFILMS

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Ureolytic bacteria produce urease enzymes that catalyse the degradation of urea resulting in formation of ammonia and carbon dioxide. The process is often accompanied by a raised pH that drives the formation of mineral precipitates. Potent ureolytic organisms include *Streptococcus salivarius* present in dental plaque biofilms and *Proteus mirabilis* implicated in catheter biofilm infections. To date laboratory investigations of enzyme activity in these species has mainly involved bulk measurements of analytes in the effluent or rough spatial mapping with pH microelectrodes. However, the chemical microenvironment can be different in the biofilm as a result of local heterogeneities in the enzyme activity and the composition of the biofilm. In addition, the presence of fluid flow may influence the pH gradient in the biofilm. Given that pH plays such an important role in processes including biomineralisation, methods are now emerging for the 3d spatial mapping of the pH microenvironment in biofilms.

Here we investigate *P. mirabilis* biofilm formation in a controlled flow environment with shear rates that reflect the urinary flow rate typically obtained in catheters. The formation of biofilms with various laboratory and clinical strains was compared and the effect of the shear rate on the biofilm density was determined. We present preliminary results on the spatial mapping of the pH in these biofilms and correlate the results with the expression of urease. Our laboratory results are supported by 3D reaction-diffusion-advection (RDA) computer simulations to determine the influence of the spatial variation of enzyme activity on the pH in the biofilms. We found that the local pH in the biofilm could reach high values of pH > 10 when the bulk solution pH remains acidic, resulting in the initiation of crystallisation before the urinary pH is raised. Our goal is to develop an early warning system which provides an indication of a local pH increase as a result of ureolytic biofilms in flow environments when visualisation is not possible (eg in a catheter tip).

[P151] IMAGING O2 DISTRIBUTION IN A NEW IN VITRO MODEL SYSTEM FOR CHRONIC INFECTION BIOFILMS

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Biofilms involved in chronic infections typically consist of relatively small (~20-200 µm wide) cell aggregates suspended in host material and surrounded by polymorphonuclear neutrophil immune cells that impose strong O_2 depletion and slow growth of the pathogenic bacteria. However, most *in vitro* studies of biofilms involve use of microtiter plate assays or biofilm growth in flow chambers, which do not reproduce the mentioned *in vivo* growth patterns. We recently introduced growth of pathogenic bacteria (*Pseudomonas aeruginosa*) in alginate beads as a simple model system for studying biofilms under *in vivo*-like conditions. The system shows *in vivo*-like growth of pathogenic bacteria, enables application of a wide range of experimental methods at high reproducibility and replication, and allows easy experimental modulation of gradients of substrates and electron acceptors and exposure to antibiotics. We present the model system, with special emphasis on novel ways to map the distribution of the key electron acceptor O_2 in the alginate bead model system, using embedded O_2 sensitive sensor nanoparticles in combination with ratiometric luminescence imaging. Such measurements can now be combined with microscopic measurements of bacterial biomass enabling novel high-resolution studies of the growth and activity of pathogenic bacteria under *in vivo*-like conditions.

[P152] PERFORMANCE OF A GLOW LUMINESCENCE-BASED ATP VIABILITY ASSAY FOR THE QUANTIFICATION OF STAPHYLOCOCCUS AUREUS BIOFILMS AND PERSISTER CELLS

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Viability determination based on ATP (adenosine triphosphate) is an established technique in microbiology work used, e.g., for antimicrobial screening. A typical assay consists of a lysis buffer and a luminogenic reagent mix of luciferase and its substrate. ATP, a cofactor for the reaction, is liberated from living cells upon lysis generating light output relative to its concentration.

Despite the widespread usage of these assays there is somewhat less experience on applying such methods for intact biofilms. In antimicrobial screening assays involving the cultivation of bacterial suspensions in microtiter well-plates, the presence of biofilm is practically a given after hours of incubation. Since a notable fraction of bacteria may have converted into biofilm growth prior to the endpoint measurement, the phenotype should be considered when validating such assays. Another relatively uncharted phenotype of interest for applying high-throughput viability determination to is known as persisters – non-reproducing cells with low metabolic activity.

Our focus has been on the development of novel methods and workflows for viability testing in biofilms and persisters. As a part of this work we have sought to explore and validate luminometric ATP assays in these more unusual settings with the common biofilm pathogen *Staphylococcus aureus*. Using previously identified anti-biofilm and anti-persister agents, we assessed the ability of a commercial ATP-based assay to detect changes in viability in both biofilms and persister cultures. We also studied the effect of mechanical disruption of the biofilms on the luminescence signal output.

Differences in viability were variably detected between test settings. The results indicate that this assay is not very sensitive for the ATP content in biofilms, and the lysis reagent cannot effectively reach the cells within. The disruption of biofilms and even the delicate manipulation of static, persistent cultures affected the measured signal levels dramatically. We conclude that assay performance depends heavily on the phenotypes present, which should be considered in routine work. Still, the impressive overall sensitivity of ATP-based assays makes them a promising tool for studying persister bacteria, in which the inherently low level of metabolism limits the usefulness of other types of indicators.

[P153] BIOFILM MATRIX VISUALISATION

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The extracellular matrix represents an essential part of microbial biofilms and bio-aggregates. The matrix acts as a multi-functional structure and is attributed to be of high importance in different stages of biofilm development. Nevertheless the matrix remains a challenge in many respects. The issues comprise the (1) biosynthesis, release and identity; (2) structural and biochemical analysis; (3) characterisation by -omics; (4) interactions and reactivity; (5) mechanics and dynamics; and (6) control and degradation. Focus of this discussion poster will be on matrix presence – Yes/No, and matrix imaging – laser scanning microscopy/optical coherence tomography. For this purpose selected 3-dimensional image data sets will be used to demonstrate several critical aspects of matrix investigations and techniques. In view of this process, these two most popular imaging approaches will be compared and evaluated. In addition, emerging ideas on matrix staining and visualisation will be elaborated and rated. In summary, this contribution intends to raise the controversy on the in situ biofilm matrix related to presence, characterisation and structure. Furthermore, it is intended to push the envelope in order to reveal thoughtful ideas and new approaches in biofilm matrix research.

[P154] DEFINING CONDITIONS FOR BIOFILM INHIBITION AND ERADICATION ASSAYS FOR GRAM-POSITIVE CLINICAL REFERENCE STRAINS

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Biofilms are formed by a complex bacterial community encapsulated by a polymeric matrix, with strong adherent and persistent phenotype. Biofilms are considered one of the most challenging areas of modern medicine, accounting for over 80% of hospital-acquired infections. Existing antibiotics have been developed against free-floating bacterial cells, thus many treatments of biofilm-linked infection fail. In this study, we compared the effects of different media on biofilm growth of clinical reference strains of *Staphylococci* and *Enterococci*, including multi-drug resistant representatives. Further we optimized the resazurin-based assay for determining the minimal biofilm inhibitory and eradication concentrations (MBIC and MBEC) of standard antibiotics. We showed that tryptone soy broth supplemented with 1% glucose was an optimum media for an average maximum biofilm growth of all strains tested, with an extended incubation time for *Enterococci*. A range of parameters was tested for the resazurin assay including concentration, temperature and time of incubation. Using quality parameters to analyze the assay's performance, the conditions for the resazurin assay were set as follows: 4 µg/mL and 8 µg/mL, with incubation at 25°C for 20 min and 40 min for *Staphylococci and Enterococci*, respectively. In summary, we defined conditions for optimum biofilm growth and for standardized resazurin assay for MBIC and MBEC determination against six Gram-positive clinical reference strains.

[P155] TARGETING INTRACELLULAR BACTERIA WITH SUPRAMOLECULAR ANTIMICROBIAL "NANOBULLETS"

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Introduction Bacterial infections are common disease in human beings. Although endless effort has been spent to combat infections, complex and persistent infections, e.g., intracellular infections[1], are still emerging. With these infections, the bacteria can survive and persist inside the professional phagocytic cells, e.g., macrophages and neutrophils[2], as well as non-professional phagocytes e.g., epithelial and endothelial cells[3]. These bacteria shield themselves from the host immune system and antibiotic treatment, which may cause antibiotic resistance and induce chronic and recurrent infections[4]. To address this problem, intracellular delivery of antimicrobial peptides via a supramolecular method is proposed to treat the intracellular bacterial infections.

Experiments and results To this end, supramolecular ureidopyrimidinone (UPy)-based materials were used as intracellular carriers since they have been successfully used for intracellular delivery purpose[5]. The antimicrobial peptide was coupled onto the UPy molecules to form functional UPy-assembly. Antimicrobial "nanobullets" were obtained through assembling of the functional UPy-assemblies in aqueous phase. The formation, ζ-potential and morphology of these nanobullets were fully characterized. These "nanobullets" are rod-like fibrils and can be internalized by HK-2 cells and THP-1 derived macrophages.

Conclusion Supramolecular antibacterial "nanobullets" containing antimicrobial peptide has been successfully prepared. These "nanobullets" can be internalized by professional and non-professional phagocytes, which indicates their potential to treat intracellular infections.

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[P156] UTILIZATION OF BIOFILM DISPERSAL AS A THERAPEUETIC STRATEGY TO ERADICATE COLISTIN RESISTANT STRAINS

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The potential of biofilm dispersal to restore antimicrobial *in-vitro* efficacy against 1-day biofilms formed by a colistin resistant strain is evaluated. *P. aeruginosa* PAO1 strain containing the arabinose-inducible p_{BAD}-*yhjH* plasmid has evolved to resist high levels of colistin and is characterized. The strain morphology, virulence, susceptibility, and resistance profile are examined as compared to the parent strain. Whole genome sequencing and analysis revealed point mutations in *phoQ* and *lpxC*, two genes involved in the biosynthesis of the lipid-A located in the bacterial membranes and the biological target of colistin. Other mutations included genetic alterations in *tpbA*, causing an alteration to the production of eDNA in the biofilm matrix. This change is suspected to reduce the penetration of the positively charged drugs; results were further confirmed with experimental data.

The colistin-resistant derivative exhibited an altered drug susceptibility profile, with increased susceptibility to carbapenems and glycopeptides: "The seesaw effect", heterogeneous resistance and biofilm dispersal principles are further explored. In this context, we demonstrated that following induced dispersal, the seesaw effect is facilitated, enabling a synergistic effect between colistin and vancomycin, to eradicate the resistant biofilms. The results show an example how the development of resistant bacteria populations could be prevented. This change is suspected to reduce the penetration of the positively charged drugs; results were further confirmed with experimental data. Of interest, colistin plus vancomycin (at clinically relevant concentrations) demonstrated a synergistic effect only against the dispersed biofilms of the colistin resistant strains.

The results of this work indicate the enormous potential for biofilm dispersal as a therapeutic strategy for efficient treatment of biofilm-associated infections. Further developments in novel biofilm dispersal agents represent a promising therapeutic strategy, against both susceptible and resistant bacterial biofilms, when used in combination with existing antimicrobial therapies.

[P157] BIODEGRADABLE AND ANTIBACTERIAL P4HB FILMS TO TREAT AND PREVENT S. AUREUS INFECTION

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For the reduction of bacterial infections in wounds it is advantageous to have a slow but sustainable release of antibacterial active compounds. Herein poly(4-hydroxybutyrate) (P4HB) produced by *Escherichia coli* was selected because it possesses excellent biocompatibility and its biodegradation takes place via surface erosion rather than burst degradation known for other often used biopolymers. P4HB was fabricated to films together with different metal protoporphyrine complexes such as gallium, copper and zinc, as antibacterial agents. The fabricated films were first analyzed for the release of incorporated metal ions. In the next step the antibacterial properties of the films against methicillin resistant *Staphylococcus aureus* were examined by in the standard method ASTM2180 where bacterial cells are in direct contact with surfaces. The antimicrobial activity of the lixiviates released from the films were also tested by Minimal Inhibitory Concentration (MIC) susceptibility test, to co-relate the released metal ion and antibacterial effect. It was found that the film incorporated with gallium complex showed excellent antibacterial activity even after 1 week incubation.

[P158] ARSENIC-RELATED MICROORGANISMS IN AQUATIC ENVIRONMENTS: DISTRIBUTION AND EXPLOITATION IN BIOREMEDIATION

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Arsenic (As) is one of the most toxic and widely distributed element in the environment. Aquatic microorganisms are able to resist to high As concentration and/or metabolize it, with metabolic potentialities suitable for biotechnological applications. In particular, microbiological As(III)-oxidation is one of the most promising application as a precursor step in As removal from contaminated groundwater, since conventional iron-based treatments are more effective in removing As(V) rather than As(III). This work is aimed to (i) investigate the identities and metabolic potentialities of microorganisms occurring in As-rich aquatic environments (e.g. groundwater, freshwater and geothermal waters) and (ii) optimize the biological pre-treatment of As-contaminated waters in column systems (biofilters). Next Generation Sequencing and CARD-FISH technique were applied to describe microbial community structure in As-contaminated waters. The analysis highlighted a marked diversity between samples and revealed the occurrence of novel thermophiles in geothermal environments. Furthermore, the abundance of functional genes involved in bacterial As-resistance was estimated by qPCR and the main microbial functional groups were evaluated by MPN (Most Probable Number) through selective growth media. Additionally, the main parameters affecting the As(III)-oxidation process in engineered systems were tightly evaluated on different filling materials (i.e., coarse sand, fine sand and sintered glass rings) under a variety of operating conditions (e.g. flow rates, column volume, different As(III) concentrations, use of biofilm at different maturation stages). Biofilm samples were taken at the end of the kinetic experiments and subsequently microbial communities composition were investigated using high-throughput sequencing. The tridimensional structure and microbial colonization on filling materials were assessed using CARD-FISH technique in combination with Confocal Laser Scanning Microscopy. This study confirmed the high potentialities of microbially-mediated As(III)-oxidation process in water treatment and assessed the impact of inflow water quality and hydraulic conditions to efficiently scale-up the laboratory set-up for long term/high volume field application.

[P159] SYNTHETIC BIOLOGY APPROACHES FOR ENGINEERING PSEUDOMONAS PUTIDA CATALYTIC BIOFILMS

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Even though biofilms are the most common form of bacterial growth in nature, industrial fermentations rely almost entirely on planktonic lifestyle. Biofilms, however, offer several advantages to be exploited in modern fermentation processes. Bacteria in biofilms are more tolerant to several stresses than free cells, including toxic chemicals and shear stress. Furthermore, the adhesion of cells to a surface can be used to operate a continuous fermentation process without excessive loss of biomass, thereby facilitating downstream processing. So far, *catalytic biofilms* have exploited the natural ability of bacteria to form such structures, and switchable genetic systems to control bacterial adherence at the user's will would be desirable. Here, we use *Pseudomonas putida* to engineer this switchable system. *P.putida* is a robust soil bacterium for biotechnology as it can thrive under harsh operating conditions, has high tolerance towards several chemicals, and is able to generate NADPH at high rates. These characteristics make *P. putida* a promising *chassis* for the production of a broad spectrum of chemicals. Biofilm formation in *P.putida* is mainly controlled by the trigger signal cyclic diguanylate (c-di-GMP). A network of di-GMP phosphodiesterases (PDE) and diguanylate cyclases (DGC) adjusts the level of c-di-GMP according to internal and external stimuli, like quorum sensing and carbon starvation. Therefore, we decoupled the activity of PDE and DGC from their native regulation network and integrated them into a synthetic circuit. Furthermore, we used a feedback resistant DGC from *Caulobacter crescentus.* Through this engineering, biofilm formation and dispersion was controllable by addition of cheap inducer molecules such as 3methylbenzoate. Upon activation of the system in *P. putida*, biofilm formation increased 8-fold after 18 hours of shaken cultivation. Future endeavors will focus on rendering biofilm formation and dispersal controllable through a toggle switch, thereby circumventing the need of permanently present inducer in the medium.

[P160] FLOW CYTOMETRY ANALYSIS OF MOUTHWASH FORMULATIONS TO INVESTIGATE THE MECHANISMS OF ROBUSTNESS AGAINST MICROBIAL CHALLENGE

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Mouthwash is a complex formulation for cleansing the mouth and teeth, acting as a liquid vehicle for the delivery of active agents to the oral cavity. As the market and regulatory agencies shift towards demand of fewer preservatives in products, a fundamental understanding of the key drivers preventing contamination by environmental bacteria is needed for the development of micro-robust products. Specifically, an understanding of how different formulations and ingredients are affected by processing conditions can help develop improved manufacturing processes that ensure the prevention of microbial contamination. Developing a clear understanding of how the micro robustness of the product is impacted is critical to improving the speed to market of quality products.

The majority of the published research on mouthwash has focused on the efficacy of oral care products in the mouth and effects on oral microbiota. However there is little publically available research on mouthwash formulation and preservation against environmental bacteria. In this study, flow cytometry was used to investigate the effect of different mouthwash formulations on environmental bacteria that could be introduced during manufacturing. Flow cytometry is a rapid technique for studying particles using scattered light and fluorescence. Using fluorescent dyes with specific binding characteristics, flow cytometry can be used to rapidly enumerate bacteria and determine bacterial physiology and viability.

Flow cytometry analysis of environmental bacterial species showed a high number of dead cells immediately after treatment with different concentrations of four different mouthwash products. There was an increase in the mean forward scatter of samples incubated with two mouthwash products, indicating an increase in particle size. These two mouthwash products had common ingredients not present in the other mouthwash products tested and likely responsible for the aggregation of bacteria. An understanding of how such aggregates impact robustness can provide direction for future processing, sanitization, and formulation recommendations.

[P161] IDENTIFICATION OF GENES INVOLVED IN BIOFILM-SPECIFIC RESISTANCE AGAINST ANTIMICROBIALS IN PSEUDOMONAS AERUGINOSA

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Bacteria living in biofilms tolerate much higher antibiotic concentrations compared to planktonic bacteria and survive long enough to evolve antimicrobial resistance (AMR). Biofilms can cause persistent, hard-to-treat infections and exhibit an intrinsic biology that promotes the development and transmission of AMR. The goal of this project is to improve our understanding of the phenotypic adaptation of biofilms against antibiotics and antimicrobial surfaces, using *Pseudomonas aeruginosa* as a model organism. *P. aeruginosa* is one of the ESKAPE pathogens, known to be multi-drug resistant bacteria responsible for many infections. In order to identify biofilm-specific resistance genes, a *P. aeruginosa* transposon-mutant library is screened in presence of antibiotics, antimicrobial surfaces and a combination of both. Gene expression of wild type *P. aeruginosa* is analyzed via RNA sequencing in relation to different antibiotic exposure in order to unveil potential pathway of resistance. Another set of screening is focused on key genes related to bacterial surface sensing and biofilm formation toward antibacterial materials. By doing so, we expect to elucidate the induction of AMR of biofilms in response to materials. This work will help us with understanding the contribution of biofilms to AMR acquisition and will facilitate the development of novel antimicrobial strategies and medical devices that are more effective in preventing biofilm-associated infection and AMR.

[P162] ESTABLISHMENT AND CHARACTERIZATION OF A LABORATORY MODEL OF A MIXED SPECIES MICROBIAL SKIN CONSORTIUM

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The commensal skin microbiota plays an active role in maintaining skin health and function. Several clinical phenotypes are associated with an imbalance in the relative proportions of these microbiota including, atopic dermatitis, psoriasis, and dandruff. For example, dandruff, a scalp disorder that is characterized by abnormal flaking and irritation, is correlated with higher incidence of *Malassezia restricta* and *Staphylococcus epidermidis*, and lower incidence of *Propionibacterium acnes* compared to healthy scalp ($p<0.05$). Despite considerable advancements in the field, we have limited knowledge on how the skin microbes interact with each other, and with the host, to establish and maintain a stable equilibrium in relative abundance that could play an important role in defining the skin/scalp phenotypes. Therefore, it is crucial to ascertain the mechanistic basis of such interactions to understand the equilibrium that will help designing novel interventional strategies.

To study the mechanisms by which skin microbes maintain a stable community, we have developed a robust and reproducible microbial community with three major skin microbial species: *S. epidermidis*, *P. acnes* and *M. restricta*. We have established growth conditions that allow co-cultivation of all three microbes that form a mixed-species biofilm, and have standardized methods to label, quantify, and track their relative abundances within this biofilm community. One intriguing observation is that the mixed-species community produces significantly higher biofilm biomass than the mono-species biofilms. Dissection of this collaborative behavior revealed both cooperative and antagonistic interactions between different members of this community. Our data further suggests that the relative proportions of each member may modulate the growth of other community members. Based on these results, we have defined the interaction network and gained a detailed understanding of the community dynamics of skin microbes, which could be further modulated by varying environmental parameters. This *in-vitro* model system forms the basis for development of a fully characterized skin microbiota model that could help develop novel microbiota-targeted therapies.

[P163] IMPACT OF SUBSTRATUM PHYSICAL PROPERTIES ON PSEUDOMONAS AERUGINOSA BIOFILM GROWTH UNDER MANUFACTURING HYDRODYNAMIC CONDITIONS

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The growth of microbial biofilms on process manufacturing surfaces is a significant industrial problem. Due to their progressive accumulation and resistant phenotype, biofilms are responsible for substantial losses in industrial productivity, as well as product and capital equipment damage. Despite their negative impact on manufacturing processes, industrial surfaces that prevent biofilm deposition and growth are lacking. There is a lack of knowledge of the key physical, biological and chemical mechanisms that drive bacterial attachment to industrial surfaces and biofilm development under manufacturing hydrodynamic conditions. To understand how the substratum physical properties affect bacterial attachment and biofilm formation under manufacturing conditions, biofilm growth on different industrially relevant materials (metal and plastics) was investigated. To mimic roughening that occurs during manufacturing processes, coupons of metal (stainless steel) and plastics (polycarbonate, polyethylene, and polypropylene) with different physical properties (categorised as smooth, rough and very rough) were used. The physical properties of the coupons (in terms of roughness, surface free energy for bacterial attachment, wettability, and coefficient of friction) were qualitatively characterised by using interferometry, goniometry and tribometry. *Pseudomonas aeruginosa* ATCC 15442 biofilms were grown on the coupons under manufacturing hydrodynamic conditions (wall shear stress of 0.07 N m^{-2}, bulk linear velocity of 0.8 m s^{-1} and Reynolds number of 6383), using the CDC biofilm reactor. The biofilms were visualised by confocal microscopy and the biomass volume per area quantified by COMSTAT2. Despite no significant differences (with 95% confidence) in the biomass volume per unit area of *P. aeruginosa* biofilms developed on the smooth, rough and very rough surfaces after one and two days of growth, after three days of biofilm growth, biomass was significantly (with 95% confidence) higher on the rough and very rough compared to the smooth coupons. Therefore, it seems that depending on the physiological state of the biofilm, the substratum physical properties (in terms of roughness) can play a role on *P. aeruginosa* biofilm growth dynamics. Further investigations on the biological and chemical mechanisms of *P. aeruginosa* attachment and biofilm growth will allow the development of a mechanistic model of *P. aeruginosa* biofilm formation under manufacturing hydrodynamic conditions.

[P164] PERFORMANCE OF NOVEL MICELLIC PLANT EXTRACT FORMULATION USING EMBEDDED HYDROGEN PEROXIDE ON EXPERIMENTAL BIOFILMS

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Background: Bacteria living in biofilm communities are protected from detrimental influences including eradication by antimicrobial compounds. In water systems as well in medical devices used in healthcare, bacterial biofilms pose a major challenge due to the possibility of accumulating and harbouring pathogenic bacteria, and spread of infection. Currently, chemical elimination of bacteria in biofilms requires environmentally toxic antimicrobial compounds or application of better tolerated antimicrobials such as reactive oxygen compounds at high concentrations and long application times. We investigated the activity of a novel micellic plant extract formulation using embedded hydrogen peroxide on environmental biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, two relevant pathogens commonly responsible for infection in healthcare settings.

Materials/methods: The efficacy of the novel formulation was tested against biofilms formed by reference strains of *S. aureus* ATCC 29213, *S. epidermidis* DSM3269 and *P. aeruginosa* PA01. The novel micellic based formulation contained 17% v/v hydrogen peroxide, 2% v/v lactic acid, 0.3% v/v plant extracts and water. The $\log_{10}$ reduction of bacterial test strains in biofilm was measured at 1%, 3%, 5%, 25% and 100% of the initial biocide concentrations and after 1, 3, 5, 15, 30 and 60 min exposure time. Subsequently, using crystal violet staining the optical density (OD) of biofilm mass eradication was recorded. The measurement of minimal inhibiting concentrations (MIC) of the new biocide on planktonic cells of the reference strains completed the investigation.

Results: The preliminary testing on mature, 24-hours old biofilms of *S. epidermidis*, *S. aureus*, and *P. aeruginosa*, achieved after 5 min exposure to 100% biocide a complete killing (log reduction > 8 log10) of viable bacteria embedded in biofilm. Further fine-tuned investigation showed that 25% dilution of the tested biocide eradicate all bacterial strains in biofilms after 1 min (log reduction > 8 log10) and a 10% dilution achieved the same result after 5 minutes of exposure. However, the biofilm thickness measured with the OD did not show any change.

Conclusions: A 10% concentration of a novel micelle based biocidal formulation was able to eradicate viable bacteria in a magnitude of 8 log10 within 5 minutes from 24-hours old biofilms.

[P165] BIOFILM REMOVAL BY VANCOMYCIN AND TOBRAMYCIN WITH DIFFERENT BIOFILM AGE, MEDIA AND EXPOSURE TIME

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Osteomyelitis is an inflammatory process with bone destruction caused by infecting microorganisms. Chronic osteomyelitis is especially difficult to treat due to biofilm formation. The common treatment includes antibiotics therapy and surgery. Although a lot are done to investigate the effect of antibiotics removing biofilms, it is difficult to compare those results due to various test conditions and different biofilm models. In this study, we investigated the possibility of eradicating biofilm *in vitro* by using vancomycin and tobramycin in clinically relevant concentrations and tested influence of biofilm age, growth media, and antibiotics exposure time on biofilm removal.

Staphylococcus aureus and *Pseudomonas aeruginosa,* the common pathogens in osteomyelitis, were tested with vancomycin and tobramycin, respectively. We used MBEC™ assay for growing both 24 hours and 72 hours old biofilms. The biofilms were exposed to different doses of antibiotics (vancomycin: 1.25 mg/L to 3000 mg/L; tobramycin: 0.31 mg/L to 80 mg/L) for 1, 2, 4 and 7 days in Tryptic Soy Broth (TSB) or Cation-adjusted Mueller Hinton Broth (CAMHB). Minimum inhibitory concentration (MIC), minimum biocidal concentration (MBC) and biofilm removal efficacy were determined in each test.

We found that choice of media influences biofilm removal, i.e. biofilms were more difficult to remove in TSB than in CAMHB ($p<0.001$ for both strains). Furthermore, 72 hours biofilms were more difficult to remove than 24 hours biofilms ($p=0.001$ for *S. aureus*, $p<0.001$ in *P. aeruginosa*). Prolonged exposure of *S. aureus* biofilm to vancomycin significantly improved removal ($p<0.001$). For *S. aureus* biofilms, two-day treatment is required to raise the biofilm removal efficacy in CAMHB, while four-day treatment is required in TSB. On the contrary, prolonged exposure to tobramycin did not affect *P. aeruginosa* biofilm removal. The data indicated that it was possible to eradicate local *S. aureus* biofilm infection with high local dose of vancomycin if required treatment duration was ensured. It was also possible to eradicate local *P. aeruginosa* biofilm infection if enough local dose of tobramycin was provided.

[P166] ASSESSMENT OF ANTI-BIOFILM TREATMENTS IN VIVO USING THE GALLERIA MELLONELLA BRISTLE IMPLANTATION MODEL

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The *Galleria mellonella* infection model has shown increasing popularity among the non-mammalian infection models in the last years. However, antimicrobial drug testing has been so far only studied in planktonic, but not biofilm-embedded bacteria in this model. We therefore expanded the biofilm toothbrush bristle implantation model established by Benthall *et al.* using a novel research grade *G. mellonella* breeding line (TruLarv™, BioSystems Technology). These age and weight defined, genome sequenced larvae overcome the variability normally associated with larvae from *e.g.* bait shops. To assess anti-biofilm effects in our model, we analysed the biofilm-eradicating activities of ampicillin, gentamicin and ceftaroline, all currently discussed for the treatment of *Enterococcus faecalis* endocarditis, a classic biofilm-associated disease.
Before implantation in the larvae, enterococcal biofilm establishment on the bristles was examined by confocal laser scanning microscopy and colony forming unit (CFU) determination of detached biofilms. Biofilm and sterile control bristles were injected via one proleg of the larvae and implanted parallel to the intestinal tract. Antibiotics were injected into the haemocoel 1 hour post-implantation using a Hamilton syringe. Biofilm treatment was assessed by an intern pathology scoring system of the larvae, plating of the haemolymph and histological sections using GFP-labeled bacteria.
Survival, movement and melanization of the larvae were not impaired by sterile bristles, whereas biofilm-overgrown bristles led to melanization and death of the larvae. None of the antibiotics in 2x the minimal inhibitory concentration were able to significantly improve survival of biofilm-burdened larvae, in contrast to planktonically infected larvae. CFU/mL values in the isolated haemolymph were not reduced by antibiotic treatment. Histological sections allowed for visualization of biofilm size and structure, but showed no difference between the different treatment groups.
The TruLarv™ bristle implantation model allows for a rapid, low cost and reproducible analysis of anti-biofilm treatments in an *in vivo* setting. *G. mellonella* larvae possess an innate immune system that is structurally and functionally similar to that of mammals, enabling biofilm bristle infections in TruLarv™ to reflect the virulence of biofilm-associated pathogens in mammals. Surfaces of toothbrush bristles can be further modified by using anti-adhesion coatings to analyze the prevention of biofilm formation.

[P167] CLINICAL ISOLATES OF THE OPPORTUNISTIC PATHOGEN STENOTROPHOMONAS MALTOPHILIA DISPLAY DISTINCT BIOFILM ARCHITECTURES

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Biofilm-producing bacteria are a major cause of morbidity and mortality in immunocompromised patients. Today in clinical settings, *Stenotrophomonas maltophilia* is considered an important multidrug resistant opportunistic pathogen that is frequently isolated from the lungs of cystic fibrosis patients (CF) and can contribute significantly to disease progression in these patients.

Here we report on the biofilm characteristics of *S. maltophilia* clinical isolates. By utilizing the microtiter plate technique, 350 clinical isolates, isolated from patients at various clinics in Germany were screened for their biofilm forming abilities. Further 42 environmental isolates were included in this study. This screen revealed a strong variation in biofilm forming capabilities among the individual isolates. Based on these results, isolates were then classified as strong, intermediate and weak biofilm formers. For a more detailed observation, the biofilms of strong and weak biofilm forming isolates were grown for 72 hours in flow cells under the same *in vitro* conditions. The biofilm 3D structures were then examined after a live/dead staining with a laser scanning microscope. It was evident that there is a high variation in the structure of biofilms formed by the various clinical isolates of *S. maltophilia* on a strain level regardless of their origin. While some isolates formed thick lawn like biofilms, other isolates formed sparse patchy like biofilms. Because of these observations, we speculate that under *in vivo* conditions *S. maltophlilia* displays varying biofilm architectures on a strain-specific level. These data are correlated with whole genome analyses.

[P168] ELUCIDATING THE POTENTIAL ROLE OF QUORUM SENSING IN SULFATE REDUCING BACTERIA AND ITS POSSIBLE LINK TO BIOCORROSION

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Sulfate reducing bacteria (SRB) are widely regarded as the dominant microbial community involved in biocorrosion within the marine environments. The high sulfate content of seawater enhances the proliferation of SRBs within the marine biofilms, which contributes to biocorrosion. Considering the major role played by SRB biofilms in biocorrosion, there is an urgent requirement to reduce its abundance within the marine environment. Conventional methodologies involve the use of non-environmentally benign biocides and antibiotics and hence, are limited by health, safety and environmental concerns. Quorum sensing is a type of cell density-dependent intercellular chemical communication mediated by a group of small signal molecules such as acyl homoserine lactones (AHLs), which play an important role in biofilm formation. Previous studies have reported on the production of several AHLs by SRB species and these signal molecules have also been implicated to be the driving force for the metabolic activities of SRB and its interspecies interactions in a highly complex natural environment such as the microbial mats. Using *Desulfovibrio vulgaris* and *Desulfobacterium corrodens* as model sulfate reducers, we showed that SRBs produce and release AHLs into extracellular space in the presence of lactate and Na_2SO_4 as electron donor and acceptor respectively. Under saline conditions, the specific AHL production rate (expressed as nmoles of AHLs produced/cells/h) at logarithmic phase was found to be ~3-folds higher for both *D. vulgaris* and D. *corrodens*. In addition, salinity also induced a higher rate of sulfate reduction (~2-folds), which correlated well with the logarithmic phase-AHL production kinetics. Taken together, the results suggest that quorum sensing play a potential role in enhancing sulfate reduction under saline conditions and hence, could lead to elevated rates of biocorrosion in seawater environments. This study could harness a relationship between quorum sensing, sulfate reduction and biocorrosion. Further, this study could serve as a platform to design and deploy environmentally benign quorum quenching approaches to control SRB and their subsequent impacts on biocorrosion.

[P169] COMPARÍSON OF THE EFFECTS OF PULP CAPPING MATERIALS ON ORAL CARIOGENIC BACTERIA: AN IN VITRO STUDY

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Background/Aims: Pulp capping agents are synthetic materials used in clinical dentistry to protect the dental pulp and facilitate its healing after being exposed traumatically or pathologically. The aim of this in vitro study was to compare three different pulp capping materials, Biodentine™, White ProRoot® mineral trioxide aggregate (MTA) and calcium hydroxide (Dycal®) with regard to their antibacterial effect against two reference strains of cariogenic bacteria, *Streptococcus mutans* and *Lactobacillus acidophilus*.

Materials and methods: The three pulp capping materials: Biodentine ™, Mineral Trioxide Aggregate (MTA) and Calcium hydroxide (Dycal) were tested on *S. mutans* (ATCC No. 25175) and *L. acidophilus* (DSM No. 20079). The samples were divided into 3 groups according to the time interval between mixing and testing. Direct contact test (DCT) was the method used in assessing the antibacterial effect at different time points and the numbers of colony-forming units (CFU/ml) on the agar plates were calculated for each tested material.

Results: All tested materials in the three groups showed significant activity against both bacteria ($P < 0.001$) compared with the control group. After 24 h and 7 days both Biodentine and MTA showed superior antibacterial effects against *S. mustans* compared to Dycal. For *L. acidophilus*, after 7 days Biodentine showed the most antibacterial effect compared to the other two.

Discussion: The antibacterial properties of certain pulp capping materials over others dictate their preferential selection in clinical settings where the dentist encounters deep dental cavities with bacterial potential. Such proper material selection raises the chances of treatment success at the long run.

[P170] EFFECTS OF AN ETHANOLIC EXTRACT OF YELLOW TAGETE (TAGETES ERECTA) ON STAPHYLOCOCCUS SPP. BIOFILMS

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Bacterial biofilms display an increased tolerance towards antimicrobial therapy when compared to their suspension counterparts, making the discovery of effective anti-biofilm molecules tremendously challenging. Due to the recognized value of natural-derived chemotypes in drug discovery, it is worth exploring plant-derived molecules as sources of novel anti-biofilm. However, so far, the studies of anti-biofilm activity among flower extracts has been very scarce. The essential oils of *Tagetes erecta*, a plant species originally from Mexico, are rich in carotenoids like lutein and terpenoids. These terpenoids have been reported to exert biocidal activity and thus it is plausible to hypothesize that they may have anti-biofilm effects. In this investigation, the ethanolic extract of the flowers of *T. erecta* was tested for biofilms inhibiting effects using orthogonal approaches.

The ethanolic extract of the flower was prepared by Sohxlet extraction. Viability of both (planktonic bacteria and biofilms) as well as biofilm biomass, were quantified using resazurin and crystal violet staining sequentially in the same plate, while biofilm matrix staining was conducted with a wheat germ agglutinin-Alexa Fluor 488 fluorescent conjugate, as in *Skogman et al. 2012*. The extract was tested against *Staphylococus aureus* ATCC 25923, *Staphylococcus aureus* Newman and *Staphylococcus epidermidis*. This quantitative data was complemented with Brightfield imaging of the microbial attachment pattern and Fluorescence microscopy of samples stained with the LIVE/DEAD™ Bac*Light*™ bacterial viability kit. The extract of *T. erecta* reduced the viability of the suspensions of all bacterial strains, with the potency (IC_{50}) values of 0.73 ± 0.18; 1.36 ± 0.11 and 1.25 ± 0.19 mg/mL when treating *S. aureus*, *S.aureus* Newman and *S. epidermidis*, respectively. The extract was also similarly effective in reducing *S. aureus* Newman biofilm biomass, with a potency value of IC_{50} of 1.35 ± 0.19 mg/mL.

In conclusion, the ethanolic extract of *T. erecta* represents displayed significant bactericidal effects against planktonic bacteria and anti-biofilm activity against *S. aureus* Newman. Even though results were not overly positive with this particular extract, this investigation does provide an example of a project devoted to natural products discovery in the field of anti-biofilms, more of which are definitively needed.

[P171] USING THE ZEBRAFISH TUBERCULOSIS MODEL TO TEST BIOFILM-TARGETING TREATMENTS COMBINED WITH ANTIBIOTICS

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The zebrafish has established itself as a convenient model to study tuberculosis, which is a difficult-to-treat infection affecting millions of people. There is *in vitro* evidence on the formation of biofilms by the causative pathogen, *Mycobacterium tuberculosis*. The formation of biofilm is a likely cause of the requirement of a prolonged treatment of tuberculosis with multiple antibiotics. We have developed an adult zebrafish-*Mycobacterium marinum* model and want to use it to study biofilm formation and its role in antibiotic tolerance *in vivo*. Anti tuberculosis drugs can be used against *M. marinum*, but similarly to human tuberculosis, their efficacy against this fish pathogen is suboptimal. We are therefore planning to test different biofilm-targeting strategies to improve the efficacy of antibiotic treatment against pathogenic mycobacteria using the zebrafish model.

[P172] COMPARISON OF IN VITRO BIOFILM PRODUCTION OF STAPHYLOCOCCUS PSEUDINTERMEDIUS STRAINS ON CONGO RED AGAR AND MICROTITER PLATE TEST

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Background:

Biofilm formation of staphylococci is a common cause of antibiotic resistance. As a virulence factor, it enables bacteria to adhere to biological and artificial surfaces and to produce extracellular matrices. Compared to non-biofilm producers, bacteria embedded in biofilms have enhanced resistance towards harmful environmental conditions such as antimicrobial agents and disinfectants. In veterinary medicine, early detection of biofilm formation is crutial and essential for effective therapy of chronic ear, skin or mammary gland infections.

Aims:

In this study, biofilm-forming *Staphylococcus pseudintermedius* strains causing chronic canine otitis externa and dermatitis were tested. The aim was to find the correspondance between the amount of biofilm biomass detected by Crystal Violet (CV) staining and the colony morphology on Congo Red Agar (CRA).

Methods:

In total 79 strains of *S. pseudintermedius* isolated from canine external ear canal infections were tested. The biofilms pruduced by each strain were evaluated on 96-well Microtiter Plates (MTP) after 24 hours of incubation in Mueller-Hinton broth at 37 °C. The biofilm biomass was detected by the application of 0,1% CV solution and absorbance was measured with spectrophotometry at the wavelength of 545 nm. Colony morphology was simultaneously examined with CRA method after 24 hours of incubation at 37 °C. Biofilm producer strains formed black, while biofilm non-producer strains red colonies.

Results:

The CRA method showed low sensitivity (83%) and negative predictive volume (5,8%) compared to MTP test. Therefore, the widely used MTP test cannot be replaced by CRA method for detection of biofilm production of *S. pseudintermedius* strains. Further investigations are needed to find a quicker and reliable method to detect biofilms.

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[173] BIOFILM FORMATION BY DIFFERENT GENOTYPES OF DAIRY STAPHYLOCOCCUS AUREUS ISOLATES FROM NORTHERN ITALY

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Staphylococcus aureus (*S. aureus*) is a pathogen that causes several serious diseases in both humans and animals worldwide. It represents a great concern in the dairy production chain, since it is often isolated from raw milk and dairy products. Furthermore, it is considered a major issue for the food processing industry due to its ability to form biofilms, that can become persisting sources of contamination.

It has been observed that different genotypes of *S. aureus* differ in their contagiosity and pathogenicity. In particular, *S. aureus* genotype B (GTB) is associated with high within-herd prevalence. This indicates an increased contagious and virulence potential compared with other genotypes (OGTs). Furthermore, in a recent study dairy *S. aureus* GTB from Switzerland showed significantly higher biofilm formation compared with OGTs.

The aim of this study was to evaluate the biofilm-forming abilities of different genotypes of dairy *S. aureus* strains isolated from Italy (Piedmont and Emilia Romagna).

A total of 49 dairy isolates were genotyped by using ribosomal spacer PCR and MLST. Biofilm formation was evaluated under static conditions on six-wells polystyrene plates (37°C, 24 h).

15 out of 49 (30,6%) isolates were found to be GTB, whereas the remaining isolates (=34) belong to other ten different genotypes. Six of GTB isolates (40%) shown biofilm forming ability. In particular, four isolates were classified as weak and two as moderate biofilm producers. As OGTs isolates concern, 13 strains (38.2%) resulted capable to form biofilm: five weak, six moderate, and two strong biofilm producers. The majority of STs found are previously reported to be bovine-associated.

In contrast with previous findings, in this work differences in biofilm forming ability between *S. aureus* GTB and OGTs were not observed. Interestingly, both the strong biofilm producers were not GTB (AA, ND).

In summary, no specific genotype or MLST could be categorized significantly into one level of biofilm formation. Further studies are necessary to confirm these initial findings.

[P174] A MOVING STORY: HOW EPITHELIUM WITH IMPAIRED CILIARY FUNCTION RESPONDS TO BIOFILM INFECTION

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Background Primary ciliary dyskinesia (PCD) is a clinically and genetically heterogeneous disorder that is characterised by abnormal ciliary function. Resultant failure of mucociliary clearance is associated with long-term bacterial colonisation of the airways, causing localised inflammation and airway damage. PCD patients also have low airway nitric oxide levels compared to healthy individuals. We hypothesised that the lack of mucociliary clearance would result in increased biofilm formation by non-typeable *Haemophilus influenzae*(NTHi), the most frequently isolated pathogen from the airways of PCD patients. To examine this we compared NTHi biofilm formation on primary respiratory epithelial cells cultured from PCD patients and healthy volunteers, and also characterised the epithelial response to colonisation.

Methods Epithelial cells isolated from nasal brushings of PCD patients and healthy volunteers were cultured at air-liquid interface to form established ciliated layers. Epithelial cells were co-cultured with a clinical NTHi isolate and biofilm formation assessed over 72 hours using scanning electron microscopy, fluorescent *in situ* hybridisation, and conventional culturing techniques. Ciliary beat frequency, antimicrobial peptide (LL-37) production, cytokine production, and nitric oxide synthase activity were measured for all co-culture models. A label-free LC/MS proteomic approach was applied to elucidate any resultant changes in cellular protein expression.

Results A significant increase in NTHi biofilm formation was observed on PCD epithelial cells compared to healthy epithelium ($p<0.001$). With the exception of impaired ciliary function both PCD and healthy epithelium demonstrated similar cytokine, LL-37 and NO production profiles. Label-free proteomic analyses revealed that NTHi biofilm colonisation of PCD epithelial cells resulted in dysregulated cytoskeletal remodelling and increased expression of S100 proteins in comparison to healthy epithelium.

Conclusions Impaired ciliary function is the primary defect in PCD and renders the airway epithelium more permissive to NTHi biofilm formation, with dysregulated cytoskeletal remodelling also suggesting enhanced cellular invasion.

[P175] EFFECT OF LED LIGHT ON PSEUDOMONAS FLUORESCENS BIOFILMS ON A HYDROPHOBIC SURFACE

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Bacteria are ubiquitous and may cause clinical infections (e.g., in catheters or needles), corrosion, loss of efficiency in different processes (e.g., water process, bleaching teeth processes). Nowadays, many antimicrobial agents (e.g., ampicillin, ciprofloxacin) are used to avoid bacterial infections. However, when bacteria attack a surface and grow as resistant biofilms, they become less susceptible to biocides and disinfectants. Current research in the field aims to provide alternative solutions to tackle this problem, by chemical and also by physical means. An alternative solution could be the utilization of light to kill or facilitate the detachment of biofilms from different consumer relevant surfaces, whether or not in synergy with different surface modification techniques. Light treatments exhibit various advantages. They are environmentally friendly and easy to use, making them extremely desirable for the food or medical industry. They could represent a new way to tackle the biofilm problematics. The use of cheaper light sources (e.g., LED - Light Emitting Diodes) represents a future perspective for the realization of household devices or small hospital devices for photodynamic inactivation. This work aims to unravel the underlying mechanism which governs the biofilm detachment or death by investigating and optimizing different light sources. This work aim to develop a protocol to grow a reproducible biofilm.

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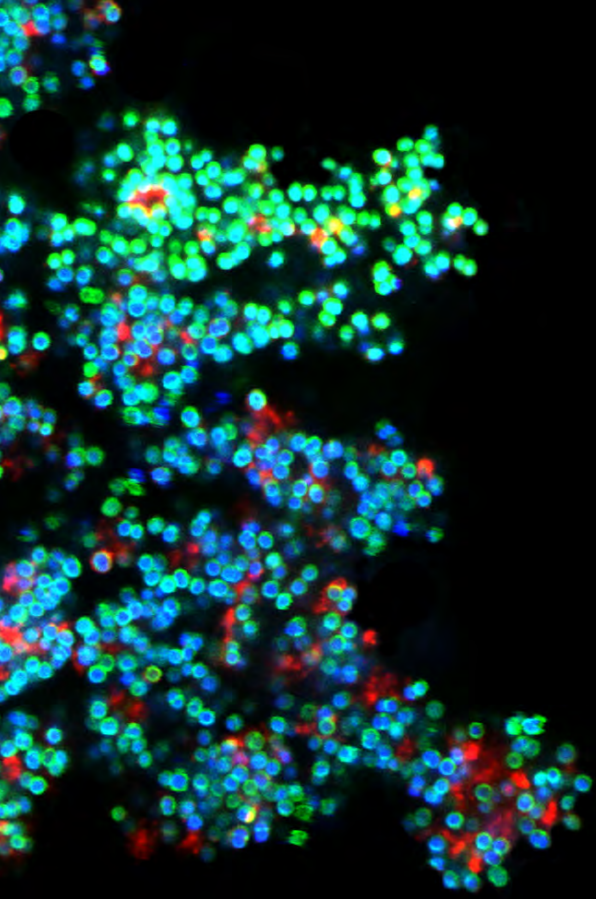

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