



BIOFILMS⁷

Microbial Works of Art



Biofilms7 – Microbial Works of Art

Final e-version

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Welcome Message

Dear participants,

We are very pleased to welcome you warmly to **Biofilms7**, one of the most important international meetings on biofilms that aims at bringing together researchers, industry experts and students interested in various aspects of biofilm science and technology. This meeting is organized by the biofilm research groups of University of Minho and University of Porto and is built upon previous meetings in Vienna (2014), Paris (2012), Winchester (2010), Munich (2008) and Leipzig (2006). **Biofilms7** will address cutting-edge issues of beneficial and deleterious biofilms in industry, environment and health organized in seven different topics:

- Biofilms and surfaces/interfaces: from nano to macro-scale.
- Biofilm detection and characterization methods (physical, chemical, biological).
- Biofilm prevention and control strategies.
- Biofilms and the environment.
- Productive biofilms.
- Biofilms, Industry and Energy.
- Modeling and Simulation.

On behalf of the Scientific and Organizing Committees we wish to thank the authors who have contributed to the high scientific standard of the program. We are grateful to the sponsors who have contributed decisively to this event. We also would like to extend our gratitude to all those who, through their dedicated efforts, have assisted us in this task. We wish you a fruitful and pleasant stay in Porto and hope that you will enjoy the scientific and social programs of this conference.

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Organizing Committee Chairs

Organizing committee

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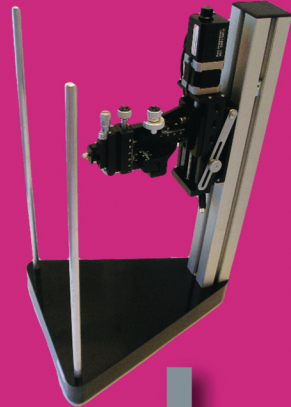


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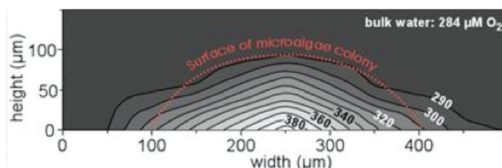


Micromanipulators

- manual or motorized (1 μ m precision)
- 1D and 2D



Application Example



pyroscience
sensor technology

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Plenary Sessions

Session 1

Biofilms and the environment

S01: 1

Birds-eye lecture

The ecology of stream biofilms

Tom Battin

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It is reasonable to argue that stream biofilms have been around on Earth for quite some time. Billions of years ago when flowing water started to shape the continental crust into drainage channels and when unicellular life developed was the cradle of stream biofilms. Over these evolutionary times, stream biofilms have had therefore innumerable occasions to adapt to an environment characterised by high shear stress, elevated UV radiation, low resource availability and high grazing pressure. Today biofilms dominate microbial life in streams and rivers, drive critical ecosystem processes and contribute substantially to global biogeochemical fluxes. In this talk, I will review the current state of stream biofilm ecology and biogeochemistry, and highlight the relevance of physical and ecological controls on their structure and function. At the heart of this talk is our new concept of biofilms as the “microbial skin” to encapsulate the functional relevance of biofilms at catchment scale. This perception is closely linked to our hypothesis that biofilms and the sedimentary environment in streams have undergone a “co-evolutionary” relationship. This assessment may pave the way towards a mechanistic understanding of climate change and global change impacts on stream biofilms and their involvement in the biogeochemistry of stream ecosystems.

S01: 2

Temporal shifts in the bacterial communities of urban, rural and forested streams throughout the year.

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The bacterial biofilm community is known to vary between streams based on region and surrounding land use for late summer sampling. This provides little insight into the variability of stream bacterial biofilm communities at different time points through the seasons. Six streams, four development (2 Urban, 2 Rural) and 2 undeveloped (forested), were sampled monthly at the same site for up to 18 months. Five samples were taken from each stream at each time. The V4 region of the 16S rRNA gene were sequenced from extracted whole sample DNA and was analysed using the primer statistical package. All stream communities were dominated by Proteobacteria with the Alpha proteobacteria most represented (mean 33% all OTUs), Betaproteobacteria (mean 8% all OTUs) and Gamma proteobacteria (mean 5% all OTUs). Planctomycetes were also important with Planctomycetia (mean 10% all OTUs) and Phycisphaerae (mean 1.5% all OTUs). Verrucomicrobiae (mean 5% all OTUs), and Cyanobacteria (mean 4% all OTUs) were also well represented. Temporal analysis by stream showed that the community, to bacterial class level, at any time point was most likely to be similar to those collected a month on either side although the communities generally showed less than 90% similarity. One urban stream showed strong similarity in communities between samples with most greater than 95% similar. Differences in bacterial community at phylum level within streams was often driven by variance in Planctomycete, Bacteroidetes, Proteobacteria and Cyanobacteria. Class level comparison of winter and summer bacterial communities across all streams suggested that between stream and year were as important as season.

S01: 3

Parallel evolution in bacterial biofilm development

Nicholas W. V. Churton¹, Raju V. Misra⁵, Robert P. Howlin¹, Robert Hull¹, Priscila Vitola¹, Raymond N. Allan¹, Johanna Jefferies⁴, Kerensa E. McElroy², Scott A. Rice^{2,6}, Staffan Kjelleberg^{2,6}, Torsten Thomas², Saul N. Faust^{1,3}, Richard J. Edwards², Stuart C. Clarke^{1,4}, Jeremy S. Webb¹

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Growth of bacteria within biofilms can quickly lead to within-population genetic diversity; but the generation of this diversity and the dynamics of evolution with bacterial biofilms remain poorly understood. By using genomic approaches, we have examined genetic diversification that occurs during biofilm formation of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. For both organisms, we discovered extensive parallel evolution between biological replicates. For *S. pneumoniae*, whole genome sequencing of 12 colonies with a small-colony variant (SCV) phenotype, each from independent biofilm experiments, revealed that all SCVs studied had mutations within the DNA-directed RNA polymerase delta subunit (RpoE). For *P. aeruginosa*, we used deep sequencing to carry out studies of within-population genetic diversification occurring during biofilm formation. Parallel evolution also occurred between biological replicates, at the level of pathways, genes, and even individual nucleotides. Biofilms therefore appear to generate very strong selection for certain mutations – this may help to identify genes that are central to biofilm development.

S01: 4

Investigation of biofilm formation at an oil-water interface in a microfluidic setup

George Kapellos

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Improved understanding of the fundamental mechanisms that control the physical and biochemical interactions between microbial communities and oily substances will enable the reliable prediction of oil dispersion and contamination risk assessment in aquatic ecosystems (e.g., deep-sea releases of crude oil). The research focus of this work is on the experimental and theoretical investigation of biofilm formation over a non-aqueous phase liquid (fuel oil) at the single-droplet scale of observation. Visualization experiments, based on a combination of video-microscopy and image analysis, have been carried out for the growth of *Marinobacter hydrocarbonoclasticus* at the oil-water interface using a custom-made glass microfluidic device. Extensive breakup of the initially stable oil-water interface into fine oil droplets as well as the entrapment of those droplets within biofilms with irregular geometry have been observed. In addition, a theoretical model has been formulated and is currently used to examine the strong interplay between multifluid dynamics, oil biodegradation and microbial proliferation. The model is based on a continuum description of all phases and tracks the spatiotemporal evolution of the interfaces developing between oil, water and biofilms using the volume-of-fluid method. The effects of key rheological and biochemical parameters on the fragmentation and biodegradation of the oily phase are systematically investigated.

S01: 5

A view from the inside: observing biofilm detachment from inside a pipe during stagnation and flow

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As more than 99% of bacteria in building plumbing live in biofilm, understanding detachment into the water phase is a critical element of drinking water risk analysis. Biofilm detachment in an intermittently operated system is often attributed to shearing with the first flush. In this work, we develop a novel approach to observe biofilm detachment in real time from directly inside the pipe. We used real-time flow cytometry with a sampling needle positioned inside a shower hose to quantify in situ suspended bacterial concentrations during and immediately after a shower event. We also measured detachment during stagnation up to 8 days. Immediately before flow, following 24 hours stagnation, bacterial concentrations are highest (up to 21 times the minimum). This decreases quickly during flow to a minimum reflective of the distribution system. With 60 minutes stagnation, concentrations linearly increase to 4 times the minimum. The rate of increase is proportional to biofilm concentration and is faster than possible for growth in the water phase. The maximum concentration of suspended cells over 8 days correlates with biofilm concentration ($R^2 = 0.94$). The flow cytometry fingerprint also reflects biofilm community differences, indicating seeding from the biofilm. Our novel approach is critical for differentiating the effects of the sloughing and stagnation. Since stagnation typically exceeds 24 hours in a shower hose and opportunistic pathogen infections typically occur with showering, it is clearly imperative to control biofilms on shower hoses. Our results shed new light on the mechanisms of biofilm detachment and highlight the problem of stagnation in building plumbing.

S01: 6

Anammox biofilms: successful improvement of the biomass retention

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The application of biofilm systems for the implementation of the Anammox process has been continuously growing since the discovery of the process. The Anammox autotrophic biomass, able to combine ammonium and nitrite to release nitrogen gas, is characterised by a low biomass yield and slow growth rate. Therefore, the use of reactors with very good biomass retention is a key factor for the start-up and stable operation. In this work, two types of Anammox biofilm biomass were used and compared with the aim of minimising sludge wash-out: granular biomass promoted by using influents with high salinity (5 and 10 g/L NaCl) and biofilm biomass growing on inorganic carriers (zeolites). In the first case, Anammox granules with good settling properties (Sludge Volume Index <60 mL/gVSS) were obtained about 20d after the addition of 5g/L NaCl to the influent. In the second case, a good development of biofilm on the zeolites (>65% particles at least partially covered) was observed 37d after their addition to the reactor. Both alternatives achieved biomass concentrations in the effluent lower than 20 mg VSS/L and biomass retention (calculation based on the theoretical biomass growth) about 73% (granular biomass) and 90% (zeolite carrier). Besides, the specific activity of the biomass was not negatively affected. There are some novel carrier materials for Anammox biofilms (e.g. plastic sponge, graphene oxide, non-woven fabric) which will also be discussed and compared with the obtained results. To conclude, biofilm Anammox reactors can be a good choice and the selection of the carrier type and material would mainly depend on the characteristics of the wastewater to be treated.

S01: 7

Learning from nature: biofilm dynamics on reactive substrates relevant to consolidated bioprocessing

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Cellulosic bacteria co-evolved with ruminants to develop specialized mechanisms to enable close associations with ingested cellulose substrates via the formation of unique, transient biofilms. These associations involve efficient cyclic biological-physical cotreatment of the cellulose fibres to yield simple sugars. In contrast, existing industrial bioprocesses typically involve expensive physical-chemical treatment, followed by enzymatic hydrolysis and fermentation – all as discreet steps. Mimicking the natural process may hold significant benefit to waste-to-value conversions. We applied scanning confocal laser microscopy to describe biofilm development on cellulose fibres under conditions simulating industrial bioreactors to assess spatial organization and migration of the adherent cells, cell yield from biofilms, and the relevance of bacterial sporulation at the interface with cellulose. The system was further adapted to measure microbial activity in real time, which enabled the analysis of carbon partitioning into cellular biomass, carbon dioxide gas and fermentation products. Finally, a mathematical growth model was linked to the product formation model, which verified measurements that showed substrate colonization by bacterial cells is an important initial rate-limiting factor, whereas substrate surface-to-mass ratio becomes the dominant factor thereafter. In the context of both the rumen and bioreactors, there is a constant dissolution of the attachment substratum and thus biofilm turnover, which is notably different from the conventional model of biofilm development on inert surfaces. This may also be of much relevance to host-pathogen interactions.

Plenary Sessions

Session 2

Biofilms and surfaces/interfaces:
from nano to macro-scale

S02: 1

Invited lecture

Biofilms and surfaces/interfaces: from nano to macro-scale

Joanna Verran

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It is generally acknowledged that an increase in surface 'roughness' results in an increase in the retention of microorganisms on that surface. However, over recent decades, the introduction into microbiology of increasingly sophisticated methods for describing surface properties emphasises the naivety of the assumption. Early work on 'adhesion assays' encompassed the incubation of standardised cell suspensions with test substrata over a short time period, typically one hour. Removal and rinsing of the surface, and quantifying the resulting amount of coverage of surface by cells, or counting cells, was subsequently deemed to be assessing 'retention' rather than adhesion. An increase in surface roughness was achieved by abrasion or blasting of the surface, and the retention assay duly showed an increase in retention with an increase in roughness, measured as the Ra value – the average departure of the surface profile from an average centre line – up to a point beyond which surface features were larger than the cells and thus failed to retain them. The atomic force microscope (AFM) enabled surface topography to be visualised and characterised on a nanometre rather than micrometre scale, and simulation of surface wear became more realistic and related to the environment under investigation. Indeed, in addition to an assessment of the amount of retention, the strength of attachment of cells within surface features could be measured. By increasing the force of the AFM probe as it scanned the surface, less firmly attached cells could be more easily removed. The coupling of amount versus strength of cell attachment facilitated consideration of the parameters required for surface hygiene, cleanability and disinfection. The development of surfaces whose topographies can be defined and controlled enables us to define lower limits of roughness where (nano-) features smaller than the microbial cells prevent attachment/retention. An understanding of the relationship between the size and nature of approaching cells, and the physical and chemical properties of the substratum enables us to customise surfaces for particular uses, but the environment in which the

surface will be use also requires consideration – how does flow affect retention? Does the orientation of surface features/defects affect retention? What are the target microorganisms? Does the presence of organic material affect the significance of a topography effect? How does the underlying topography affect subsequent biofilm formation and surface cleanability? Much research has resulted from an initial and somewhat simplistic premise!

S02: 2

Early fouling of surfaces by drinking water organic polymers and bacteria

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Accumulation of organic matter onto surfaces in early contact (within a few hours) with natural waters (forming a so-called conditioning film) has been reported since the 70s. However, the impact of the conditioning film on bacterial adhesion is currently a source of debate and no studies have been carried out in conditions relevant to drinking waters. Our pioneer study explored the conditioning film formed on a set of 17 materials (15 hydrophobic, polymeric materials and 2 hydrophilic hydrogel-like materials) using atomic force microscopy (AFM) and chemical force microscopy (CFM) as well as the counting of bacterial cells. After 24 h immersion in drinking water, organic polymers accumulated on 12 to 63% of the material surfaces and bacteria from 0 to 10^4 cells/cm², according to the tested materials. We showed that the potential facilitation of bacterial cell adhesion due to the conditioning film should be minimal because of the scattered distribution of the sticky polymers on the surface and the relatively high distances between them (in many cases longer than the bacteria cell dimensions). Additionally, statistical analysis (regression, correlation analyses and hierarchical cluster analysis) were performed and allowed the objective classification of the materials in four classes. The materials with the best anti-biofilm properties for use in drinking water systems were characterized by low conditioning film, low bacterial colonization, and ease to clean by chlorine and water flushing.

S02: 3

Amyloid production increases the hydrophobicity and stiffness of *Pseudomonas* biofilms

Guanghong Zeng¹, Brian Stougaard Vad¹, Morten Simonsen Dueholm², Gunna Christiansen³, Per Halkjær Nielsen², Tim Tolker Nielsen⁴, Carl Martin Peter Nilsson⁴, Daniel Erik Otzen¹, Rikke Louise Meyer¹

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The matrix of biofilms consists mainly of polysaccharides, DNA and proteins. Recently, amyloid protein fibers expressed by the *fap* operon were found in *Pseudomonas*, but their function is yet to be understood. We investigate the properties that production of amyloids conveys to the mechanical and physico-chemical properties of *Pseudomonas* sp. UK4 biofilms. Using atomic force microscopy (AFM) and force spectroscopy, we compared the cell morphology and mechanical properties of the wildtype (WT), a Δfap mutant, and the same mutant with controlled *fap* expression from on a plasmid (pFab). WT and Δfap cells collapsed when drying, while the over-expression of amyloids in pFab enabled cells to retain their morphology. Force spectroscopy of single cells adhering to hydrophilic or hydrophobic surfaces revealed that over-expression of amyloids affect the adhesion pattern, promoting adhesion to hydrophobic surfaces and abolishing the distinct adhesion pattern of the major adhesin LapA. Over-expression of amyloids led to strong aggregation in planktonic cultures, and the biofilms formed were extremely robust. Nanoindentation revealed that the stiffness of the biofilm increased by 20 fold compared to the wild type. Amyloid production in *Pseudomonas* led to a phenotype which promoted biofilm formation and increased the mechanical robustness of single cells as well as biofilms. The *fap* operon is found in many Proteobacteria and may contribute to biofilm formation in many other species from this Phylum. We are yet to discover the ecological significance of amyloid production for the survival of bacteria in the diverse habitats of Proteobacteria.

S02: 4

Measuring adhesion between uropathogenic *E. coli* and bladder-epithelial cells

Gerald Fuller, Emily Hollenbeck, Lynette Cegelski

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Bacterial adhesion to host cells is often a first step in the infection process. For example, uropathogenic *Escherichia coli*, the major causative agent of urinary tract infection, bind to host bladder-epithelial cells and initiate cell invasion. This triggers a subsequent pathogenic cascade characterized by recurrent infection. There is currently growing interest in developing new antimicrobials that, instead of targeting bacterial survival and placing high selective pressure for drug-resistant mutations, target mechanisms promoting infection such as binding to host cells. This new therapeutic strategy requires a detailed understanding of the factors that contribute to bacterial adhesion. To address this issue, we adapted a novel live cell monolayer rheometer recently developed in the Fuller lab to measure adhesion between a monolayer of bladder-epithelial cells and a layer of bacteria. The bacterial strain used in this study is UT189, a uropathogenic strain of *E. coli* that is capable of expressing several different extracellular components such as type 1 pili, curli, and cellulose. Using our adapted device, we can quantitatively compare the extent to which these different extracellular components affect bacterial adhesion to the cell monolayer. Additionally, we can use these measurements to assess the effectiveness of various small molecules in preventing binding to host cells.

S02: 5

Comparative genomics reveals a vast novel network of secreted protein domains involved in eDNA binding and dynamics in biofilm contexts.

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Extracellular DNA plays an important role in the formation and dynamics of biofilms, but very little is known about the diversity of proteins participating in these processes. Using comparative genomics, and sensitive sequence and structure analysis of proteins from 2774 complete prokaryote genomes and the NR database at Genbank in NCBI, we have uncovered a network of domains from secreted proteins predicted to be conserved players of biofilm dynamics across diverse bacteria. Using contextual information from protein domain architectures, conserved operons, and phyletic patterns, we have discovered novel domains and systems involved in binding and recognizing eDNA, an important component of biofilms. Furthermore, we have also identified domains involved in the clearance of eDNA and in remodeling of other macromolecular components of the extracellular matrix such as polysaccharides. These domains can be used as predictors of an organism's capacity to form dynamic eDNA-rich biofilms. We have also discovered an intersection of this system with interorganismal biological conflict systems involving secreted toxins and their cognate immunity proteins as well as links to phosphate nutrition and competence systems involved in uptake of DNA from the environment.

S02: 6

Dissecting the regulatory network of biofilm formation by *Pseudomonas putida*

María Isabel Ramos-González, Óscar Huertas-Rosales, María L. Travieso, Laura Barrientos-Moreno, Víctor G. Tagua, María Antonia Molina, Manuel Espinosa-Urgel

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Pseudomonas putida KT2440 is an efficient colonizer of the root system of different plants, having an applied interest since it activates induced systemic resistance in the plant. *Pseudomonas putida* KT2440 can develop biofilms on biotic and abiotic surfaces and molecular determinants participating in these processes have been described, including two large secreted proteins, LapA and LapF, which have different roles: LapA is mainly involved in the first stages of biofilm formation, facilitating cell-surface interactions, whereas LapF mediates cell-cell interactions, providing support for microcolony formation and maturation of the biofilm. Both proteins are also likely part of the extracellular matrix of mature biofilms, along with exopolysaccharides (EPS) and DNA. In this strain, four different EPS have been described, two of them (Pea and Peb) species-specific. We have started to identify and analyze the different players that regulate all these elements. As in many other bacteria, the intracellular levels of the second messenger cyclic di-GMP influence the multicellular lifestyle of *P. putida*. Increasing these levels by means of the diguanylate cyclase CfcR in multicopy has an effect on expression of *lapA*, *lapF* and EPS, and causes a pleiotropic phenotype that includes flocculation in liquid medium, crinkly colony morphology, increased biofilm formation and reduced detachment. CfcR and the control of expression of structural elements of the biofilm architecture are part of a complex network ultimately controlled by the two-component system GacS/GacA that involves transcriptional and post-transcriptional regulators, metabolic signals and the alternative sigma factor RpoS.

Plenary Sessions

Session 3

Biofilms, industry and energy

S03: 1

Birds-eye view

Biofilms in engineered systems

Hans-Curt Flemming

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Natural, semi-natural or engineered systems - biofilms grow everywhere. Their outstanding ability to capture resources even in most oligotrophic environments, to cooperate and adapt to extreme conditions and their tolerance towards biological, chemical and physical stress allow them to dwell in practically all engineered systems, provided sufficient amounts of water. Biofilms can lead to filter clogging, membrane biofouling, increased drag resistance in pipes and on ships, coating of functional surfaces, e.g., of sensors and cause contamination in purified water. Moreover, they can attack their substratum enzymatically or by biocorrosion. However, the problem is not the existence of the biofilms but the extent of their effects. Once they have reached an operationally defined threshold of interference ("pain threshold"), the bell rings. We have to learn how to live with biofilms; we can learn from handling biofilms on teeth – there is no way to prevent them once and forever. They are a form of life, which cannot be hit by magic bullets, but have an extreme ability to come back; killing is not cleaning, and dead biomass is cannibalized by survivors. Coping with unwanted biofilms requires management, including key aspects such as nutrient limitation, good housekeeping, easy-to-clean surfaces and systems, advanced means to weaken biofilm matrix cohesion and adhesion, and early warning capacity. Moreover, we can use "unwanted" biofilms in creative ways - an example is the improvement of membrane separation characteristics by biofilms as secondary membranes.

S03: 2

Invited speaker

May electroactive biofilms be even more efficient, more robust, more widespread than initially thought?

Alain Bergel¹, Elise Blanchet², Mickael Rimboud², Raphaël Rousseau², Marie-Line Délia², Benjamin Erable¹

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Electroactive biofilms are fascinating research objects, which should be a source of great fundamental advances and should pave the way to processes that “abiotic electrochemists” would never have dared to imagine before. Three recent studies will be presented showing how outstandingly efficient, robust and widespread electroactive biofilms are. Multispecies electroactive biofilms were formed on graphite anodes with garden compost used as the inoculum and acetate as the substrate. At first glance, the biofilms displayed electron transfer rates slower than would be found for reversible (Nernstian) kinetics. Actually, careful electroanalytical characterization of the bioanodes showed that the biofilm implemented different redox systems, each ensuring fast reversible electron transfer around a different redox potential: each redox system was fully efficient in its own potential range. Similar biofilms were successively exposed to only acetate (electron donor) and then only oxygen (electron acceptor), without changing the applied potential. The biofilms successively catalysed acetate oxidation and then oxygen reduction. As acetate-oxidizing bioanodes are generally thought to be composed of anaerobic species, such robustness was unexpected and may be linked to the dominant presence of *Chloroflexi* spp. Finally, bioanodes were formed using salt marsh as the inoculum in media that contained 45 g/L NaCl (1.5 times seawater concentration). Current densities of up to 80 A/m² were produced. The possibility of forming such efficient electroactive biofilms in such unusually saline conditions was shown to be linked to the association of *Marinobacter* and *Desulfuromonas* spp.

S03: 3

Impaired performance of pressure retarded osmosis due to irreversible biofouling

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Pressure retarded osmosis (PRO) is an emerging membrane technology that utilizes salinity gradients to generate sustainable energy. Next-generation PRO approaches aim to harness the energetic potential of streams with high salinity differences such as wastewater and seawater desalination brine. In this study, we evaluated biofouling propensity in PRO and estimated the effect on system efficiency. Dynamic bench-scale experiments were carried out for 24 hours using a model wastewater feed solution inoculated with *Pseudomonas aeruginosa* bacterium and artificial seawater brine containing the model bacterium, *Pseudoalteromonas atlantica*. Permeate flux and pressure losses were measured during the experiment, while a full membrane autopsy was conducted at the end including confocal, scanning and transmission electron microscopy as well as other biochemical assays. Our results indicate that at the wastewater stream, irreversible biofilm developed throughout the spacer and the membrane support layer, resulting in ~50% permeate water flux decline. We also observed an increase in the pumping pressure required to force water through the spacer-filled feed channel, with pressure drop increasing from $6.4 \pm 0.8 \text{ bar m}^{-1}$ to $15.1 \pm 2.6 \text{ bar m}^{-1}$ due to spacer blockage from the developing biofilm. In contrast, no biofilm was found attached to the spacer or membrane at the seawater brine stream. We estimate the energetic losses due to biofouling may be greater than 40%, posing serious doubts regarding the feasibility of wastewater-brine pairing in PRO. We conclude that generating energy by PRO using wastewater and seawater brine may become possible mainly by using a new membrane design.

S03: 4

Which role for minority microbial species in bioelectrochemical devices?

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In the majority of studies interested in the microbiology of bioelectrochemical systems (BESs), greater importance is assigned to most abundant species settled in electroactive biofilms (EABs). Here, experiments in three-electrode devices involved conservation or removal of BESs' planktonic bacteria and addition of exogenous species. The impact of those bacterial communities on both the electroactivity and the microbiology of EABs was investigated. Deep analysis of experimental data (chronoamperometric monitoring and high-throughput sequencing) followed by statistical calculations allowed to propose explanations regarding the microbial ecology of EABs. Best coulombic efficiency (CE - 34%) was obtained with the conservation of planktonic bacteria. Joint reading of electrochemical data and species relative abundances highlighted two distinct communities settlement: predominance of *Desulfuromonas* in EABs (42 to 53%), concomitant with the presence of planktonic species belonging to Mollicutes and Actinobacteria classes; or coinciding settlement of *Geobacter* and *Clostridium* in EABs. This study showed that exogenous species brought into contact with established biofilms were not necessarily likely to induce the loss their electroactivity. Relationships which exist between strictly speaking electroactive bacteria and other species present in BESs were identified as key for the sustainability of efficient biofilms.

S03: 5

Reversing methanogenesis to capture methane in archaeal biofilms

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No pure microbial culture that grows on methane anaerobically has been isolated. Hence, to make advances in our understanding of methane capture in global cycles and to produce biofuels from methane, it is necessary to be able to culture the microorganisms responsible for methane capture. Here, we engineered the archaeal methanogen *Methanosarcina acetivorans* to grow anaerobically on methane as a pure culture in biofilms. To capture methane, we cloned the enzyme methyl-coenzyme M reductase (Mcr) from an unculturable organism, anaerobic methanotrophic archaeal population 1 (ANME-1) from a Black Sea mat, into *M. acetivorans* to effectively run methanogenesis in reverse. Starting with low-density inocula, *M. acetivorans* cells producing ANME-1 Mcr consumed up to $9 \pm 1\%$ of methane after six weeks of anaerobic growth on methane and utilized 10 mM FeCl_3 as an electron acceptor. Accordingly, increases in cell density and total protein were observed as cells grew on methane in a biofilm on solid FeCl_3 ; planktonic cell numbers did not change, and the biofilms were imaged via SYTO9 staining. When incubated on methane for five days, high-densities of ANME-1 Mcr-producing *M. acetivorans* cells consumed $15 \pm 2\%$ methane, and produced 10.3 ± 0.8 mM acetate. We further confirmed the growth on methane and acetate production using ^{13}C isotopic labeling of methane and bicarbonate coupled with nuclear magnetic resonance and gas chromatography/mass spectroscopy, as well as RNA sequencing. We successfully reversed methanogenesis in a pure culture archaeal biofilm by cloning an archaeal enzyme from unculturable microorganisms that is responsible for capturing up to 300 Tg of methane per year.

S03: 6

The impact of food surfaces on *Escherichia coli* colonization in single- and dual-species biofilms

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Biofilm formation on food-contact surfaces is a major factor in pathogen persistence in food processing environments and *Escherichia coli* is one of the most common foodborne pathogens. It has been reported that surface properties, nutrient load and hydrodynamic conditions affect the biofilm onset in industry. In this work, the relative importance of the surface material (glass, copper or stainless steel), the culture medium (high or low nutrient) and the shear stress (0 or 0.27 Pa) on *E. coli* biofilm formation was analysed. It was verified that the most important factor under the tested conditions was the surface properties and that biofilm formation was correlated with the surface hydrophobicity. Moreover, higher biofilm development was observed in static conditions. Considering these previous results and knowing that *E. coli* typically colonize food surfaces in presence of other strains, the impact of two Diamond-Like Carbon (DLC) coated stainless steel surfaces, SICAN and SICON[®], on the biovolume and spatial structure of single- and dual-species biofilms was evaluated. It was found that these surfaces reduced single-species biofilm formation by *Pseudomonas grimontii* 13A10 (a non-pathogenic strain isolated from a fresh-cut salad industry) compared to stainless steel. For dual-species biofilms formed by a model *E. coli* pathogen in the presence of *P. grimontii*, confocal laser scanning microscopy also revealed a significantly reduced *E. coli* colonization after 24 h in the DLC-coated surfaces when compared to the monoculture biofilms. Results obtained after 72 h of biofilm development also suggest a protective effect of *P. grimonti* against surface colonization by the pathogen.

Plenary Sessions

Session 4
Productive biofilms

BIOFILMS ⁷ 

S04: 1*Invited lecture*

Biofilms in product driven biocatalysis

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In biocatalysis, traditional bottlenecks such as biocatalyst instability, toxicity issues, and difficulties regarding continuous processing are still prevailing. The exploitation of biofilms for producing industrially relevant compounds constitutes a most promising approach to counteract such shortfalls. Advantages of cells naturally immobilized in biofilms as compared to their planktonic counterparts include their physical robustness, self-immobilization, and long-term stability.^[1] In the recent years, we have developed a number of different biofilm forming biocatalysts for the synthesis of value added compounds. Examples include the synthesis of epoxides, alcohols, and polymer precursors from fossil carbon, as well as from glucose or CO₂. Biofilm-specific challenges have been tackled by reactor and strain engineering to address clogging,^[2] insufficient mass transfer,^[2] and biofilm adhesiveness.^[3] This presentation will discuss the potential and myths of biofilm catalysts. Different reaction and reactor concepts will be presented drawing the bow from heterotrophic to photoautotrophic^[4] biofilms. We will present solutions for the prevention of clogging, extensive biomass formation, and insufficient mass transfer, enabling optimized reaction rates. As an outlook, first results in the area of photoautotrophic biofilms fuelled by sunlight, CO₂, and water will be presented.

[1] Halan et al, TiBtech (2012) 30:453; [2] Karande et al B&B (2014) 111:1831; [3] Schmutzler et al AMB (2016) 100:347; [4] David et al, JIMB (2015) 42:1083

S04: 2

Does forced cooperation lead to improved productivity in a multispecies biofilm?

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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 crossfeeding of by-products allows a cooperative syntrophic relationship to be established. We have recently demonstrated that *M. maripaludis* exhibits taxis toward hydrogen, or hydrogenotaxis, as well as showing that biofilm helps optimize the carrying capacity of the two populations. In order to better understand the interactions between *M. maripaludis* and *D. vulgaris* Hildenborough, RNA-Seq and deuterium-labeled proteomics was used to characterize the coculture biofilm as compared to the planktonic mono- and co- culture states. Our results suggest that key steps in methanogenesis are down-expressed for *M. maripaludis* and electron transfer related genes are down-expressed for *D. vulgaris* Hildenborough. Many of the up-expressed genes include hypothetical proteins but also include cell surface modifications, communication via small metabolites, N-cycling, and metal homeostasis. This is in direct contradiction with results published for work done with similar coculture systems in the planktonic growth mode, and the results suggest mechanisms that enable the biofilm to increase productivity and increased carrying-capacity via resource sharing in a methanogenic biofilm.

S04: 3

Patterned biofilms for synthetic microbial consortia

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Over the past decade, synthetic biology has developed increasingly robust gene networks within single cells, but relatively few systems have demonstrated engineered multicellular behaviour. In contrast, naturally existing terrestrial bacteria primarily live in complex surface-attached communities known as biofilms. Within these biofilms, multiple distinct microbial sub-populations form intricate spatial structures such as growth co-localization. This spatial organization allows bacterial communities to achieve cooperative behaviours such as metabolic division-of-labour, and conversely, ecological interactions between different microbial subpopulations in turn influence the spatial patterning within the biofilm. Using optogenetic and metabolic tools from synthetic biology, we have developed a biofilm culture platform that can generate optically patterned microbial biofilms that provides spatiotemporal control of cell-surface attachment with sub-millimetre resolution. These represent new tools to investigate the relationship between intercellular interaction and patterning in microbial biofilms, as well as to engineer synthetic microbial consortia capable of complex tasks requiring biological division of labour.

S04: 4

Electron transfer mechanisms and microscale gradients in electrochemically active biofilms

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The exact mechanisms of extracellular electron transfer between microorganisms growing as biofilms and solid substances remain a matter of debate in the literature. Recent work in our group demonstrated that microscale gradients reflect the electron transfer mechanisms operating in biofilms. Therefore, the goal of this presentation is to discuss how microscale gradients are generated by electron transfer in biofilms and what can be learned by measuring them. Operationally, biofilms grown on electrodes are electrochemically active biofilms (EABs). Further, EABs that donate electrons to the electrode are anodic and those that accept electrons from the electrode are cathodic. Both anodic and cathodic biofilms generate microscale gradients during electron transfer. Over the past decade, our research group has developed and integrated novel microelectrodes and *in situ* Nuclear Magnetic Resonance (NMR) imaging techniques with polarized electrodes to investigate electron transfer mechanisms in biofilms. Both NMR and microelectrode technologies allowed us to quantify metabolic and chemical variations in biofilms while the cells respired on polarized electrodes. Combined with mass transfer investigations on rotating electrodes and biofilm attachment assays on an electrochemical quartz crystal microbalance, we generated a fundamental understanding of microscale gradients in EABs. Microscale gradients identified metabolically inactive layers within EABs respiring on electrodes. Using electrochemical impedance spectroscopy, we determined that inactive layers could be identified as a biofilm pseudocapacitance. We discuss the implications of these findings on electron transfer in EABs.

Plenary Sessions

Session 5

Biofilm prevention and
control strategies

S05: 1

Invited lecture

How dead is dead when considering biofilm control strategies?

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Many biofilm species may have experienced stress e.g. iron sequestration in the host or environment, exposure to host defences, solar irradiation, desiccation, oligotrophic environments, disinfectants *in vitro* and antibiotics *in vivo*. While Gram-positive bacteria respond to environmental stressors through sporulation, non-spore formers must adapt their physiology to one of several increasingly severe stress states, requiring careful resuscitation. Importantly, many have also evolved an alternative strategy of becoming viable but non-culturable (VBNC) where resuscitation proves difficult although species remain metabolically active and capable of infection in amoebae or higher organisms. So, how dead is dead? New physiological and molecular biology tools have been developed to monitor the transition into the VBNC state and track the location and persistence of these “dormant” bacteria. In biofilms a sub population of persister cells become quiescent and resilient to antimicrobial treatment, making chronic biofilm infections difficult to treat with antibiotics. This “persistence” occurs through toxin-antitoxin modules and other dormancy pathways which are now proving amenable to the development of new classes of antibiotics. Recent microscopy and biochemical techniques measuring cell membrane integrity, respiration, energy generation and cell growth, as well as eukaryote infectivity, shed new insight into the importance of the VBNC state in biofilm communities.

S05: 2

Fast emergence of phage-resistant *Pseudomonas aeruginosa* biofilm cells in response to the pressure exerted by bacteriophage treatment

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Antibiotic resistance constitutes currently one of the most serious threats to the global public health and it urgently requires new and effective solutions. Bacteriophages are bacterial viruses increasingly recognized as an attractive alternative to the conventional antibiotic therapies. In the present study, the efficacy of phages against *Pseudomonas aeruginosa* PAO1 biofilm and planktonic cell cultures was evaluated over the course of 48 hours. Although significant reductions in the number of viable cells were achieved for both cases, the high adaptation capability of bacteria in response to the selective pressure caused by phage treatment, resulted in the inevitable arising of phage-resistant variants. In most cases, those variants appeared later in planktonic cultures than in biofilms. Given the interest in further understanding their genetic makeup and possible mutations accumulated, some were selected for further phenotypic and genotypic characterization. The complete genomes of five *P. aeruginosa* PAO1 phage-resistant variants were sequenced and all revealed to carry mutations in the *galU* gene, which is involved in lipopolysaccharide core biosynthesis, as well as in one *pil* gene, which is involved in type IV pilus synthesis. Three of the *P. aeruginosa* PAO1 variants further revealed large deletions (> 200 kbp) in their genomes. Overall the results of this study reveal that the selective pressure caused by phages while targeting biofilms results in a faster emergence of resistance compared to planktonic cultures, probably due to the high genetic diversity of cells within biofilms. Furthermore phage-resistant variants seem to be quite adapted to the biofilm phenotype.

S05: 3

Evidence of pleiotropic effects of silver nanoparticles upon interaction with *Pseudomonas aeruginosa* (PAO1) biofilms

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Nosocomial infections due to multi-drug resistant *Pseudomonas aeruginosa* biofilms are being reported all over the world. It therefore becomes imperative to develop newer strategies to prevent biofilm formation. In our laboratory silver nanoparticles (SNP, size 7-20nm) have been synthesized by a patented process (USA Patent No.:75146000). Earlier studies using SNP have indicated a broad spectrum antibacterial, antifungal activity at low concentrations whilst exhibiting good biocompatibility and wound healing characteristics. Formulations containing SNP are being promoted as the next generation therapeutics for the control of multi-drug resistant microbes (S-gel[®], Silveron[®], MegaNano[®]). A genome-wide transcriptional analysis (using RNA-Seq) was performed to elucidate the cellular responses of *Pseudomonas aeruginosa* (PAO1) biofilms to SNP. Our results indicate that SNP exhibit pleiotropic effects; affecting biofilm adhesion, dispersion and vital cellular processes. Genes associated with exopolysaccharide synthesis [viz., polysaccharide synthesis locus, pellicle forming polysaccharides, alginate], adhesion lectin B and small regulatory RNAs were down-regulated. A substantial number of the genes encoding chemotaxis, biofilm dispersion (bdIA), DNA repair and amino acid degradation were up-regulated. Interactions of SNP with established biofilms disturbs the expression of genes encoding quorum sensing, virulence, attachment (Type IV pili), efflux systems, DNA degradation, oxidative stress etc. These findings provide insights into the mechanism of action of silver nanoparticles and may lead to designing rational strategies to prevent bacterial adhesion onto abiotic surfaces.

S05: 4

Listeria monocytogenes: how biofilms adapt to desiccation

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Listeria monocytogenes is a foodborne pathogen able to adhere and form biofilms on various types of surfaces. Associated with a high mortality rate, it is one of the major biological concerns in food hygiene. Since few years, industries attempt to reduce the environmental impact of hygiene operations in the workshops of refrigerated food processing, through optimized use of dehumidification after cleaning disinfection treatments. Our study was focused on the adaptation of *L. monocytogenes* biofilms in response to desiccation stress mimicking food plants conditions. The intracellular subproteome was analyzed both by in-gel (two-dimensional gel electrophoresis, 2-DE) and off-gel (LC-MS/MS) approaches while the cell envelope subproteome (surfaceome) was studied through three beforehand optimized extraction methodologies (enzymatic shaving, biotinylation and cell fractionation) and LC-MS/MS analyses. *L. monocytogenes* biofilms were first adapted to low temperature before submitted to air relative humidity decrease (75% Relative Humidity). The different subproteomes were analyzed after 3 h and 24 h desiccation by comparison with non-stressed sessile cells. The analysis of 2-DE intracellular proteins patterns revealed 46 differentially expressed spots corresponding to 28 different proteins. These results are being completed with a label-free relative quantitation approach by LC-MS/MS. The comparative analyses of surfaceome composition of *L. monocytogenes* biofilms consecutively to air RH decreasing is underway and should contribute to a better comprehension of mechanisms involved in the resistance and persistence of this pathogen in food plants despite the daily hygiene procedures.

S05: 5

Rechargeable nanoparticles for antibiofilm applications

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Biofilm formation is a serious problem due to the increased resistance of biofilms to killing compared to free-living bacteria. This has prompted the search for agents that can inhibit bacterial growth and biofilm formation. In the current study, N-halamine-derivatized cross-linked polymethacrylamide nanoparticles (NPs) were synthesized by copolymerization of methacrylamide (MAA) and the cross-linker monomer N,N-methylenebisacrylamide (MBAA), and were subsequently loaded with oxidative chlorine, using NaOCl. The chlorinated NPs demonstrated remarkable stability to organic reagents and to repetitive bacterial loading cycles as compared with the common disinfectant NaOCl, which was extremely labile under these conditions. The antibacterial mechanism of the cross-linked P(MAA-MBAA)-Cl NPs involved the generation of reactive oxygen species (ROS) only upon exposure to organic media / bacteria, revealing that the mode of action is target-specific. Further, a unique and specific interaction of the chlorinated NPs with bacteria was discovered, whereby these microorganisms were all specifically targeted and marked for destruction. This bacterial encircling was achieved without using a targeting module and represents a highly beneficial, natural property of the NPs. Finally, as a proof-of-concept of our technology P(MAA-MBAA)-Cl NPs embedded within irrigation drippers were shown to prevent fouling on them compared with the control, hence providing the drippers with 'self-cleaning' and 'self-sterilizing' properties. In summary, our findings underscore the potential of developing sustainable and rechargeable NPs-based devices for inhibiting bacterial colonization and growth.

S05: 6

Nitric oxide mediated control of microbial fouling of reverse osmosis membranes

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Bacteria form biofilms on almost all surfaces, ranging from ship hulls to cooling towers, to indwelling biomedical devices. The biological fouling of membranes used for water purification or reuse, such as reverse osmosis membranes, decreases water production, incurs increased energy costs and increases costs associated with membrane cleaning or replacement. Coupled with the fact that such membranes are not compatible with many traditional biocides, novel strategies are needed to increase efficiency of these systems. Here, we present the application of nitric oxide (NO) as a strategy to delay membrane fouling that is compatible with reverse osmosis (RO) membranes. We have selected a slow release NO donor and demonstrated it can achieve a long-term, steady state NO concentration in solution and that the NO concentration is maintained the RO reactor. A mixed species community was collected from previously fouled membranes from the NEWater plant in Singapore and was used to foul the freshly prepared RO membranes in the laboratory under operational conditions. Repeated dosing studies were performed using a mixed species community, where fouling was reduced on successive treatments and the impact on the microbial community was determined by 16S rRNA gene sequencing at each stage of treatment. Addition of the NO donor was effective at controlling the transmembrane pressure change, associated with fouling and did not significantly alter the mixed species community, suggesting there was no strong selection for NO insensitive species in the biofilm.

S05: 7

Influence over aqueous foam flow on biofilm removal towards food industries sustainable cleaning conditions

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Nowadays, we are facing important energy and water saving challenges. These ones are making scientist all over the world finding innovative solutions to improve industrial processes efficiency. One of many original ideas is the use of “not-regular” fluids over regular applications. Aqueous Foam Flow presents several unusual rheological properties when put inside a horizontal channel: low density, visco-elasto-plastic behaviour, and high wall shear stress. These ones give this type of fluid interesting capacities and uses: Assisted oil extraction, lubrication and food or pharmaceutical industries Cleaning In Place (CIP) operations. An innovative set-up was built to study the biofilm removal following a previous study using conductimetry and polarographic methods at different velocities ($2 \text{ cm}\cdot\text{s}^{-1}$, $4 \text{ cm}\cdot\text{s}^{-1}$ and $6 \text{ cm}\cdot\text{s}^{-1}$) and void fractions (from 55% to 85%) to identifying the wall shear stress in relation to the liquid film thickness. Preliminary data were obtained on the consequence of the wall shear stress over the removal of *Bacillus cereus* spores adhered to stainless steel surfaces of two industrial finishes: 2B and brushed. The removal kinetics observed were compared to those obtained with similar mean wall shear stress values for standard CIP conditions. The bubbles passage over the walls generates an oscillation of the slip-layer thickness which directly affects the removal phenomenon. However, as we increased the foams' velocity this influence diminishes. A good balance between these oscillations and the increase in the wall shear stress has to be optimized for efficient spores' removal regarding the stainless steel finishes tested.

Plenary Sessions

Session 6

Modeling and simulation

BIOFILMS⁷ 

S06: 1
Invited lecture

Impact of cell cluster size on apparent half-saturation coefficients

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The competition for oxygen between ammonia- (AOB) and nitrite-oxidizing bacteria (NOB) has been identified as the key mechanism to achieve stable nitrification in granular sludge reactors. This is important for developing strategies for nitrogen removal with anammox in the mainstream of wastewater treatment plants. It is believed that a key factor for outcompeting the NOB is the higher intrinsic affinity for oxygen that AOB have compared with NOB. However, other experimental reports apparently contradict this fact. We therefore present a model-based study to clarify conditions in which either AOB or NOB can have the better apparent (“observed”) affinity for oxygen. A three-dimensional diffusion-reaction model explains how the presence of microcolonies can lead to higher apparent oxygen affinities for NOB than for AOB, and how neglecting the floc organization in microcolonies could lead to misleading conclusions. However, for biofilms, stratification of AOB and NOB in different layers can still lead to higher apparent oxygen affinities for AOB. We also show that the traditional one-dimensional models are unable to explain these trends.

S06: 2

Impact of a new model of phenotypic transition rates between normal and persister cells in bacterial biofilms on their regeneration capacity after an antibiotic stress: an individual-based model study

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We constructed an individual-based model of bacterial biofilm that supports a new model of transition rates between normal and persister cells based on substrate and antibiotic concentrations. Persistence lately emerged as an important factor of the survivability of bacterial populations exposed to temporary disturbances, particularly to antibiotics, and gained interest in environmental and human health studies. Persisters are bacteria able to tolerate stresses in a susceptible isogenic population. This temporary phenotype enables part of bacterial populations to survive and switch back to actively growing normal cells after stress-removal. Persister formation can be stochastic or triggered by environmental factors, such as the lack of substrate or sub-inhibitory antibiotic concentrations. Our transition model links the micro-environments of cells, i.e. the local substrate and antibiotic concentrations, to their probability of phenotypic switch between the normal and the persister state. The use of an individual-based biofilm model enables to take into account the spatial and phenotypic heterogeneity of bacterial biofilms. The transition model was validated with experimental data of persisters' population dynamics in planktonic and biofilm cultures and confocal microscopy of biofilms of *Klebsiella pneumoniae*. In addition, we simulated the dynamics of biofilms' viable cells when subjected to an antibiotic. The simulations show that although the former biofilm's biomass is usually recovered, the biofilm undergoes changes in its structure and diversity.

S06: 3

Cell shape as a determinant of biofilm structure and ecology

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Bacterial cells come in a huge variety of shapes, but we have yet to understand the importance of these shapes for biofilm biology. How are multi-species biofilms affected by the morphologies of constituent cells? Which morphologies might the biofilm environment select for in turn? To address these questions, we combine individual-based modelling and experiments to investigate the effects of cell shape on patterning and evolution within bacterial biofilms. We have developed a flexible hybrid simulation framework, coupling a continuum model of the biofilm chemical environment to a cellular-level description of biofilm growth mechanics. Improving on previous ad-hoc models, this system allows competitions between different bacterial cell shapes to be rapidly simulated and explored. We show that cell shape can strongly affect patterning within bacterial biofilms. Rod cells do better at colonising surfaces and the expanding edges of colonies, while round cells are better at dominating the biofilm surface. Our predictions are supported by experiments with *Pseudomonas aeruginosa*, which show that elongated cells gain a competitive advantage at the colony edge. We argue that cell shape is a major neglected determinant of patterning and evolutionary fitness within bacterial biofilms.

S06: 4

A spatially explicit model for inverse colony growth of cellulosic biofilms

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Cellulosic ethanol is produced from non-edible plants and plant materials, such as wood, switchgrass, and corn stover. It can have a positive net energy output with a reduction in green house gas emissions that is drastically lower than that of corn based ethanol and fossil fuels. *Clostridium thermocellum* is a bacteria that is able to convert cellulose into ethanol. These bacteria colonize the cellulose directly and utilize the immobilized carbon substrate. This leads to the formation of biofilm colonies that are quite different from those that are typically observed in aqueous environments where nutrients are supplied by convection and diffusion. In particular colony formation is controlled by colonization site availability rather than substrate diffusion gradients. We formulate a spatially explicit mathematical models for this process. Our computer simulations show that the model can predict biofilm structures similar to those in the experimental literature for *C.thermocelum* and *Caldicellulosiruptor obsidiensis*. This includes inverse colony growth and crater-like depressions, suggesting that these structures can be explained by few basic ecological principles such as limitation of and competition for colonization space and substrate limitations. It also supports the conjecture that hydrolysis is rate limiting. Furthermore, we have been able to fit an earlier, spatially implicit, experimentally validated (for *C.thermocellum*), lumped reactor scale model to our spatially explicit model. This suggests that overall biomass production and activity follows a Richards growth model, which might be a useful insight for the design of cellulosic biofilm reactors.

Plenary Sessions

Session 7

Biofilms and the environment:
microbial ecology

BIOFILMS⁷ 

S07: 1

Invited lecture

Next generation water and wastewater treatment processes are taking advantage of heterogeneous biofilm structures

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Improved understanding of microbial interactions in biofilms can lead to more resource efficient or more robust processes for the treatment of water and wastewater. Historically, biological processes for wastewater treatment were developed based on empirical observations and engineering intuition. Treatment processes depend on exposing bacteria to variable environmental conditions (i.e., variable redox conditions, variable substrate availability) to select for a desired microbial community and to force this microbial community to perform the desired functions. In activated sludge systems (with suspended small microbial flocs) systems the environmental conditions of the bacteria can well be approximated based on bulk phase measurements – activated sludge can be considered as homogeneous systems. On the other hand, biofilms are difficult to monitor as they are characterized by significant gradients of environmental conditions and gradients of microbial communities – biofilms are heterogeneous systems. Even though biofilm systems are more complex to monitor and to understand, there can be significant benefit in the inherent heterogeneous conditions. This presentation will review the development of engineered systems for biological water and wastewater treatment and will highlight the opportunities of using heterogeneous biofilms.

S07: 2

Biofilm formation on chitinous surfaces protects *Vibrio cholerae* from protozoan grazing

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It has been reported that biofilm formation on chitinous surfaces (e.g. exoskeletons of marine copepods) plays an important role in the long-term persistence of *Vibrio cholerae*. Protozoan grazing is one of the major mortality factors faced by bacteria in the environment and previous studies from our laboratory have shown that biofilm formation protects *V. cholerae* from predation by heterotrophic protists. We have shown that quorum sensing (QS) regulates the production of anti-protozoal factors produced by *V. cholerae* biofilms. Here, we investigated the role of chitin association in *V. cholerae* grazing resistance. Data show that both *V. cholerae* WT and QS mutant strains formed more biofilm biomass in the presence of chitin. The growth of the protozoan, *Rhynchomonas nasuta* was inhibited by WT biofilms grown on chitin flakes compared with non-chitin controls, while this inhibition was attenuated on QS mutant biofilms. The anti-protozoal activity was also observed when *V. cholerae* WT biofilms developed in flow cell chambers containing chitin, or formed on chitinous surfaces of *Artemia*. Ammonium, a result of chitin metabolism, accumulated in supernatants of *V. cholerae* biofilms grown on chitin flakes, and the supernatants of WT biofilms and medium with ammonium supplementation were inhibitory against *R. nasuta*. RNA-Seq revealed that the majority of genes involved in chitin metabolism and chemotaxis were down-regulated in QS mutant biofilms. Therefore, chitin association not only provides *V. cholerae* nutrient for growth, but is also a protective niche for long-term persistence of *V. cholerae*, where QS regulation is critical for chitin metabolism and anti-protozoal activity.

S07: 3

Predation resistance of bacterial biofilms: a matter of predators traits

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Biofilm formation in bacteria is considered to be a mechanism to avoid protozoan grazing. However, a closer look shows that this assumption is mainly based on experiments with suspension feeding protozoans. The large diversity of protozoan feeding traits has not been considered adequately so far. We hypothesize that biofilms do not form a grazing resistance in general; instead specialization among feeding preferences (i.e., either plankton or biofilm cells) can occur. We assume that the presence of such specialized predators leads to the biomass accumulation of the non preferred prey phenotype, which can be either plankton or biofilm bacteria (depending on predator type). We performed short-term batch experiments with the bacterium *Pseudomonas putida* and either the suspension feeding ciliate *Paramecium tetraurelia* or the surface-feeding amoeba *Acanthamoebae castellanii*. We conducted two types of experiments whereby plankton and biofilm prey was introduced to one or both predators, only the development stage of biofilm prey varied between the experiments. The results show consistently that either bacterial biofilm or plankton maintain in the experiments as grazing defended phenotype whereas the biomass of the corresponding phenotype was strongly reduced. Interestingly, on the one hand biofilm formation was even stimulated in relation to the grazer free control when exposed to the suspension-feeding grazer. On the other hand the densities of planktonic cells are not stimulated above the control level when exposed to the surface feeder. Our results show that grazing resistance is a matter of the functional feeding trait of the grazer rather than a biofilm-specific property.

S07: 4

Co-infecting microbes modulate the *S. aureus* essential genome in vivo

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Staphylococcus aureus is a leading cause of both acute and chronic bacterial infection worldwide. Chronic *S. aureus* infections often exist as multi-species biofilms recalcitrant to antibiotic treatment. Despite the importance of *S. aureus* to human health, little is known about the genetic requirements for survival *in vivo* or how microbial community composition modulates these requirements. In this study, we used transposon insertion sequencing (Tn-Seq) to determine the essential genome of *S. aureus* during mono-infection in three murine infection models: abscess, osteomyelitis, and chronic wound. We found that overall the genetic requirements of *S. aureus* were highly similar across infection sites with the chronic wound impacting the essential genome to the greatest extent. Co-infection with *P. aeruginosa*, the most common bacterium found in chronic wound infections with *S. aureus*, dramatically shifted the essential genome of *S. aureus* and alleviated therequirement of 130 *S. aureus* genes that were essential in all three mono-infections. Collectively, these findings indicate the presence *P. aeruginosa* exerts a greater selective pressure on *S. aureus* than infection site and underlies the need to pursue co-culture studies of this bacterium to understand its pathogenesis.

S07: 5

Developing timely insights into *Pseudomonas aeruginosa* quorum sensing therapeutics through text mining

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The pervasive growth of antibiotic-resistant is pressing the development of novel strategies to control infectious diseases. Quorum sensing (QS) is a key communication mechanism that allows bacteria to regulate gene expression, and thus many physiological activities e.g. virulence, motility, and biofilm formation. Hence, QS inhibition or quorum quenching is being pursued as a promising strategy to control clinical pathogens. Most available information about drug interactions with QS genes and molecules is scattered in the vast and ever-growing biomedical bibliome. So, text mining and network mining are attractive solutions to identify relevant interactions and generate new hypothesis for antimicrobial research. Here, we describe the implementation of such an automated workflow that extracts key information on *P. aeruginosa* QS-focused antimicrobial strategies from PubMed records. The workflow produces an integrated network, capturing the effect of antimicrobial agents over QS genes, QS signals and virulence factors. Interactions are contextualised by information on the conducted experimental methods and details on the antimicrobials and QS entities retrieved. The public Web-based interface (<http://pcquorum.org>) enables users to navigate through the interactions and look for indirect, non-trivial antimicrobial-QS associations. Currently, the *P. aeruginosa* antimicrobial-QS network contains 439 interactions encompassing 170 different drugs and 72 different QS entities; but it is in continuous, semi-automated growth. It offers a comprehensive picture of emerging anti-QS findings and thus may help in gaining novel understanding and prioritising new antimicrobial experiments.

Plenary Sessions

Session 8

Biofilm detection and
characterization methods

S08: 1

Invited lecture

Lectin-barcoding of the biofilm matrix

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Microbial biofilm systems are defined as interface-associated microorganisms embedded into a self-produced matrix. The biofilm matrix represents a continuous challenge in terms of characterization and analysis. The tools applied in more detailed studies comprise extraction/chemical analysis, molecular characterization and visualisation using various techniques. Imaging by laser microscopy became a standard tool for biofilm analysis and allows 3-dimensional multichannel characterization of hydrated biofilms. In combination with fluorescence lectin-binding analysis, the glycoconjugates of the matrix can be assessed. By employing this approach a wide range of biofilms from different habitats were examined. For this purpose all commercially available lectins (≈ 70) were applied. From the results a barcode pattern of lectin binding can be generated and fine-tuned according to signal intensity. The barcode can be used to investigate the biofilm matrix characteristics at various levels, e.g. gross biofilm, microcolony, bacterial cell surface, adhesive footprints. Other applications include: comparison of different habitats, effect of physicochemical factors, characteristics of mutants, microbial interactions, microbe-eukaryote interactions, in situ versus in vitro, etc.. In fact the lectin approach represents still the only tool to characterize the heterogeneous glycoconjugate makeup of environmental biofilms in situ. Lectin-barcoding has the potential of combining it with other fluorescence approaches. This includes matching fluorochromes for other biofilm matrix constituents, fluorescence in situ hybridization, fluorescent proteins and innovative sensor probes.

S08: 2

Micro to nanometric optical imaging resolution to ascertain antibiotic target localization: why are these antimicrobials not effective?

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The extensive research devoted to understand biofilms resistance/tolerance to antimicrobials has been oriented on mechanisms involving specific genetic or physiological cell properties but also on antibiotic sorption and/or reaction with biofilm components that may lessen the antimicrobial bioavailability, decreasing its efficiency. This latter hypothesis can be now explored using a set of advanced fluorescence imaging devices with microscopic (time-lapse imaging and Fluorescence Recovery After Photobleaching (FRAP)) and nanoscopic (Single Molecule Localization Microscopy (SMLM)) resolutions. Both FRAP and time-lapse imaging can highlight the diffusion limitation related to the interaction of a fluorescent antimicrobial with its biological environment, but only SMLM techniques are well-suited for retrieving the localization of antimicrobials at the nanometer scale. In this study, we used these techniques to characterize the dynamics of fluorescently-labeled daptomycin combined or not with rifampicin in biofilms formed by *Staphylococcus aureus* clinical isolates. This correlative dynamic fluorescence microscopy approach has allowed discarding a lack of antibiotic bioavailability and specific interaction with its bacterial target to explain exposed biofilms persistence. Similar results were obtained under host immune system action, on a prosthetic vascular mouse model graft infection, revealing that antibiotic resistance/tolerance phenomena must probably be related to the specific physiology of adhered bacteria.

S08: 3

Architectural transitions in *Vibrio cholerae* biofilm development at single-cell resolution

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Many bacterial species colonize surfaces and form dense three-dimensional structures, known as biofilms, which are highly tolerant to antibiotics and constitute one of the major forms of bacterial biomass on Earth. Bacterial biofilms display remarkable changes during their development from initial attachment to maturity, yet the cellular architecture that gives rise to collective biofilm morphology during growth is largely unknown. Here, we use high-resolution optical microscopy to image all individual cells in *Vibrio cholerae* biofilms at different stages of development, including colonies that range in size from 2 to 4500 cells. From these data, we extracted the precise three-dimensional cellular arrangements, cell shapes, sizes, and global morphological features during biofilm growth on submerged glass substrates under flow. We discovered several critical transitions of the internal and external biofilm architectures that separate the major phases of *V. cholerae* biofilm growth. Optical imaging of biofilms with single-cell resolution provides a new window into biofilm formation that will prove invaluable to understanding the mechanics underlying biofilm development.

S08: 4

Visualization of particle deposition onto biofilm surfaces and its impact on oxygen mass transfer

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It is supposed that particles depositing onto the biofilm surface reduce the availability of oxygen in the biofilm and in turn decrease its metabolic activity. To shed more light on the impact of the deposition of wastewater (WW) particles onto biofilm surfaces, optical coherence tomography (OCT) imaging and oxygen microsensor measurements were combined. OCT visualized biofilm and particles whereas oxygen microsensors revealed the impact of the deposited particles on the oxygen flux into the biofilm. Raw municipal WW was separated into four particle size fractions (28 to 500 μm). A heterotrophic biofilm was fixed in a lab-scale flume operated in recirculation mode. After two hours of aeration WW particles were added. OCT and oxygen microsensor measurements were conducted after 0, 20, 60, and 240 min after particle addition. For the first time OCT imaging data allows to discern clearly between deposited WW particles and biofilm. In addition, oxygen microsensor profiles point to a 10 to 30 % reduced oxygen flux into the biofilm due to the particle layer forming above the biofilm. The results were confirmed by two-dimensional mathematical simulations using OCT data as structural templates (biofilm + particles) and calculating a reduction of the oxygen flux by 15 to 30 %. By combining OCT imaging and oxygen microsensor measurements it was shown, that the oxygen transport into the biofilm is physically hampered by WW particles depositing onto the biofilm surface. In addition and even more generally, the combination of imaging and modelling evolves the knowledge about substrate gradients at single spots as for microsensor measurements to multi-dimensional information.

S08: 5

Streamer-like extracellular matrix of *Pseudomonas aeruginosa* facilitates rolling biofilm migration

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The means to quantitatively resolve rheological gradients in biofilms are described and used to illustrate the interplay between exopolymers and the behaviour of key morphotypes. Magnetic tweezing was used to demonstrate that *Pseudomonas aeruginosa* biofilms grown at low Reynolds number were dominated by streamer-like biofilms. Rheological heterogeneity was an inherent property of the streamers, with the compliance spanning four orders of magnitude including the highest compliance for a biofilm reported in the literature. Despite this, the matrix material nonetheless retained significant elasticity, as shown by the dominance of the elastic component in the four-element creep model, suggesting that the matrix exceeds the critical cross-linking density. The compliance distribution suggests that the viscosity of the material will be similarly broadly distributed within the streamer-like biofilms arising either from gradients in either the concentration of individual EPS components or a distribution of the molecular weights of those biopolymers. Deletion of the Pel gene operon prevented streamer formation, which was coincident with a thousand-fold reduction in rheological heterogeneity. A high degree of material compliance appeared to facilitate time-dependent biofilm fortification. Conversely, biofilms without highly compliant representation were limited in their ability to thicken. Additionally, we describe a new mechanism for increased spreading of streamer-like biofilms, consisting of flow-induced detachment and reattachment of viscoelastic tethers, with alginate likely mediating their attachment to the substratum, resulting in rolling migration of the biofilm downstream.

S08: 6

Analysis of biofilm-nanoparticles interaction using microscopy (fluorescence, MEB, STEM, MET, EDS)

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Among biofilm's properties, the ability to interact with/catch pollutants can have applications in bioremediation. Here, biofilm interactions with metals (as iron nanoparticles (NanoFer 25S)) was evaluated using various approaches in microscopy. For this, biofilm growth, sampling, labelling and treatment were developed for each type of microscopy to access the surface or inside of the biofilm, biofilm composition, and metal location. Multispecies biofilms were grown on sand or in PVC tubes inoculated with aquifer water spiked with a nutritive solution to enhance denitrification, and then put in contact with nanoparticles. According to the targeted microscopy, biofilms were (i) sampled as flocs or attached biofilm, (ii) submitted to cells (DAPI) and/or lectins (PNA and ConA coupled to FITC or Au nanoparticles) labelling, and (iii) prepared for observation (fixation, cross-section, freezing...). Fluorescent microscopy revealed that nanoparticles were embedded in the biofilm structure as 0.5-5µm size aggregates. SEM observations also showed NP aggregates closed to microorganisms but it was not possible to conclude a potential interaction between nanoparticles and the biological membranes. STEM-in-SEM analysis showed NP aggregates could enter inside the biofilm over a depth of 7-11µm. Moreover, microorganisms were circled by an EPS ring that prevented the direct interaction between NP and membrane. TEM(STEM)/EDS revealed that NP aggregates were co-localized with lectins suggesting a potential role of exopolysaccharides in NP embedding. The combination of several approaches in microscopy is thus a good tool to better understand and characterize biofilm/pollutant interaction.

S08: 7

Development of a membrane separated flow cell for simultaneous detection of electroactive biofilm formation by EIS and CLSM

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Understanding the attachment of electroactive bacteria to electrode surfaces and their subsequent biofilm formation is one of the major challenges for the establishment of bioelectrochemical systems (BES). These can be used for current production (microbial fuel cells, MFC) and product formation (microbial electrosynthesis, MES). The wide variety of monitoring techniques for electroactive biofilm formation is given in literature, ranging from classical electrochemical techniques to different optical and spectroscopical methods. In this work, the simultaneous application of two powerful methods of analysis - electrochemical impedance spectroscopy (EIS) coupled with confocal laser scanning microscopy (CLSM) - in a membrane separated flow cell, is presented. The custom-built cultivation system provides a reproducible flow at the substratum surface and makes macro- as well as microscopic online observation of biofilm growth feasible. For electrochemical reactor characterization, cyclic voltammetry and abiotic EIS were done. Moreover, in simultaneous abiotic EIS and CLSM measurements, no influence of the imaging process on the EIS measurement or vice versa was found. Biotic experiments were performed to demonstrate the practical application of the flow cell for: (a) electrochemical monitoring of microbial activity and adhesion via chronoamperometry as well as EIS with (b) simultaneous imaging of the adherent biomass to the transparent electrode via CLSM. Therefore, the flow cell was run in MFC mode cultivating an engineered *Shewanella oneidensis* MR-1 producing eGFP. Results show a typical current curve of a MFC complemented by confocal time series images demonstrating adhesion.

S08: 8

Hardening of *Bacillus subtilis* soft mater during biofilm formation

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In sucrose rich medium *Bacillus subtilis* forms a pellicle that is especially rich in polyfructan levan. Purified levans from several bacteria were characterized by Small Angle X-Ray scattering technique (SAXS) and differential interference contrast microscopy (DIC). The experimental data obtained were analysed by string of beads modelling, which enables one to obtain levan structural parameters on nano to micro scale. We have shown for the first time that levan can form disperse intermolecular suprastructures composed of semi-flexible segments. Macrorheological measurements confirm that levan is unusually low viscous polymer. However, when levan interacts with eDNA its viscosity can increase significantly as confirmed by microrheology with optical-tweezers. In dilute *B. subtilis* bacterial cultures in sucrose rich medium, which were previously thought to be purely planktonic, we have demonstrated that cells mechanically couple forming a fragile intercellular fractal network as determined by optical tweezers and TEM. The chemical nature of the network at present is not known, but seems to be different from the network composition in mature biofilms, where the EpsA-O polysaccharide governs the mechanical properties. This is consistent with the results of single cell fluorescence microscopy which indicates that *epsA-O*, a hall mark of mature biofilm matrix, is expressed later at the end of the exponential growth phase. This work brings novel insights into how different matrix components contribute to mechanical properties and hardening of the biofilm.

Plenary Sessions

Session 9

Biofilm control strategies

S09: 1

Anti-biofilm activity of *Lactobacillus*

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Knowledge of the different competitive strategies deployed by bacteria when forming mixed biofilms should lead to the development of innovative strategies against biofilms. In this context, we evaluated the anti-biofilm activity of beneficial *Lactobacillus* strains against opportunistic pathogens, *Klebsiella pneumoniae* (Kp) and *Staphylococcus epidermidis* (Se). Initial screening (crystal violet staining) of neutralized supernatants from 140 *Lactobacillus* led to the selection of 31 and 35 of them able to induce significant biofilm biomass reductions of Kp and Se, respectively, or both strains (13). Further measurement of the viable Kp biofilm biomass (Colony Forming Unit numbering) showed statistically significant decreases when the biofilms were formed in the presence of extracts of *L. plantarum* 432 (53.8% diminution, $p=0,0186$), independently of a bactericidal effect. Addition of this strain's supernatant to already formed (2h-old) Kp biofilm also led to a significant reduction of the pathogen biofilm biomass after 4 hours of incubation (67.4%, $p = 0,006$). Observations of co-biofilms formed by *L. plantarum* 432 and Kp in continuous flow devices (flow cells under confocal microscope and microfermentors) showed reduced tridimensional structures and a decrease in the pathogen biomass compared to the Kp monospecies biofilm. Transcriptional analysis of Kp cultured in *L. plantarum* 432 supernatant or in mixed biofilms showed that the type 3 pili usher encoding gene, *mrkC*, was up regulated in both cases. Type 3 pili are key factors in biofilm formation by Kp, and the underlying mechanism due to the presence of *L. plantarum* 432 still remains to be determined.

S09: 2

A novel quinoline derivative that targets non-dividing bacterial cells is effective against biofilms of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, in vitro.

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Biofilms possess a physiological subpopulation of non-dividing cells that contribute to increased tolerance to antibiotics. A novel quinoline derivative called HT61 has demonstrated efficacy towards non-dividing planktonic cultures of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The aim of this work was to assess the ability of HT61 to subvert biofilm tolerance mechanisms associated with the presence of a non-dividing subpopulation of cells and increase antibiotic mediated biofilm killing. Minimum inhibitory and bactericidal concentrations, (MIC and MBC respectively) were determined for planktonic, stationary planktonic and biofilm phases of bacterial growth using HT61 and selected antibiotics against *S. aureus* UAMS-1, *Pseudomonas aeruginosa* PAO1-UW and *S. epidermidis* ATCC 35984. Biofilms of *S. aureus* and *P. aeruginosa* were visualised after treatment with HT61 with confocal laser scanning microscopy and scanning electron microscopy. The chequerboard method was used to assess potential interactions between HT61 and either tobramycin or vancomycin, against cultures of *P. aeruginosa* and *S. aureus*. Against biofilms of *P. aeruginosa*, HT61 was shown to be more effective, relative to planktonic cultures, reducing thickness by 80% ($P \leq 0.01$) and total live biomass by 99.9%, ($P \leq 0.0001$). HT61 was more effective than vancomycin toward biofilms of *S. aureus* and *S. epidermidis* and chequerboard analysis suggested partial synergy between HT61 and vancomycin. SEM suggested a potential

mechanism of action towards the bacterial membrane. To conclude, this work strengthens evidence that compounds targeted against non-dividing cells could be useful against biofilms.

S09: 3

Fluorescence in situ hybridization for the evaluation of anti-biofilm activity of antibiotic-loaded microparticles.

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Bone infections are one of the most devastating complications in orthopaedics and biofilm-forming bacteria, such as *Staphylococcus* spp and *Streptococcus* spp, are the most common pathogens. These infections are recalcitrant towards antibiotic, due to the presence of biofilms. High concentrations of antibiotics are required for biofilm eradication and polymeric microparticles have emerged as a valuable strategy for controlled drug delivery directly into biofilms. However, the characterization of the microparticles effect on polymicrobial biofilms is still a challenge. In this context, fluorescence *in situ* hybridization (FISH) has been gaining interest as a powerful diagnostic tool for polymicrobial biofilm due to its high specificity and ability to identify the presence of different bacteria within the same biofilm. The aim of our work was to develop novel antibiotic-loaded polymeric microparticles (plain, loaded with vancomycin or daptomycin) and assess their effect against mature biofilms of methicillin-resistant *Staphylococcus aureus* (MRSA) and polysaccharide intercellular adhesin-positive *S. epidermidis* by FISH. Results showed that daptomycin-loaded microparticles presented the highest antibiofilm activity against both tested strains. In addition, adhesion of microparticles to biofilms may be a crucial aspect for the enhancement of their antibiofilm activity. Finally, FISH proved to be an important tool to provide insights on the interaction of microparticles and staphylococcal biofilms.

S09: 4

Exploiting microbial glycoside hydrolases to prevent and disrupt fungal and bacterial biofilms

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Exopolysaccharides are a key component of the biofilm matrix where they are involved in initial colonization as adhesins, provide 3-D structure to the biofilm, and offer protection against antimicrobials and immune defense mechanisms. Our structure-function studies to determine how the exopolysaccharides Pel and Psl from *Pseudomonas aeruginosa* and galactosaminogalactan (GAG) from the fungus *Aspergillus fumigatus* are synthesized has led to the identification of glycoside hydrolases in each biosynthetic operon or gene cluster. As our analyses revealed that the recombinant enzymes, PelA, PslG and Sph3, were able to hydrolyze their respective polysaccharides, we hypothesized that these enzymes could be exploited as a therapy to prevent and disrupt microbial biofilms, thereby increasing the susceptibility of the microbes to antimicrobial therapies. We found that low nanomolar concentrations of these enzymes abrogate biofilm formation, and disrupt mature biofilms from lab, clinical, and environmental isolates on abiotic surfaces. Furthermore, the enzymes can potentiate neutrophil killing of bacteria by up to 50% and the activity of both antibiotics and antifungals. We found that the enzymatic activity of the fungal and bacterial proteins, Sph3 and PelA, respectively, both prevent the cell damage caused by the hyphae of *A. fumigatus* providing further evidence that the Pel and GAG polysaccharides have a similar carbohydrate composition. The enzymes are non-cytotoxic and do not alter human cell morphology at concentrations 100-fold above their active concentrations. Our findings suggest that anti-biofilm enzymes are promising agents for the treatment of microbial infections.

Plenary Sessions

Biofilms7-SPM
Young researcher award

BIOFILMS7 

SPM: 1

Designed peptides for inhibition of amyloid fibrillogenesis in medical biofilms

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Nosocomial infections affect hundreds of millions of patients worldwide each year, and approximately 60% of these infections are associated with biofilm formation on an implanted medical device. Biofilms are dense communities of microorganisms in which cells associate with surfaces and each other using a self-produced extracellular matrix (EM) composed of proteins, polysaccharides, and genetic material. Proteins in the EM take on a variety of forms, but the most common structure is the amyloid fold. Amyloids have long been associated with protein misfolding and neurodegenerative diseases, but recent research has demonstrated that an increasing number of bacteria utilize the amyloid fold to fortify the biofilm matrix and thereby resist antibiotic treatments. Consequently, these functional amyloids, in particular the soluble amyloid intermediate oligomers formed early in amyloidosis, represent a novel target to interrupt biofilm formation. We hypothesize that this intermediate stage of fibril production in the EM is characterized by the creation of α -sheet structures; therefore, molecules designed to bind these oligomeric intermediates may be capable of disrupting colonization and biofilm formation in a variety of microbial systems. This work describes the design, synthesis, and testing of anti- α -sheet peptides for inhibition of amyloid fibrillogenesis in *Staphylococcus aureus*, which will ultimately lead to new biofilm prevention strategies for improved patient outcomes.

SPM: 2

Extracellular polymeric substances of anammox granular sludge contain glycoproteins and have a gel-forming property

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Anammox (anaerobic ammonium oxidation) sludge granulation is applied in full scale wastewater treatment plants. To understand the formation and structure of the granules enriched with anammox bacteria, it is important to elucidate the composition of extracellular polymeric substances (EPS). However, due to the limitation in the methodologies of extraction and characterization, the EPS of anammox granules are still a black box. The aim of this study was to investigate the composition and rheological property of the extracted EPS. An alkaline extraction was used to extract EPS. The EPS were characterized by elemental composition analysis. Proteins and polysaccharides were quantified by the BCA and phenol-sulphuric acid assay, respectively. The proteins were further analysed using SDS-PAGE. Coomassie blue, periodic acid-Schiff (PAS) and Alcian blue staining were applied to detect respectively proteins, glycoproteins and acidic glycoproteins. The monomer composition of polysaccharides was determined by using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The mechanical properties of the extracted EPS were investigated by rheometry. The results showed that 205 ± 21 mg/g VSS EPS was extracted from anammox granules, in which the main component is proteins. A glycoprotein of 80 kDa was found to be dominant. Multiple sugar monomers were detected by HPAEC-PAD. The rheological measurements showed that the EPS form a gel at pH 8 and 25°C. The strength of the gel increased with multiple frequency sweeps. This research provides first insights into glycoproteins in EPS of anammox granules, and their gel-forming property.

SPM: 3

What happens to *Gardnerella vaginalis* when growing as a biofilm: a comparative transcriptomic analyses by RNA-seq

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Bacteria assume distinct lifestyles during the planktonic and biofilm modes of growth. In biofilms, they are more tolerant to antibiotics and can evade the immune system response more effectively. However, little is known regarding the molecular determinants involved in biofilm formation by *Gardnerella vaginalis*, the predominant species found in the polymicrobial condition bacterial vaginosis (BV), the most common vaginal disorder of women in reproductive age. Hence, to gain insight into the pathogenesis of *G. vaginalis*, we carried out a comparative transcriptomic analysis between planktonic and biofilm phenotypes, using RNA-sequencing. Significant differences were found in the expression of 815 genes. A detailed analysis of the results obtained was performed based on direct and functional gene interactions. In biofilm bacteria, the cell envelope appeared to be very active since genes encoding binding proteins and proteins involved in the synthesis of murein were significantly up-regulated. In addition, our data showed that *G. vaginalis* reflects the typical adaptation to stress and starvation conditions. Interestingly, genes associated with glucose and carbon metabolism, as well as oxidoreductase activity were found down-regulated in biofilms. Furthermore, gene-regulated processes in *G. vaginalis* biofilms resulted in a protected form of bacterial growth, characterized by low metabolic activity, which is appropriate to guarantee long-term survival during BV recurrence. Therefore, our data suggested that *G. vaginalis* adjust its lifestyle during colonization and infection by means of an extensive change of gene expression.

Poster Sessions

Session 1

Biofilms and the environment

Biofilms and surfaces/interfaces;
from nano to macro-scale

P1: 1

Aerobic granular biomass versatility applied for industrial wastewater treatment

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Application of aerobic granular systems for wastewater treatment is increasing in interest from the beginning of this century due to their potential to treat high loads and compactness. The development of this special form of biofilm, without carrier material, relies on the operational conditions of the systems they are grown in and the composition of the treated effluents. Among the possible aerobic granular biomass three types stand out, capable to simultaneously remove: (i) organic matter, nitrogen and phosphorous, (ii) organic matter and nitrogen, and (iii) nitrogen autotrophically via partial nitrification-anammox. Mostly these three kinds of aggregates are developed in sequencing batch reactors (SBR) in order to provide the biomass with the required stress conditions to force the microorganisms aggregation. The aim of this research work is to define the optimal conditions for the obtaining of the three different kinds of aerobic granules treating industrial effluents. Obtained results indicated that in all cases imposed operational parameters like sludge settling velocity (> 10 m/h), feeding regime (length and aerobic/anaerobic conditions), oxygen availability (over 2 mg O_2/L) and applied loads (higher than 50 mg P/L for (i) 500 mg COD/L for (ii) and 100 mg N/L for (iii)) are required. Once aerobic granules are formed optimal settling properties are expected to facilitate their retention inside the system. In this way, values of volumetric sludge index equal or lower than 60 mL/g TSS are recommended to be able to accumulate enough biomass (larger than $3-4$ g VSS/L) to treat the desired loads significantly larger than in conventional systems.

P1: 2

An in vitro dynamic model of catheter-associated urinary tract infections to investigate the role of uncommon bacteria on the *Escherichia coli* microbial consortium

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About 9% of nosocomial infections are attributed to catheter-associated urinary tract infections (CAUTIs). Uncommon bacteria (*Delftia tsuruhatensis*) have been isolated in CAUTIs in combination with well-established pathogenic bacteria such as *E. coli*. Nonetheless, the reason why *E. coli* coexists with other bacteria instead of outcompeting and completely eliminating them are unknown. As such, a flow cell reactor simulating the hydrodynamic conditions found in CAUTIs (shear rate of 15 s⁻¹) was used to characterize the microbial physiology of *E. coli* and *D. tsuruhatensis* individually and in consortium, in terms of growth kinetics and substrate uptake. Single-species biofilms showed that up to 48 h the CFU counts significantly increased for both species ($p < 0.05$). After 48 h, both species stabilized with similar CFU values reaching log 6.24 CFU.cm⁻² for *E. coli* and log 6.31 CFU.cm⁻² for *D. tsuruhatensis* ($p > 0.05$). The assessment of spatial distribution of dual-species biofilms by LNA/2'OMe-FISH revealed that *E. coli* and *D. tsuruhatensis* coexist and tend to co-aggregate over time, which implies that bacteria are able to cooperate synergistically. Substrate uptake measurements revealed that in artificial urine medium the bacteria metabolized lactic acid, uric acid (*E. coli* and *D. tsuruhatensis*) and citric acid (*D. tsuruhatensis*). In the consortium, *D. tsuruhatensis* consumed citric acid more rapidly, presumably leaving more uric acid available in the medium to be used by *E. coli*. In conclusion, metabolic cooperation between *E. coli* and

uncommon species seems to occur when these species share the same environment, leading to the formation of a stable microbial community.

P1: 3

Biofilm and resistance to metals in *Thiomonas* strains

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Bacteria of the genus *Thiomonas* are found ubiquitously in Acid Mine Drainages (AMD) and contribute to the precipitation of arsenic. To survive, these bacteria have developed different mechanisms that allow them to extrude the metals out of the cell but also to repair the damage due to oxidative stress caused by these metals in the cell. Biofilms can also allow microorganisms to better withstand toxic metals. We study the processes by which *Thiomonas* strains resist to arsenic and the role of biofilms in this resistance. We have compared the genomes of seven strains isolated from an AMD contaminated with arsenic. Our results suggest that these bacteria have evolved differently and have acquired genomic islands conferring different abilities to survive in toxic environments. We showed that these bacteria are capable of forming different biofilm structures in the absence or in the presence of arsenic. Within these biofilms, we observed the appearance of cells with survival skills superior to those of the original population, called variants. To identify the mechanisms involved during the biofilm formation and the emergence of variants in the biofilm, we have identified by RNAseq the genes induced or repressed during these processes. We sequenced the genomes of these variants and observed that their resistances may be due to point mutations or genomic duplications. These studies allowed to determine the main factors favoring the emergence of stress-resistant variants and to understand the contribution of the biofilms in bacterial adaptation processes in extreme environments.

P1: 4

Biofilm formation and antibiotic-resistance of bacteria isolated from wild animals

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The emergence and dissemination of antibiotic-resistant bacteria with ability to form biofilms is a growing concern for public and animal health. In fact, antibiotic resistance is consistently increasing and many resistance determinants remain poorly understood, particularly the wild reservoirs. The aim of this study was to determine the antibiotic resistance patterns and the biofilm production ability of four bacteria (*Shewanella putrefaciens*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens* and *Acinetobacter* spp.) isolated from different species of wild animals. Antimicrobial resistance was screened by the standard Kirby-Bauer disc-diffusion method. Monolayer adhesion was measured by a thermodynamic approach and by the polystyrene (PS) microtiter plate assay. Biofilms were also formed in microtiter plates. The four species were multiresistant, i.e. resistant towards at least to two antibiotics belonging to different chemical classes. Of particular concern was the resistance of all the bacteria to imipenem, an intravenous β -lactam antibiotic. All the isolates had hydrophilic surfaces and exhibited different abilities to adhere on PS and form biofilms. *P. fluorescens* had the highest adhesion ability. However, *K. pneumoniae* was the bacterium producing the highest biofilm amounts. The overall results propose that wild animals are potential reservoirs of persistent antibiotic resistant bacteria with strong ability to form biofilms and therefore might represent important vehicles for the dissemination of pathogens and resistance determinants.

P1: 5

Biofilm sloughing in integrated fixed-film activated sludge (IFAS) systems

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Biofilm sloughing is an integral aspect, and a dynamic activity of biofilm development. This study focused on biofilm sloughing on carriers in integrated fixed-film activated sludge (IFAS) systems in both laboratory (LS; synthetic feed) and full-scale (FS; municipal) systems. During biofilm development in the LS system the relative hydrophobicity of flocs and biofilm increased with an increase in carrier bound biomass. There was a decline in relative hydrophobicity of the flocs and attached biomass upon sloughing. The protein to polysaccharide ratio in the extracted EPS of the suspended biomass (flocs and sloughed biomass) and biofilms decreased and increased, respectively, which corresponds to confocal imaging showing that micro-colonies with polysaccharide rich content sloughing from the biofilms. Sloughed biomass rapidly associated with flocs to form large and compact structures that led to a decrease in SVI from 60-70 ml/L to 34 ml/L. The α and β diversities determined by 16S rRNA gene sequencing (Illumina) of FS samples revealed greater diversity of the biofilm community compared to flocs. The α diversity of biofilms and suspended solids decreased and increased, respectively, during sloughing. The shift in community structure was in part due to the loss of *Comamonadaceae* from the biofilm. At the class level, after biofilm sloughing, *Alphaproteobacteria*, *Gammaproteobacteria*, *Flavobacteria*, and *Acidobacteria-6* dominated on the remaining biofilm. The relative abundance of *Rhodocyclaceae* decreased in biofilms and flocs, after biofilm sloughing. Live-dead staining revealed areas of the biofilm where viability of biomass is an additional factor in sloughing.

P1: 6

Biofilms and disinfection by-products in a water distribution system in Colombia

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Biofilms in drinking water distribution systems (DWDSs) a major concern because they are related to deterioration of drinking water quality. Drinking water and biofilm samples were collected in 9 sampling points within a DWDS in a city in Colombia. Samples of pipes were taken during leakage repair activities to enable recovery of the biofilm, while drinking water samples were collected from the nearest household to each site. Microbial communities were characterized in both biofilm and water samples by pyrosequencing of extracted DNA. Content of dry biomass and total organic carbon (TOC) were also measured in biofilm samples. Temperature and concentrations of chlorine, TOC and total trihalomethanes (TTHMs) were measured in drinking water samples. Sampling points were characterized by water age, pipes age, and pipes material. Statistical tests were carried out to identify associations between microbiological data, water quality variables and DWDS properties. *Proteobacteria* was the dominant phylum in water and biofilm samples, similar results have been obtained in other areas with temperate weather, which may indicate that conventional water treatment processes act like a “filter” that favours the presence of specific microbial communities to phylum level. Microbiological data were not associated with any water quality parameter, which may be related to the complexity of the studied DWDS. Relative abundance at species level in both water and biofilm samples were correlated to water age, habitat, and water age and habitat combined. Results of sequencing analysis represent a step forward on the application of suitable tools to study microbiological aspects of DWDSs.

P1: 7

Development of an in-vitro oral mucosal tissue model for biofilm infection

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Denture-associated stomatitis presents as areas of inflammation on the palatal mucosa, and results from biofilms on the denture-fitting surface. Our previous research has shown that bacteria in *Candida* biofilms influence *Candida* virulence and pathogenicity, but associated effects on host cells and responses remain poorly understood. Therefore, this study aimed to develop cost-effective 3D tissue models representative of oral palatal mucosa to allow evaluation of biofilm infections. Single cell-type models were cultured over 3-10 d. Co-culture models were established by preparing a collagen mixture containing fibroblasts, and culturing for 2-3 d, after which keratinocytes were added. The tissues were incubated for 2 d before being lifted to air-liquid interface (ALI). Subsequently, tissues were maintained at ALI with medium changes every 2-3 d, and then infected with acrylic biofilms to evaluate host cell responses. A number of cell seeding densities and culture periods were investigated. Single culture fibroblast and keratinocyte growth conditions were optimised ensuring good tissue thickness, cell morphology and structural integrity. Co-culture was achieved with establishment of fibroblasts in collagen followed by addition of keratinocytes and lifting to the ALI for maturation. Host cell responses to biofilm infections included up-regulation of known infection response genes, and increased production of pro-inflammatory cytokines. Single and co-culture 3D tissue models were established which correspond with commercial constructs. This allows for a more cost-effective and therefore wide ranging/comprehensive evaluation of host-cell responses to biofilm infection.

P1: 8

Dynamics of biofilm development in porous media: bioclogging, preferential flow pathways and anomalous dispersion

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Biofilm growth in porous media is driven by interactions between the local hydromechanics and mass transport. The developing biofilm changes the structure of the pore network, leading to an increase of non-Fickian mass transport of solutes – the so-called anomalous dispersion. There is a lack of understanding of the mechanisms that are at the root of anomalous dispersion and conflicting claims in the literature about whether preferential flow pathways caused by the growth of biofilms are at the root of the anomalous dispersion observed. The latter fact represents a challenge for the modelling of flow and mass transport for practical applications such as the bioremediation of contaminated aquifers, geothermal injection wells or tertiary oil recovery. This study presents experimental evidence that sheds light on the existence of the preferential flow pathways and their link with anomalous dispersion. To tackle this question, three-dimensional millimodels of porous media are inoculated with wildtype bacteria and biofilm cultivated for 60 h. Flow in the increasingly bioclogged medium is quantified every 12 h in a Lagrangian framework using 3D particle tracking velocimetry. An increase of anomalous transport is observed with biofilm growth and is reflected in earlier arrival times and stronger tailing in breakthrough curves of passive tracers and by the intermittency exhibited by the pore scale Lagrangian velocities. The spatial correlation length of the flow increases with biofilm growth, indicating a strong channelization. Finally, an statistical analysis of the distribution of the velocity field allows the identification of preferential flow pathways.

P1: 9

Effects of hydrodynamics on the behavior of free-living amoebae in freshwater biofilms

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Naturally presents in waters of river which feed cooling circuits (CRFs), free-living amoebae (FLA) can meet conditions favorable to their multiplication during the warm-up of waters. So, the temporary occurrence of the planktonic pathogenic *Naegleria fowleri* represents a potential public health risk. Bacterial density and temperatures over of 32°C are necessary conditions but not sufficient to explain the growth of amoeba *N. fowleri* in biofilms (Goudot *et al.*, 2012). Indeed, microbiological balances are still unknown and under certain conditions can favor or not the installation, growth or survival of *N. fowleri*. The complexity of cooling circuits, particularly in terms of hydrodynamic conditions, may also influence the dynamics of FLA in biofilm. This study tends to better understand the effects of the representative hydrodynamic conditions of the CRFs on the behavior of the FLA, and *N. fowleri* in particular, in freshwater biofilms. A rotating disc reactor was optimized for a new study of the development of microorganisms (> 20 µm) in biofilms; in turbulent environment and under large parietal hydrodynamic stresses representative of the CRFs (large Reynolds number and large shear rate from 136 to 63,698 s⁻¹ at 42°C) (Mathieu *et al.*, 2014). Analytical monitoring of biofilms over time at different shear rates, includes measurements of *N. fowleri*, FLA and total flora by microbiological and molecular biology approaches. This work showed that this reactor is a suitable tool to assess the impact of hydrodynamics in near-wall found in CRFs of FLA in biofilms generated at 42 °C. The dynamics of colonization of surfaces by FLA under the effect of hydrodynamics will be presented.

P1: 10

Epilithic biofilm on the ancient subsurface Kupferschiefer black shale (Poland) – the underground field studies

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The exploitation of the Lopingian Kupferschiefer black shale leads to the exposition of the enormous surfaces of this underground polymetallic and organic-rich rock. These surfaces are inhabited by microorganisms both indigenous and foreign, in the form of biofilms. The aim of presented study was to characterize the epilithic bacterial biofilm on the surface of black shale exposed 12 years ago within Lubin mine at 760 m depth. The biodiversity, metabolism and physiology of biofilm as well as its role in the underground environment were determined. The studies included the 16S rDNA metagenomics, metaproteomics, physiology profiling and the geochemistry of the environment. The biofilm was dominated by γ -Proteobacteria and Actinobacteria but also contained α - and β -Proteobacteria, Bacilli and Sphingobacteria. The number of enzymes contributing to the oxidation of organic matter of the black shale such as mono- and dioxygenases, dehydrogenases, hydrolases and peroxidases were identified. Proteins responsible for the oxidation of sulfur, uptake of iron as well as those responsible for the resistance to toxic elements, oxidative stress and biofilm formation were also detected. To conclude, bacteria use the fossil organic matter of black shale as a source of carbon and energy. Its activity leads to the mobilization of organic carbon from primary organic matter (mainly aliphatic and aromatic hydrocarbons) and formation of secondary oxidized organic compounds (alcohols, aldehydes, organic acids). In addition, the mobilization of sulfur from primary minerals and precipitation of secondary biominerals on the surface of black shale were detected.

P1: 11

Estimating the impact of flow regime and sample volume on changes in bacterial community diversity in a low biomass aquatic environment.

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During treatment processes and all the way to consumers' taps, the diverse drinking water (DW) microbiome varies in abundance and in composition as a result of a large number of variables. A quantitative understanding of relevant variables is essential towards ensuring better control of DW quality and biofilm dynamics. The goal of this study was to understand the impact of sample volume and sampling flow rate at the tap on the structure and membership of DW bacterial communities, using high-throughput sequencing technology. This is especially critical in DW systems, which represents a low biomass but high diversity aquatic environment. To do this, we sampled at five different residential sampling locations in the City of Glasgow, UK. Sample volumes, ranging from 1 to 20 litres, were filtered to harvest microbial cells at each sampling location, under laminar and turbulent flow regimes. DNA extracts from each samples were PCR amplified using three different barcoded Golay primers targeting the V4 hypervariable region of the 16S rRNA gene followed by Illumina MiSeq sequencing. Bacterial community structure and membership change in bulk DW samples as a function of sampling flow regime. This is likely caused by sloughing of biofilms from water pipes under turbulent flow conditions, as compared to erosion under laminar flow regime. We will provide an overview of findings that stress the importance of ensuring similar flow regimes for DW microbial community investigations involving multiple sampling sites.

P1: 12

Flow-enhanced biofilm initiation on flat and corrugated surfaces

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Bacteria are ubiquitously exposed to fluid flow in natural environments, the human body, and artificial systems. However, the influence of flow on the transport and attachment of bacteria to surfaces and the formation of biofilms remains poorly investigated and understood. We have used microfluidic technology and mathematical modelling to study the role of fluid shear on surface colonization by pathogenic bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*, under clinically relevant flow rates. In a first set of experiments, we discovered a novel and counterintuitive phenomenon by which the coupling of motility and shear results in a higher cell concentration near the walls of a channel and consequently in a strong enhancement of bacterial surface attachment compared to quiescent conditions. A crucial step in obtaining these results was the use of a multi-channel microfluidic device, which allowed the simultaneous monitoring of bacterial surface coverage under different shear conditions while avoiding potential confounding factors stemming from variability among cell cultures. In a second set of experiments, we show how the topological features of the flow can promote the attachment of bacteria to specific regions of a corrugated surface, which will ultimately influence the formation of biofilms. Taken together, these results underscore the importance of flow in triggering biofilm initiation under common environmental conditions and represent one step towards the ability to mechanistically predict and thus ultimately either prevent or induce biofouling and the formation of biofilms.

P1: 13

Hydrogeological, chemical, and microbiological study of a massive biofilm development induced by fluid mixing in fractured rock

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Biofilms play a key role in groundwater biochemical processes. They are of a primarily importance for understanding the fate of contaminants in aquifers, for designing efficient bioremediation techniques, and preventing biofouling and bioclogging of artificial recharge systems and geothermal fields. The mechanisms controlling the development of biofilms in soils and rocks derive from the interactions occurring at the micro-scale that depend on mineral compositions, the biota of subsurface environment, but also fluid mixing, which determines the local concentrations of nutrients, electron donors and electron acceptors. In this presentation we report the results of a multidisciplinary field campaign for understanding the origin of a massive biofilm observed in the fractured granite aquifer at the Ploemeur observatory (H+ network hplus.ore.fr). The observed rust-colored biofilm, composed mainly of ferro-oxidizing bacteria, forms decimeter scale structures that initiate at about 60 meters depth and invades the observation borehole upwards until the surface. The presented dataset includes an *in situ* characterization of the vertical distribution of flow properties of fractures at different depth and the associated chemical and microbiological composition of water, obtained through pyrosequencing techniques. At this site, the mixing of oxygen rich and iron rich groundwater at a depth of about 60 meters is shown to represent a hotspot of microbial activity, which explains the presence of the observed massive biofilm. This study brings new insights on the dynamics of biofilm across fluid mixing interfaces in natural environments.

P1: 14

Interaction between physical heterogeneity and microbial processes in subsurface sediments: a laboratory-scale column experiment

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Subsurface biofilms offer the potential for biotransformation of nutrients and organic compounds. Physical heterogeneity in porous medium modifies hydraulic conductivity, as well as microbial growth and activity in subsurface sediments. Up to now most studies focusing on grain size avoid considering the effect of heterogeneity. This study goes further, by analyzing the conjunction between grain size heterogeneity and spatial heterogeneity of different grain size layers and their relation with biological and physico-chemical responses. To this aim, a laboratory-scale column experiment was performed with infiltrating sand columns. Our physico-chemical and biological results reinforce the idea that not only grain size but also spatial heterogeneity are a driver factor of the processes occurring in it. Water in slowest infiltration systems receives light for a longer time period which may promote photochemistry reactions favoring degradability of dissolved organic compounds and increasing microbial catabolic diversity. Spatial heterogeneity in infiltration systems where layers of coarse grain size are superimposed over layers of fine grain size causes organic matter accumulation in the interface between the two layers, creating hot-spots of microbial activity and favoring biodegradability. Infiltration systems with coarser grain sizes to facilitate infiltration may result in lower photo-degradation, lower biodegradability and lower phosphorous retention. It is important to account for the implications of grain size and spatial heterogeneities in subsurface sediments to improve the efficiency of organic matter degradation and nutrient recycling processes.

P1: 15

Iron availability modulates biofilm formation by *Staphylococcus epidermidis*

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Iron is regarded as essential to virtually all microorganisms, although the role of this nutrient on biofilm formation by many bacterial species is unknown or poorly explored. This is the case of *Staphylococcus epidermidis*, a major inhabitant of the human skin, which has also become an important nosocomial pathogen. Interestingly, biofilm formation has been regarded as a pivotal feature in both commensal and clinical isolates. Recent results from our group have pointed out iron uptake as an important mechanism for *S. epidermidis* biofilms survival. The present work was therefore aimed at elucidating the effect of iron availability in *S. epidermidis* biofilm formation. To achieve that, biofilm formation of three *S. epidermidis* isolates was evaluated when cultured in medium presenting different iron availability levels. Interestingly, under physiological iron concentrations, biofilm formation and planktonic growth were not affected but suprphysiological concentrations displayed an inhibitory effect both on biofilm and planktonic growth. Importantly, biofilm formation and planktonic growth was also inhibited by chelation of the iron present in the culture medium, which was completely restored after iron addition in a dose-dependent manner. Our findings provide clear evidence that iron plays a pivotal role on *S. epidermidis* biofilm formation, and this seems to be primarily related with its effect on the bacterial growth rate. Additionally, the iron concentration range supporting bacterial growth and further biofilm development was found to be very narrow, a feature that may be explored in the future for biofilm control purposes.

P1: 16

Microbial attachment and biofilm development in microbiologically influenced corrosion

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The initial attachment of microbes and the subsequent development of a biofilm are typically reported as key steps in microbiologically influenced corrosion (MIC). However, a wide variety of factors (e.g. microbiological, environmental and substrate material) can affect the initial microbial attachment stage as well as the type of biofilm that forms and hence influence MIC. The extent to which changes in individual test parameters (e.g. temperature) can influence processes relevant to MIC are sometimes relatively well known within certain disciplines. However, the multidisciplinary nature of MIC studies means that researchers in this field have often been unintentionally ignorant of some of the test parameters which have major impacts on test outcomes. At present there are no standard laboratory test procedures for MIC. Even a brief scan of the relevant literature will show that a wide range of different test media, microorganisms and substrates are commonly reported. The question therefore arises as to whether it is possible to draw any comparisons between individual MIC tests undertaken without standard testing procedures and whether it is indeed possible to develop any global understanding of the processes taking place. The purpose of this paper is to provide information for MIC researchers to help avoid some of the potential pitfalls that can occur in these studies. We present results of tests that show how changes to substrate material and test media/conditions can affect the initial bacterial attachment and biofilm development and subsequent MIC, where the bacteria *Escherichia coli* and *Desulfovibrio desulfuricans* have been used as model organisms.

P1: 17

Microbial biofilm communities in changing conditions of a pilot distribution network

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Deep understanding of the microbial communities and factors affecting the microbiome composition and activity in the complex drinking water system requires analyses of the entire microbial community. Microbial biofilm communities' stability were studied in changing conditions (extra disinfection treatment, contamination with *E. faecalis* and *E. coli*, magnetic water treatment) with two commonly used materials in water plumbing (copper and high density cross-linked polyethylene (PEX)). Pilot scale network studies simulating regular water consumption (flushed five minute four times during normal office hours) were performed with four horizontal copper and PEX pipelines containing five pipe collectors. After nine months 36 biofilm samples of the novel pilot scale water distribution system were analyzed by total microbial counts (DAPI), heterotrophic plate counts (HPC), total bacteria and alpha-, beta- and gammaproteobacteria (qPCR), active bacterial biomass (ATP) and microbial communities by next-generation sequencing using 16S ribosomal RNA genes (rDNA) and ribosomal RNA (rRNA). All biofilms consisted mainly of Alphaproteobacteria (*Sphingomonas* spp., *Methylobacterium* spp, and *Zymomonas* spp.). Higher amounts of HPC, ATP and total 16sRNA were found in copper (vs. PEX) pipeline biofilms whereas DAPI and beta-diversities (rDNA and rRNA) showed similarity. High bacterial contamination load with short one hour contact time as well as magnetic water treatment seemed to have negligible effect on biofilm microbiome. As a conclusion, our study deepened the understanding of different factors affecting water microbiome in plumbing systems.

P1: 18

Ornithine lipid-mediated modification of biofilm formation and virulence in *Pseudomonas aeruginosa*

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Ornithine lipids (OLs) are bacteria-specific lipids that are widely found in outer membrane of many Gram (-) bacteria, but not detected in Eukarya and Archaea. *Pseudomonas aeruginosa* produces OL under phosphate-limiting conditions and has *olsBA* operon encoding acyltransferases that functions the OL biosynthesis. The *olsBA* operon is highly expressed under phosphate-limiting condition. OlsB has similar structure with LasI, an acyl-homoserine lactone synthase. We addressed how OLs modulate the biofilm formation and virulence of *P. aeruginosa* during the infection to host cells. Cellular OL level was controlled by the overexpression of *olsBA* operon and confirmed by TLC and HPLC analyses. The virulence of *P. aeruginosa* was investigated by using two host models, *Tenebrio molitor*, an insect and *Caenorhabditis elegans*, a nematode. The OL effect on biofilm formation was analyzed in flow cell system. The host response to OLs was investigated by measuring the expression of inflammatory factors in mouse macrophage cells. The overproduction of OLs alleviated the virulence of *P. aeruginosa* by reducing the quorum sensing activity. We suggest that the *olsBA* overexpression sequesters the cellular acyl-group pool toward the OL synthesis from acyl homoserine lactone synthesis. OLs enhanced the biofilm formation of *P. aeruginosa*. Since the hydrophobicity of cell surface increased by the OL overproduction, this may facilitate the attachment of cells to surface. Finally, OLs reduced the production of inflammatory factors such as iNOS, COX-2, PGE₂, and nitric oxide in host cells, suggesting that OL can modulate the host immune response.

P1: 19

Structural features of *Campylobacter jejuni* biofilms

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Campylobacter jejuni is continuously reported as the leading cause of bacterial foodborne infections in developed countries, with a significant increase in the prevalence observed over the past years. Despite its fastidious growth requirements, *C. jejuni* is able to survive in the environment without permanent loss of viability and virulence. The mechanisms responsible for its survival remain unknown, but one of the survival strategies might be linked to the biofilm formation. Recent studies indicate that *C. jejuni* is able to adhere to inert surfaces and develop biofilms. In this work, confocal laser scanning microscopy (CLSM) with fluorescent lectin binding analysis (FLBA) were used in order to visualize and characterize *C. jejuni* biofilm structure and matrix composition. The biofilm architecture differed between the two strains used in the study, ranging from a finger-like structure with voids and channels to a compact multilayer-like structure. Biofilms of both strains contained motile cells. Exposure of cells to oxygen enriched conditions enhanced biofilm development, although the architecture of the biofilms differed between the strains. Finally, glycoconjugates of *C. jejuni* biofilm matrix were partly described using FLBA. Screening performed with 73 different lectins revealed strain-specific patterns with only 6 lectins interacting with the biofilm matrix of both strains. Taken together, these data provide new insights on structuralization and composition of *C. jejuni* biofilm that probably serves as a protective niche facilitating the survival of *C. jejuni* in the environment.

P1: 20

The chemical nature of extracellular polymeric substances (EPS) is linked to the hydraulic resistance of a biofilm in a structurally dependant manner.

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The hydraulic resistance of a biofilm during ultrafiltration reduces permeate production. An understanding of what governs this hydraulic resistance is required to identify a solution for improved filtration performance. Past studies have separately linked the biofilms hydraulic resistance to either EPS accumulation or to distinct biofilm physical structures. But the interplay between these two variables and their joint impact on hydraulic resistance is unclear. EPS are agglomerations of biopolymers with defined functional and structural properties. Thus, will specific accumulations of EPS form distinct biofilm physical structures with characteristic hydraulic resistances? We investigate this question by growing biofilms under contrasting growth conditions to produce different accumulations of EPS-anticipating formation of different biofilm physical structures with characteristic hydraulic resistances. Over 30 days of biofilm growth, accumulation of polysaccharide (Ps) and eDNA-associated with dense biofilm physical structure-was linked to higher hydraulic resistances. Conversely, the accumulation of protein-associated with permeable biofilms-had limited influence on hydraulic resistance. Reduction of the biofilms physical structure to a simple cake layer led to a loss in the hydraulic resistances dependency on EPS type. In conclusion, our results indicate specific EPS types can form distinct biofilm physical structures, which have a characteristic hydraulic resistance. Given hydraulic resistance is dependent on EPS type only when part of a structured biofilm, it is proposed that EPS accumulation effects biofilm hydraulic resistance in a structurally dependant manner.

P1: 21

The effect of anthranilate on microbial biofilms

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Anthranilate is an important intermediate for the syntheses of tryptophan and *Pseudomonas* quinolone signal, and degradation of tryptophan toward TCA cycle in *Pseudomonas* spp. While some bacteria, including *P. aeruginosa*, degrade tryptophan to anthranilate through a kynurenine pathway using the *kynBAU* genes, other bacteria harboring *tnaA* gene encoding tryptophanase that converts tryptophan into indole, pyruvate, and ammonia produce indole from tryptophan degradation (for examples, *Escherichia coli*, *Haemophilus influenzae*, and *Vibrio vulnificus*). Therefore, bacterial community that exists in tryptophan-rich environments contains anthranilate or indole (or both) necessarily. It has been recently reported that anthranilate deteriorates the biofilm structure by reducing the level of intracellular c-di-GMP and modulating the expression of Psl, Pel, and alginate in *P. aeruginosa*. We investigated the anthranilate effect on biofilm formation of various bacteria and the underlying mechanism of the anthranilate effect. Static and flow-cell systems were used for biofilm formation and direct confocal microscopic observation was carried out to monitor the anthranilate effect on biofilm formation. We also measured bacterial motility and intracellular c-di-GMP levels with the anthranilate treatment. Anthranilate has a significant inhibitory effect on biofilm formation of *Vibrio vulnificus*, *Bacillus subtilis*, and *Staphylococcus aureus*. Since anthranilate significantly enhanced swimming and swarming motility of *V. vulnificus* and *B. subtilis*, we suggest that the biofilm inhibition by anthranilate may be caused by the enhanced motility.

P1: 22

The HigB/HigA toxin/antitoxin system of *Pseudomonas aeruginosa* influences the virulence factors pyochelin, pyocyanin, and biofilm formation

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Toxin/antitoxin (TA) systems are prevalent in most bacterial and archaeal genomes, and one of the emerging physiological roles of TA systems is to help regulate pathogenicity. Although TA systems have been studied in several model systems, few studies have investigated the role of TA systems in pseudomonads. Here, we demonstrate that the previously uncharacterized proteins HigB (unannotated) and HigA (PA4674) of *Pseudomonas aeruginosa* PA14 form a type II TA system in which antitoxin HigA masks the RNase activity of toxin HigB through direct binding. Furthermore, toxin HigB reduces production of the virulence factors pyochelin, pyocyanin, swarming, and biofilm formation; hence, this system affects the pathogenicity of this strain in a manner that has not been demonstrated previously for TA systems.

P1: 23

β -lactamase production as a public good in *E. coli* biofilms

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Biofilms, which are a favoured bacterial lifestyle in many environments, have been implicated in recurring nosocomial infections and high mortality rates. They have also been shown to be highly resistant to commonly used antibiotics such as β -lactams. In this work an *in vitro* static biofilm model was used to investigate antibiotic penetration, as well as the social contribution of β -lactam resistance in dual-strain *E. coli* biofilms. We hypothesized that resistant, β -lactamase-producing cells could protect their susceptible neighbours efficiently in biofilms. In order to test this hypothesis, an isogenic pair of β -lactam-resistant and susceptible biofilm-forming *E. coli* strains was engineered, expressing different coloured fluorescent proteins. The resistant strain expressed a clinically relevant extended spectrum β -lactamase, encoded by the CTX-M-14 gene. We investigated the ampicillin-resistance of biofilms comprising one or both of these strains by a combination of competition experiments and confocal microscopy. In our models, biofilms were intrinsically 1000 times more resistant than their planktonic counterparts even when no β -lactamase-producing bacteria were present. Public good cooperation increased the resistance of the dual-strain biofilms beyond this intrinsic level. The protective percentage of resistant cells was found to equilibrate below 15% in almost all conditions studied. The β -lactamase-producing cells did not protect the biofilm by blocking antibiotic penetration, although their presence did reduce the penetration rate. Interestingly, we found that exposure to antibiotics also affected the positioning of resistant cells when present in low numbers.

P1: 24

Adhesion and biological characteristics of marine biofilms grown in flow

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Marine biofilms are a prevalent fouling challenge on marine vessels. Such biofilms are dynamic and heterogenic communities of primarily marine bacteria and microalgae encompassed in a sticky extracellular polymeric matrix, which mediates the adhesion to a vessel's hull. Though the financial and environmental consequences of vessel biofilm fouling are globally significant, characterisation of fouling marine biofilms is limited in scope. Much of the literature relates to biofilms grown in static conditions and most fouling control coating industry bioassays are likewise static, but fouling biofilms grow on and adhere to the hulls of even very fast moving vessels. This study investigates how hydrodynamic growth conditions affect marine fouling biofilm adhesion and biological characteristics, focusing on two hypotheses: that biofilms grown in higher flow are more adherent than those grown in static conditions, and that the metabolic cost of stronger adhesion incurs a growth trade-off. Time-dependent growth and adhesion of natural, multispecies marine biofilms on coated microscope slides in flow ranging from 0.1-1.4 knots will be presented, as well as additional detailed biological biofilm characterisation data. Though modest in comparison to the 15 knot average shipping industry vessel speed, the addition of flow nevertheless affects fouling biofilm characteristics. Implications for the fouling control coatings industry will be discussed.

P1: 25

An old dog with new tricks: functional characterization of a widely conserved t1ss-specific anchor domain

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Type-1 secretion system (T1SS)-secreted adhesins containing the repeat-in-toxin motif (RTX-Adhesins) are among the largest and most diverse proteins encoded by gram-negative bacteria. LapA-like proteins range from 280 kDa (*Vibrio cholera* FrhA) up to an astounding 900 kDa (*Pseudomonas putida* LapA) in size and contain multiple “sticky” repeating domains that are organized in seemingly countless combinations to optimize irreversible attachment to a preferred substrate. Studies from our lab on the archetypical RTX-Adhesin, LapA of *Pseudomonas fluorescens*, defined a widely conserved biofilm-promoting gene network comprised of three nodes that are required to faithfully regulate biofilm formation: a cell surface associated adhesin node (LapA), a T1SS translocation node (LapEBC), and an inside-out signaling protein pair (LapDG) that coordinate LapA cell surface release through a periplasmic cleavage event in response to intracellular cues. Here, we coupled bioinformatic analysis with molecular genetics to reveal a novel N-terminal anchor domain required for LapA cell surface retention, targeted periplasmic proteolysis, and protein stability. We also show that, despite their low-to-undetectable sequence similarity, anchor domains are functionally interchangeable, suggesting a previously unappreciated and broadly conserved T1SS-specific cell surface localization strategy. Interestingly, our recent studies suggest the LapA anchor domain can be fused to non-T1SS cargo for coordinated cargo release dependent on the intracellular state of the cell. These findings cast new light on conventional T1SS thinking and may be valuable for engineering protein delivery systems.

P1: 26

Biofilm formation by *Leptolyngbya mycoidea*

LEGE 06118: surface effect

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Biofouling is a huge problem for the marine sector as biofilm formation in ship hulls increases fuel consumption and surface corrosion. Additionally, detrimental impacts are also found in aquaculture (both in tanks as well as in off-shore fish farming). Cyanobacteria are early surface colonizers which promote the onset of surface macrofouling by barnacles, mussels and oysters. This work assesses the biofilm formation behavior of *Leptolyngbya mycoidea* LEGE06118 (a filamentous cyanobacteria isolated from Portuguese coast) during a three week period. Two surfaces were studied (perspex and glass) and results indicate that higher biofilm development occurred in perspex, as confirmed by thickness, wet weight and microscopy analyses.

P1: 27

Characterization of endoglucanases involved in bacterial cellulose synthesis

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With 52 subfamilies gathering more than 4500 enzymes, the family 5 is one of the largest glycoside hydrolase families. GH5 members, although they share a (β/α)₈ TIM-barrel fold and similar catalytic machineries, are endowed with a wide range of activities essential for the deconstruction of diverse polysaccharides. The GH5 cellulase RBcel1 is potentially involved in the synthesis of bacterial cellulose. This enzyme has, beside its hydrolytic activity, the particularity to catalyze the reverse reaction and synthesize cello-oligosaccharides via a transglycosylation. The main objective of our project is to understand molecular factors driving the reaction towards oligosaccharides synthesis rather than hydrolysis. In this work we determine the number of substrate binding subsites by biochemical and structural analysis.

P1: 28

Characterization of the bacterial population in a NiriTox fermenter

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Ammonia oxidizing bacteria (AOB) convert the first step of biological nitrogen removal in wastewater treatment, a sensitive microbial process that can be disturbed by toxic substances. A failure of nitrification in wastewater treatment plants can cause discharge of ammonia into rivers and lakes. The NiriTox fermenter (LAR Company) monitors incoming loads of toxicity by measuring oxygen consumption by bacterial fermenter community. The bacterial community populating the fermenter, however, is almost unknown. Dynamics and structure of the bacterial community were monitored over several months via denaturing gradient gel electrophoresis. In addition, size and bacterial colonisation into present flocks were analyzed by fluorescence in situ hybridization (FISH) combined with confocal laser scanning microscopy. High-throughput Illumina MiSeq of 16S rDNA (V1-V2) amplicon sequencing was applied to reveal the community composition. This study suggested that dynamics and structure of the bacterial fermenter community are stable over a period of time. Flocks seem to be predominantly inhabited by AOB and their size varied from small cell aggregates to a size of about 200x40x650 µm. Illumina MiSeq of 16S rDNA (V1-V2) shows *Nitrosomonas* as the main group and remaining bacteria belonging to heterotrophic genera within *Bacteroidetes* phylum. Sequencing of a broader-range 16S rDNA revealed a 99% similarity to *Nitrosomonas stercoris* strain KYUHI-S. For this recently described species, a specific primer set for qPCR and a FISH probe were designed. These tools achieve an easy monitoring of bacterial population, depending on existing technical and growth conditions within the fermenter.

P1: 29

Chemotaxis is required for organized biofilm formation in *Campylobacter jejuni*

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Campylobacter is the leading cause of bacterial food-borne gastroenteritis in the developed world. The ability of *Campylobacter* to survive in the food chain is key to its transmission and pathogenic potential. *Campylobacter* readily forms a biofilm under laboratory conditions and pre-existing biofilms shed large numbers of viable cells. The flagella make an important contribution to biofilm formation, and in this study we have investigated the role of the chemotaxis system of *C. jejuni* in biofilm formation. Chemotaxis mutants and complemented mutants were made and biofilm formation was visualised using both microscopy and by utilizing a microarray scanner. Following staining of the biofilm, use of a microarray scanner, employing both lasers (635 and 532 nm) permitted high-resolution scanning of the biofilm with good signal-to-noise ratio. Inactivation of the core chemotaxis genes resulted in significant morphological changes to the biofilm: while wild-type cells showed a robust biofilm at the air-media interface, chemotaxis mutants showed a disorganized biofilm below the air-media interface. Complementation with the wild-type chemotaxis genes restored robust biofilm formation at the air-media interface. Organized biofilm formation in *C. jejuni* appears to be highly dependent on a functional chemotaxis system. All chemotaxis mutants maintain flagellar motility, suggesting that the ability of *Campylobacter* to sense the environment plays a role in structured biofilm formation. These findings suggest new avenues to control *Campylobacter* in the environment.

P1: 30

Coliphages accumulate in surface water biofilms and sediments

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Biofilms are ubiquitous in aqueous environments and may act as reservoirs for pathogenic microorganisms. Accumulation of hygienically relevant bacteria in aquatic biofilms has been studied in detail before. However, little is known about the occurrence of viruses in biofilms. The aim of this study was to elucidate the distribution of somatic coliphages between water, sediment and epilithic biofilms in a river environment. Moreover, the occurrence of *Escherichia coli* as a commonly used faecal indicator and host for coliphages was assessed. Samples of water, sediment and epilithic biofilms were collected weekly in the period from July to September 2015 at three different sampling sites along the river Ruhr in Essen, Germany. *E. coli* was assessed using the Colilert-18/Quanti-Tray/2000 system in a most probable number (MPN) format. Somatic coliphages were quantified by a plaque assay according to DIN EN ISO 10705-2. *E. coli* was detected in all water samples with a mean concentration of 7.1×10^2 MPN/ml, while the mean concentration in sediment and epilithic biofilms was 2.1×10^3 MPN/g and 5.6×10^3 MPN/g, respectively. For coliphages, mean concentrations were 1.0×10^{-2} pfu (plaque-forming units)/ml in water, 8.2×10^2 pfu/g and 4.9×10^2 pfu/g in sediment and epilithic biofilms, respectively. In conclusion, the results show that not only faecally-derived *E. coli*, but also somatic coliphages accumulate in river biofilms compared to the bulk water. This indicates that surface water sediments and epilithic biofilms may play an important role as a reservoir for bacteriophages of faecal origin in surface water environments.

P1: 31

Comparative proteomics of different strains *Staphylococcus aureus* in planktonic and biofilm cultures

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Biofilm production represents an important virulence factor for *Staphylococcus aureus* (*S.a*). Its biofilm persistence in the environment of food industry poses a serious risk of food contamination. The biofilm formation mechanism in *S.a* is poorly understood and proteomics studies to understand the species-specific mechanisms of its formation are still missing. The aim of this study was to compare the proteomic profile of the planktonic and sessile form of five *S.a* strains with different behavior to form biofilm. The experiment was conducted on five strains: *S.a* ATCC 35556 (strong biofilm producer), *S.a* ATCC 12600 (moderate biofilm producer), *S.a* ATCC 29213 (weak biofilm producer), and two *S.a* wild isolate (strong and moderate biofilm producer). All these strains have been grown both in the planktonic and in the sessile form and analyzed through 2D electrophoresis coupled with MALDI-TOF MS. The data analysis has been performed in order to discover the mechanisms of biofilm formation common to all analyzed strains. Results highlighted 17 differentially expressed proteins between planktonic and sessile forms. Among the dataset, at least three proteins were found to be involved in stress response. This result provides new insights for the comprehension of mechanisms behind higher resistance of biofilm conformation to, for example, alcohol-based disinfectants. The discovered pathways could represent useful targets to counteract biofilm formation and to improve food safety.

P1: 32

Degradation of pharmaceuticals by iron/manganese depositing bacteria in biofilms at solid-water interfaces

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Pharmaceuticals are detectable in groundwater/surface water interface. Aquatic environments are impacted by these organic compounds and concern exists about their presence and the influence they exert on biological activities. This mainly occurs because pharmaceuticals are not completely degraded after application and they enter the ecosystem as metabolites, and some as unchanged forms. The aim of this study is to identify and to assess the microbial degradation potential for pharmaceuticals, especially by iron/manganese depositing bacteria (IMDB), as these bacteria species exist in aquatic environments and technical water systems. The degradation potential of IMDB in the presence of Mn^{2+} has been shown by Zhang et al. (2015). Therefore, further IMDB strains from freshwater environments were selected for laboratory degradation batch tests with pharmaceuticals carbamazepine (CBZ) and diclofenac (DCL). Preliminary degradation batch tests demonstrated that the presence of Mn^{2+} had an impact on degradation rates of CBZ and DCL. When no manganese ions were present in the media, degradation rates amounted to less than 20%. The degradation potential of pure cultures of IMDB bacteria, such as *Leptothrix* species and *Pedomicrobium* spec., were investigated in laboratory batch tests under aerobic conditions with a concentration of 1 $\mu\text{g}/\text{mL}$ CBZ and DCL. The degradation rates were analyzed by liquid chromatography-mass spectrometry (LC-MS). The study results indicated a degradation potential of IMDB for organic compounds.

Zhang, Y., Zhu, H., Szewzyk, U., & Geissen, S. U. (2015). Removal of pharmaceuticals in aerated biofilters with manganese feeding. *Water Research*, 72(0), 218-226

P1: 33

Extracellular matrix-associated proteins of microbial mats and their role in the underground environment

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The proteinaceous components of extracellular matrix of two microbial mats discovered in the endemic underground mines at 760-1000 m depth were characterized. Both mats were recognized in terms of biodiversity and metabolism. In these mats methanotrophic, sulfate reducing and sulfur oxidizing bacteria represented by *Methylomonas* sp., *Desulfovibrio* sp., and *Thiothrix* sp., were dominating. The metabolism of mats was strictly related to the geochemistry of the Lopingian, polymetallic and organic rich Kupferschiefer sedimentary rock. A common feature of these mats were numerous extracellular polymeric substances containing single- and multilayer membrane structures. Based on the metaproteomics, the origin and potential functions of extracellular material were proposed. In the proteome of the extracellular matrix structural and enzymatic proteins derived from every subcellular location and having different functions were detected. Beside typical proteins of biofilm matrix such as those responsible for biofilm formation, a group of specific proteins evidently related to the environmental conditions was identified. Among them enzymes participating in the degradation of fossil organic matter and methane metabolism were detected. A number of enzymes involved in assimilatory and dissimilatory sulfur metabolism and nitrogen assimilation were also identified. The proteomic characteristics of extracellular polymeric substances has provided a new insight into the potential functions of matrix and suggests that it may take part in the metabolic processes of microbial mats and also play a role in biogeochemical transformations occurring within the underground deep environment.

P1: 34

Identification of novel curli regulators in *Escherichia coli*

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Curli are thin fimbriae that determine adhesion of *E. coli* to biotic and abiotic surfaces. They constitute a major proteinaceous component of a complex extracellular matrix produced by many Enterobacteriaceae. Curli share structural properties with amyloid fibers and bind Congo red (CR) dye. In clinical and natural isolates, curli and other exo-polysaccharides such as cellulose direct the so-called RDAR phenotype on agar plate containing CR. To identify novel genetic determinants of biofilm formation and novel regulators of curli expression, we have screened the entire single gene knock-out collection of *E. coli* K12 strain BW25113. The mutation of about a hundred transcriptional units was shown to reduce CR-binding. The selected mutants belong to a wide variety of functional classes and include numerous transcriptional regulators. We show that curli fibers control most of the red colony-staining by CR together with LPS components. However, neither both cellulose and other exopolysaccharide are major determinant for CR-staining, nor outer-membrane proteins (except for CsgG). We confirmed the critical role of the low growth temperature for curli secretion and the involvement of at least twelve transcription factors to activate their expression. Surprisingly, many CR-binding defective mutants were able to promote biofilm formation (as a pellicle at the liquid- air interface). Finally, we have identified 8 novel mutations that specifically affect *csgBA* transcription and biofilm formation.

P1: 35

Influence of a dietary change involving consumption of simple carbohydrates on the composition of the microbial community in the supragingival dental biofilm

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Caries is associated with a shift in the microbial community in dental biofilms. Cariogenic plaque involves acidogenic bacterial species increasing in numbers due to frequent carbohydrate consumption. Few studies have analyzed the microbial community of environmentally induced bacterial shifts in the oral cavity. Therefore the influence of a defined dietary change on the dental biofilm has been studied. Eleven study participants wore splint systems containing six bovine enamel slabs for 3x7 days with 7-day intervals to obtain dental plaque samples, while keeping their regular diet. Subsequently, they went through a dietary change, sucking rock candy 5x per day for 3 months. Plaque samples were collected at the end of this phase. The regular diet of the study participants was continuously monitored. The microbial community of the oral biofilm was analyzed with microbiological culture and 16S rDNA cloning technique. Individual dental biofilm communities were dominated by oral streptococci and other members of the healthy oral flora before the carbohydrate-rich diet. Microbiological culture results showed a 6-27% increase of the viridans streptococci in the "rock candy" phase. The results of the cloning technique showed 36 different species for the first and 31 for the second phase with slightly more *Streptococcus tigurinus* and *S. salivarius/vestibularis* plus *S. parasanguinis* (19.2%, 8.2% versus 10.3%, 5.2%). An increase in viridans streptococci reflects a higher ability of the bacterial community for acid production. In recent years, next to *S. mutans* other streptococci, e.g. *S. salivarius/vestibularis* or *S. parasanguinis* have been suspected of contributing to cariogenesis.

P1: 36

Leaching and speciation of arsenic from polluted sediments: role of epipsammic biofilms

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In the Anllóns River (Galicia, NW Spain) high concentrations of arsenic (As) were found in surface and subsurface sediments attributed to natural geogenic origin exacerbated by gold mining activities. The objective of this study, conducted at the level of microcosm, was to evaluate the role of epipsammic biofilms developed onto the Anllóns riverbed sediments on the concentration and speciation of As released from As-polluted sediments. As mobility and bioavailability using different extractants, the distribution of As in extra- and intracellular compartments within the biofilm and As volatilization was also evaluated. Total As concentrations and speciation were determined by ICP-MS and HPLC-ICP-MS, respectively. The presence of epipsammic biofilm inhibited, by about half, the release of As to the water column in comparison with control sterilized sediments, as well as that of P, Mn and Fe. As^V was the predominant As species in the overlying water. As^{III} concentrations were higher in sediments without biofilm, which is of important toxicological relevance due to the usually higher mobility and toxicity of As^{III}. As solubility in different extractants followed the order: phosphate > sulphate ≈ TCLP > water. Significantly higher As concentrations were released in control samples in comparison with biofilm samples in sulphate and TCLP, as well as As bioavailability measured by DGT device. As retained by the biofilm was predominantly as intracellular forms, and inside cells significant concentrations of As^{III}, MMA^V and DMA^V were detected, which suggested active methylation (detoxification) processes. In summary, epipsammic biofilm play a key role in the As biogeochemistry.

P1: 37

Relationship between biofilms and metal corrosion: elucidation of the metal corrosion mechanism of *Phaeobacter* sp. FT01

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The metal corrosion caused by activities of the microorganisms is called a MIC (Microbiologically influenced corrosion). Because MIC causes damage to the metal substrata unexpectedly, even at normal temperature and neutral environment, MIC prevention is desired. Sulfate-reducing bacteria is known as the bacterium causing MIC. Sulfur-oxidizing bacteria, iron-oxidizing bacteria, and methane-producing bacteria are also known to be involved. However, the occurrence of metal corrosion has been reported in spite of the absence of these well-known bacteria. Interestingly, on the corroded metal surface, the presence of biofilm is often reported. Biofilm bacteria are physiologically distinct from free-swimming bacteria of the same species. Therefore, it is considered that the formation of biofilm plays an important role in MIC. Nevertheless, relationship between them is not well known. *Phaeobacter* sp. FT01 was isolated from seawater of Futtu in Japan, and biofilm formed on the metal surface and pit corrosion was observed, using Confocal reflection microscopy (CRM). Interestingly, the formation of biofilm changed in the medium that does not contain iron. Moreover the corrosion hole generated under the biofilm was easily observed by CRM imaging. In this study, we report the case biofilm formation in metal surface that leads to corrosion.

P1: 38

Role of small colony variants in reduced susceptibility of *Pseudomonas aeruginosa* biofilm to high-level disinfectants

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The occurrence of small colony variants (SCVs) in bacterial biofilms has been variously described. In this study, the impact of SCVs on the susceptibility of *Pseudomonas aeruginosa* to peracetic acid (PAA), a high level disinfectant commonly used in the disinfection of endoscopes was examined. SCV was isolated from 48 hours old *P. aeruginosa* PA14 wild type biofilm. The susceptibility of the 48 Hrs biofilm of the SCV to PAA was compared to that of 48 Hrs biofilm of the wild type *P. aeruginosa* through the evaluation of their metabolic activity after exposure to different concentration of PAA for five minutes using Alamar blue resazurin dye. The EPS producing ability of the SCV was also compared to that of the wild type *P. aeruginosa* through a crystal violet quantification of the total biomass of their 24 Hrs biofilm. The impact of age on the formation of SCVs was also examined. The SCV showed a significantly reduced susceptibility to PAA (P value <0.0001) compared to the wild type biofilm and also showed a significantly higher total biomass (Pvalue-0.001) indicating higher EPS formation ability. The proportion of the SCV in *P. aeruginosa* wild type biofilm also significantly increased with biofilm age. Result from this study indicated that population diversity phenomenon such as the formation of SCV in *P. aeruginosa* biofilm may play a significant role in bacterial biofilm survival of biocides.

P1: 39

The role of prophages on virulence transduction in *Acinetobacter baumannii* biofilms

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Recent years have observed an alarming increase on bacterial resistance to antibiotics. Many factors contribute to this, mainly antibiotics misuse but also an intrinsic capacity of bacteria to trade genetic material. These exchanges are emphasized in biofilms due to bacteria proximity, and involve several mechanisms including prophage-mediated transduction. Prophages are bacteriophages that incorporate into the bacterial genome, being able to excise and enter other bacteria. They are found in many bacterial species, being particularly frequent in *Acinetobacter baumannii*. This bacterial species is emerging as an important nosocomial pathogen worldwide especially due to a rapid acquisition of antibiotic resistance, in which prophage-mediated transduction may play a key role. The aim of this work was to evaluate the role of prophages on virulence transduction in *A. baumannii* biofilms. For this, an *A. baumannii* strain (ANC 4097) enclosing a prophage codifying a beta-lactam resistance gene and a receptor *A. baumannii* strain (NIPH 146) were selected based on biofilm-forming capacity. Strain susceptibility was tested for selecting a beta-lactam antibiotic to assess transduction. Both strains were genetically modified to follow transduction by fluorescence microscopy (mCherry inserted in the prophage and gfp in 146) and 146 was further modified to allow strain distinction on plate (lacZ). Levels of transduction were evaluated in mixed biofilms under different stress conditions (sub-MIC, light, and temperature). This work provides new insights into the importance of prophage transduction in virulence acquisition in mixed *A. baumannii* biofilms.

P1: 40

The transmembrane hybrid histidine kinase *bmsA* with a sensory chase3 domain is required for multicellular biofilm formation by *Pseudomonas alkylphenolia*

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Pseudomonas alkylphenolia KL28 can exhibit diverse multicellular behaviors such as aerial structures with *p*-cresol vapor, circular biofilm pellicles on the surface of LB liquid medium and spreading on the semi-solidified LB agar medium. This strain can also show swimming motility in liquid medium. A collection of transposon mutants was defective of multicellular behaviors including biofilm formation. The genetic analysis of those mutants revealed that all mutants contained a transposon insertion in a gene tag PSAKL28_21690 in *P. alkylphenolia* chromosome. It encodes a multi-sensor hybrid histidine kinase that consists of 1,164 amino acids with a predicted molecular weight of 130.3 kDa. The gene was named *bmsA* for biofilm formation and motility sensor. Protein domain analysis in BmsA revealed that it contains a sensory CHASE3 domain between two transmembrane domains at the N-terminal and consecutively, GAF, histidine kinase and three CheY-like response regulator domains to the C-terminal of the BmsA. In addition, the gene is co-transcribed with genes encoding proteins with CheR and CheB domains. Deletion of these genes, however, did not show apparent changes in surface related phenotypes. RNAseq analysis showed that the expression of genes coding for flagella synthesis is reduced in the *bmsA* mutant. This result suggests that BmsA is a sensor for surface-associated multicellular behavior necessary for biofilm growth. Results also showed that BmsA transduction system is not overlapped to the Gac regulon, mutation of which also results in the same phenotypes as that of *bmsA*.

P1: 41

Vibrio cholerae coordinates bile-salt promoted biofilm dispersal and colonization

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Vibrio cholerae can cause the diarrheal disease cholera when ingested, but is often associated with chitinous surfaces as a biofilm in its natural aquatic habitat. These biofilms are likely a natural route of infection, and may also be hypervirulent as both intact and manually disrupted biofilms out-compete planktonic cultures in a murine model of infection. While formation of *V. cholerae* biofilms has been studied in great detail, little is known about dispersal responses, particularly upon during transition into the host gut environment. In this study, we find that mature biofilms exposed to physiologic levels of the bile salt taurocholate (TC) show an increased number of planktonic cells with a concomitant decrease in biofilm mass. TC is also a host signal for *V. cholerae* virulence gene induction, yet we find that induction can only occur only after exit from the biofilm. Scanning electron microscopy images of TC-treated biofilms reveal an altered appearance, with less apparent matrix than controls. Furthermore, media from TC-exposed biofilms contains greater free polysaccharide than unexposed samples, consistent with degradation of the matrix during dispersal. TC does not affect polysaccharide production, and inhibition of protein synthesis does not alter dispersal rates, suggesting that *V. cholerae* is passive in this egress. Enhanced dispersal is specific to taruine-conjugated bile salts, leading us to hypothesize that this sulfonic acid-containing side group may contribute to direct degradation of the matrix. We thus propose a model in which *V. cholerae* ingested as a biofilm can co-opt the host bile salt signal to coordinate dispersal and virulence activation.

P1: 42

Bacterial adhesion driven by mechanical properties of plasma polymer coatings

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Different strategies are described in literature to limit bacterial adhesion without any use of biocide, mainly consisting in modifications of topographical, chemical or hydrophobic properties of the surface. Recently, a new approach was proposed, based on a judicious design of surface mechanical properties. Here, we investigate the potential of a plasma polymer functionalization to finely tune the mechanical properties of material surfaces. A monomer was selected for its capacities to undergo hydrogen bonding or van der Waals bonding in the plasma polymerized thin film, providing it hydrogel properties. By playing with plasma parameters, it was possible to obtain series of plasma polymer surfaces with the same chemistry but a wide range of mechanical properties, which were characterized in bacterial culture medium. Antibacterial properties were evaluated by cultivating *Escherichia coli* in selective medium, before *in situ* observation in the last rising medium by confocal microscopy. For short-term adhesion time, results show a ten-fold decrease of bacterial retention on the smoothest surfaces against the hardest ones. Moreover, more than 70% of bacteria on the smoothest surfaces did not adhere but were mobile (less than 5% for the hardest surfaces). Despite absence of flow, mobile bacteria had various displacement speeds as a function of the surface stiffness. Mean speeds were highest when substrate was soft. These results associated to others obtained after long-term incubation times demonstrate that soft surfaces can slow down the development of biofilm and may have influence on the structure, the density and the thickness of the mature biofilm.

P1: 43

Bacterial behavior on from hydrophobic to superhydrophobic coatings - consequences for their self-cleaning properties

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Superhydrophobic coatings have recently emerged as a new strategy to prevent biofilm development by acting on the adhesion of pioneer bacterial cells. Superhydrophobic coatings can be divided in three types: partial wetting coatings described by the Wenzel theory, non-wetting coatings described by the Cassie-Baxter theory and a mix of these two states. The aim of this study is to understand the link between the superhydrophobic state and the bacterial adhesion in air and aqueous surroundings. For this purpose, a range of coatings with six different levels of wettability (I) and coatings with a gradient of wettability (II) were produced by plasma (co)polymerization (I) and by a combination of nanoparticles and overcoating by plasma polymerization (II) respectively. Chemical composition and topography of the coatings were analyzed by XPS and AFM. Superhydrophobic properties were characterized by an innovative water droplet analysis. Two experimental approaches were used for the evaluation of the effect on *Escherichia coli* adhesion, both conducted by live imaging with confocal microscopy: i) coatings were completely immersed either in mineral or in Lysogeny broth culture medium; ii) coatings are contaminated in air by a droplet of culture medium. The results show a significant antibacterial (i.e., preventive) efficacy for coatings in Cassie-Baxter (I, II) and Wenzel (I) regimes. They point out that the primary steps of biofilm formation are affected by the self-cleaning effect resulting from the superhydrophobic propriety in a similar manner in both water and air surroundings. Bacterial adhesion on these coatings is now under investigation under flow conditions.

P1: 44

Biofilm development influences the migration of organic carbon from plumbing material

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Synthetic polymeric materials in household installations release organic carbon (OC) into drinking water. This carbon can be used by bacteria as a food source in the otherwise nutrient-limited drinking water, promoting both considerable biofilm formation on the material's surface and increased cell concentrations in the bulk water phase. Uncontrolled microbial growth potentially affects the water quality regarding sensory characteristics and consumer health in the case of pathogen establishment. So far, migration of OC from materials into the water was considered to be mainly influenced by temperature. We tested more than 100 materials for their OC migration and biofilm formation potential. Results unexpectedly revealed that the presence of a biofilm on a material's surface can enhance the migration of OC. An impressive example is the inner plastic tube of a shower hose that showed "normal" migration values if incubated for 2 weeks at 30° C under sterile conditions (OC = 6.2 µg/cm²), but more than 10 times elevated OC concentrations under the same conditions with bacteria present (OC = 91 µg/cm²). This increased amount of OC can further promote biofilm growth, or in the presence of other growth limiting factors (e.g. iron or phosphate limitation), it remains in the water phase as unknown compounds, potentially impacting consumer health. A microbial-enhanced migration was observed for several materials used in household plumbing. To our knowledge, this phenomenon has not been reported previously in the current context. If neglected, this effect can lead to a serious underestimation of a material's potential chemical and microbiological impact on drinking water quality.

P1: 45

Chlorella vulgaris (SAG 211-12) biofilms: assessment of the ability of adhesion to different substrates and of biofilm formation

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In large scale microalgal production the harvesting and processing steps of biomass from suspended cultures is a difficult and costly process. One way of obtaining biomass naturally concentrated for biotechnological processing is the production of organisms as biofilms (Gross et al., 2015). So, there is an interest in developing biofilm reactors. The microalga *Chlorella vulgaris* has been largely used in commercial production systems, essentially as suspended culture. Nevertheless, this species has several strains whose capacity of living as a biofilm is not known. Thus, evaluating strain capability of adhesion and biofilm formation is important to promote more economical biomass production and harvesting for the downstream production of biofuels and bioproducts. In the present work it was studied the capacity of *Chlorella vulgaris* (SAG 211-12) to grow as a biofilm. This strain is not often referred to in the literature, particularly in what concerns biofilms, but is promising for biofuel production. Simple protocols to test its capacity of initial adhesion to different substrates as Stainless steel (Inox), Polypropylene (PP), Polyethylene (PE) and Polyvinyl chloride (PVC) and to test the conditions for biofilm formation, were developed and validated. It was concluded that *C. vulgaris* (SAG 211-12) forms biofilms and PVC was the substrate promoting the highest adhesion and biofilm formation, followed by PP, PE and Inox. These findings will enable the development of new phototrophic biofilm reactors, needed for biorefinery applications of microalgae.

Gross, M, Jarboe, D, Wen, Z 2015 – Biofilm-based algal cultivation systems. Appl Microb Biotechnol, 99:5781-5789

P1: 46

Deciphering bacteria-surface interaction in *Pseudomonas aeruginosa*

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We seek to understand the role of surface-sensing in the motile-to-sessile transition of biofilm-forming bacteria. Using a unique combination of experimental approaches, we investigate *in situ* the dynamics of *Pseudomonas aeruginosa* bacteria adhesion to a surface under flow, as individual cells go from attaching to forming microcolonies. We combine reflection interference contrast microscopy (RICM), to measure the precise position and angle of each bacterium with respect to the substrate, and fluorescence microscopy, to visually detect the level of c-di-GMP within the cell. Post-acquisition image analysis provides the motility of each cell. Together, these measurements give a complete picture of the dynamics of a population of bacteria upon surface attachment, and of the phenotypic modifications taking place, with high spatial and temporal resolution. Our results highlight the co-existence of several subpopulations of cells with radically different phenotypes, and the role of each of them in early biofilm development.

P1: 47

Determining the spatial parameters of *P. aeruginosa* cell-cell signalling in cystic fibrosis using micro-3D-printing

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Chronic bacterial infection, such as that found within the Cystic Fibrosis (CF) lung, involves the formation of complex multispecies biofilm communities composed of small, dense aggregates ($\sim 10^1$ - 10^4 cells). However, knowledge is limited as to why these structured communities form or the role they play in chronic infection. The common CF pathogen *Pseudomonas aeruginosa* (*PA*) grows and forms aggregates in sputum in the CF lung. As a highly social organism, *PA* interacts both with itself and other microbes that co-inhabit the same environment. One manner in which *PA* communicates is via short-range chemical signals known to mediate quorum sensing (QS), a phenomenon thought to be critical for emergence and persistence of *in vivo* microbial communities. What remains unanswered is whether such signaling occurs as a localized event (inter-aggregate) or at the population level (intra-aggregate). To answer this question, we have developed a system to create finely tuned environments relevant to CF that contain a mixture of micro-3D-printed and naturally formed aggregates to explore the role of spatial structure in infection at the micro-scale. These findings will elucidate the importance of the biogeography of *PA* during CF infection.

P1: 48

Electrodeposition of polypyrenes with tunable hydrophobicity, water adhesion and fluorescence properties for anti-microbial adhesion and anti-biofilm applications

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The control in surface hydrophobicity and water adhesion is extremely important for anti-microbial adhesion or anti-biofilms properties. For the first time we will show that the use of fluorescent monomers such as Pyrene with various substituents differing by their hydrophobicity, size or rigidity/flexibility can lead to surfaces with tunable hydrophobicity, water adhesion and fluorescence properties by a direct electropolymerization process [1]. Seven original monomers with fluoroalkyl, alkyl, phenyl, adamantyl and triethyleneglycol substituents were synthesized and studied. The surface roughness is highly dependent on the substituent and it seems that the fluorescence signal correlates well with the surface roughness. Superhydrophobic properties and highly oleophobic properties are obtained using fluoroalkyl chains due to the presence of nanostructured microparticles. In comparison to the structured absorption and emission bands of Pyrene monomers, the Pyrene polymers exhibit a broad structureless spectral shape, where the loss of vibronic structure arises from the Pyrene oligomerization and loss of aromatic Pyrene core structure. This work is a first tentative to combine superhydrophobic and fluorescent properties using an innovative strategy. In order to investigate and demonstrate that such superhydrophobic and oleophobic surfaces have anti-microbial adhesion and anti-biofilms properties, results on two pathogenic bacteria (*S. aureus* and *P. aeruginosa*) will be presented.

[1] J. Phys. Chem. C, 2016, 120 (13), pp 7077–7087

P1: 49

Evaluation of potential resistance development by cells adhered to antimicrobial surfaces

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Immobilization of antimicrobials onto a surface has been proposed as a promising approach to fight biomaterial-associated infections (BAI). In this study, three antimicrobials, currently under investigation for use in medical devices, were evaluated for the risk of inducing bacterial resistance after their immobilization. An antibiotic, a quaternary ammonium compound (QAC) and an antimicrobial peptide (AMP) were immobilized onto polydimethylsiloxane (PDMS) using a mussel-inspired coating strategy. Results showed that antimicrobial surfaces exhibited contact-killing activity and were able to impair biofilm establishment. However, and similar to previously reported studies, a complete biofilm eradication was not achieved. The potential development of resistance towards these antimicrobials immobilized were then evaluated by continuously recovering the cells adhered to these antimicrobial surfaces and allowing them to adhere to new modified surfaces for a total of 10 passages. As a control, the same procedure was performed for unmodified PDMS. After 10 days, the cells recovered from the un- and modified surfaces were used to determine the MIC and MBC of antimicrobials. No propensity for developing bacterial resistance was found for immobilized QAC or AMP as the same susceptibility pattern was obtained for cells recovered from unmodified or modified surfaces. Cells recovered from the surfaces modified with antibiotic, exhibited a higher MBC as compared to cells recovered from unmodified PDMS. This study highlighted the risk associated to the immobilization of antibiotics and the promising potential of QAC and AMP to be used in the design of materials able to prevent BAI.

P1: 50

Impact of biofilms on performance of membranes applied for water production

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Biofilms growth in membrane systems hampers membrane performance. The occurrence, extent and time-scale of biofilm compaction and relaxation (decompaction) caused by permeate flux (water production) variations was studied. The impact of flux changes on biofilm thickness, structure and stiffness was investigated non-destructively with Optical Coherence Tomography using monitors operated at constant crossflow velocity with permeate production. The permeate flux was varied sequentially from 20 to 60 and back to 20 $\text{Lm}^{-2}\text{h}^{-1}$. The average biofilm thickness on the membrane decreased after elevating the permeate flux from 20 to 60 $\text{Lm}^{-2}\text{h}^{-1}$ while the biofilm thickness increased again after restoring the original flux, indicating biofilm compaction and relaxation. Within a few seconds after the flux change the biofilm thickness was changed and stabilized, biofilm compaction occurred faster than relaxation after restoring the original permeate flux. The initial biofilm parameters were not fully reinstated: the biofilm thickness was reduced by 21%, biofilm stiffness had increased, and the hydraulic biofilm resistance was elevated by 16%. Biofilm thickness was related to the hydraulic biofilm resistance. Membrane performance losses are related to the biofilm thickness, density and morphology, which are influenced by (variations in) hydraulic conditions. A (temporarily) permeate flux increase caused biofilm compaction, together with membrane performance losses. The impact of biofilms on membrane performance can be influenced (increased and reduced) by operational parameters. A (temporary) pressure increase leads to more compact biofilms with a higher hydraulic resistance.

P1: 52

Methylobacteria; keystone species in biofilm succession?

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The succession of bacterial biofilms in drinking water distribution systems is poorly understood. The first stage in this process is when bacteria colonise a surface. We show that the flow regime has a strong influence on the colonisation patterns that emerge over short periods of time. Laminar flow promotes a more distributed colonisation of the surface predominantly by individual bacteria that are spatially separated. Whereas in turbulent flow surfaces are colonised by microcolonies, which suggests that the formation of aggregates in the bulk phase is an important precursor to colonisation of the surface. Methylobacteria (Mtb) have been implicated in the formation of aggregates in drinking water. Thus we used 16S rDNA amplicons to identify a strain of Mtb that was present in drinking water from a domestic tap in Glasgow. Its abundance and growth kinetics were characterised. We inoculated drinking water microbial communities with Mtb at different relative abundances in Petri dishes and test tubes and recorded statistics on the motility and formation of aggregates. In Petri dishes, we tested different nutrient and moisture conditions. Test tubes were studied under both static and shaking conditions, and under both oligotrophic and eutrophic conditions. The motility of Mtb was assessed as a factor that may account for its ability to promote aggregation. We show that even at low relative abundances of 0.01 Mtb significantly enhance aggregation. This was proved by the positive correlation between the relative abundances of Mtb and the number of aggregates, the size of aggregates and the surface area that they occupied. Thus Mtb may be keystone species in biofilm succession.

P1: 53

Staphylococcus aureus adhesion on prosthetic vascular graft: an *in vivo* visualization and characterization of antibiotics efficiency.

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Dynamic fluorescence imaging combined with innovative and specific fluorescent probes have greatly improved the research concerning biofilms antimicrobial resistance: it gives the possibility to study non-invasively and *in situ* antimicrobials localization and activity according to the bacterial physiological state. However, this research currently concerns *in vitro* models of biofilms grown on polystyrene or glass: it remains debatable whether it is relevant for describing *in vivo* infections. In particular, *in vivo* biofilms may greatly differ from the *in vitro* ones not only from a structural point of view but also because of the presence of the host immune system that is difficult to mimic *in vitro*. In this context we have developed an original mouse model allowing to assess the antibiotics efficacy against vascular prosthesis-related *Staphylococcus aureus* (*S. aureus*) infections using the performance of 3D-fluorescence imaging: removed infected prosthesis implants, treated or not with antibiotics, were observed by fluorescence confocal microscopy. To control cellular viability and identify immune cells, bacteria and immune cells were labelled with a live-dead kit and specific antibodies respectively. Conclusions from this *in vivo* study are consistent with data from the *in vitro* model using the same strains and surfaces, indicating the relevance of the joined approach to examine drugs activities on infectious biofilms. Another interesting result is that living bacteria were internalized within the macrophages at the infection site, protecting them from antibiotics unable to penetrate these cells: a new insight in the recurrence of implant-related *S. aureus* infections?

P1: 54

The effect of blood conditioning films on the retention of bacteria

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Biofouling within the food industry is a critical problem posing vast economic costs upon the manufacturers and serious health risks to consumers. These hazards are particularly pertinent for those who are at risk; the elderly or immunosuppressed. Bacterial attachment and adhesion to surfaces is an important step in the process associated with foodborne infection and formation of biofilms, and is largely governed by the properties of both the surfaces and bacterial cells. Determination of these parameters could help the production of new antimicrobial surfaces. A TiNAg coating was produced using “unbalanced” magnetron sputtering and its characteristics defined, alongside stainless steel (304 2R), for roughness parameters, chemistry and physicochemistry (PC). Microbiological analysis was performed to determine the antimicrobial efficacy and mode of action against *Staphylococcus aureus* and *Escherichia coli*. Retention assays performed demonstrated that there were no significant difference in the numbers of retained bacteria, although a species-specific trend was observed. A bacterial respiratory detection assay, was successful in producing results against *E. coli* and *S. aureus* whilst zone of inhibition assays were only successful against *E. coli* suggesting different modes of antimicrobial action. Physicochemical analysis of both the bacteria and surfaces demonstrated that hydrophobicity was a main driving force for bacterial attachment. Roughness parameters did not have any effect upon retention. This work demonstrates that active antimicrobial coatings can be produced, and that their PC was important. Further, the method of antimicrobial testing used is important.

P1: 55

An extracellular self-assembling protein facilitates biofilm formation and resistance in *Clostridium perfringens*

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Clostridium perfringens is a Gram-positive spore-forming anaerobic pathogen, which causes gas gangrene, food or non-food borne poisoning. This obligate anaerobe forms biofilms and/or spores, and is therefore widely distributed in the environment. Previously we showed that *C. perfringens* forms biofilms with different structures in response to different temperature. At 37°C, cells adhere densely to the substrate surface, forming a thin biofilm. In contrast, at 25°C, cells produce a characteristic threadlike extracellular matrix, forming a viscous, thick pellicle biofilm. In the present study, we identified an extracellular protein BsaA as a biofilm matrix in pellicle biofilm. We detected high-molecular weight polymers (over 400 kDa) of native and recombinant BsaA proteins in addition to the monomers (predicted molecular mass of 21 kDa) by western blotting. Confocal immunofluorescence microscopy revealed that BsaA proteins extracellularly formed filamentous structures, which were localized to the biofilm surface layer, covering the entire biofilm. The absence of BsaA or an antibody against BsaA inhibited pellicle biofilm formation. Taken together, we propose that BsaA is a self-assembling protein that functions as a biofilm matrix in *C. perfringens*. Moreover, BsaA facilitated the biofilm resistance to oxidative stress. We suggest that temperature-regulated biofilm formation, associated with matrix production, is a strategy for adaptation to the environment, as temperature is an environmental signal that alternates between the outside and inside of the mammalian host.

P1: 56

An O-linked protein glycosylation system modulates attachment and biofilm development in *Vibrio cholerae*

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O-glycosylation in bacteria has recently been linked to essential and beneficial roles such as protection of glycosylated proteins against proteolytic degradation, protective immunity, cell adhesion and motility and cell-cell interactions. *Vibrio cholerae* encodes two putative oligosaccharyltransferases involved in O-glycosylation (O-OTases), which are integral inner membrane proteins and contain a conserved Wzy_C motif. For one of these enzymes O-OTase activity has been reconstituted in a heterologous expression system in *Escherichia coli*, showing that it has a relaxed glycan and target specificity. However, in *V. cholerae* the glycan substrate, target proteins as well as the physiological function of these enzymes remain to be elucidated. Here, we observed biofilm formation of single or double O-OTase deletion mutants under static and dynamic conditions. We could show that deletion of O-OTases alters initial attachment of bacterial cells to abiotic surface, results in enhanced biofilm formation and modulates biofilm architecture of *V. cholerae*. We hypothesize that O-glycosylation might be a feedback mechanism controlling biofilm formation by reducing attachment efficiency. Indeed, our experiments indicate that the activity of the O-OTases affect the secretion of adhesive proteins in the biofilm matrix, which mediate cell-cell or cell-surface adhesion.

P1: 57

Analysis of biofilm production in *Enterococcus faecium* strains depending on the clinical sources

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Enterococcus is one of the most important etiological factors of nosocomial infections. Although these bacteria are natural inhabitants of the gastrointestinal and genitourinary tracts, they can lead to serious infections, such as bacteremia, endocarditis, infections of the urinary tract and wounds. The aim of this study was to analyze the ability to produce a biofilm in *Enterococcus faecium* strains, depending on the patient's clinical material. A total of 66 strains of *E. faecium* (including vancomycin-susceptible and -resistant) were selected from the bacterial collection of the Department of Microbiological Diagnostics and Infectious Immunology. Automated system Vitek2 and the ID-GPC card was used for species identification, and the AST-P516 card for susceptibility testing. The biofilm production was determined using the tube method and the Congo red agar method. Among 66 strains of *E. faecium* 72.7% was biofilm-positive BIO+ and 27.3% was biofilm-negative BIO-. The majority of strains were from rectal swabs (30.4%), blood (18.3%) and urine (13.6%). BIO+ strains causing infections constituted 31.8% (52.4% of them isolated from the blood) and from colonization 40.9% (48.2% of them from rectal swabs). 91.7% of Blood Group strains produced biofilm, and 68.5% of Other Group strains (the difference nonsignificant). Strains from the Colonization Group produced biofilm in a similar proportion as in Infection Group (about 75%). There were no statistically significant differences in antibiotic resistance except vancomycin (more resistant BIO+ Other than BIO+ Blood Group and BIO+ Colonization than BIO+ Infection Group) and teicoplanin (BIO+ Colonization than BIO+ Infection Group).

P1: 58

Biofilm formation and curli production by Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 isolated from beef cattle in Canada

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Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is an important foodborne pathogen. *E. coli* O157:H7 strains are able to form biofilms on abiotic and biotic surfaces in the food industry and are often difficult to eradicate. Curli are proteinaceous fibrous structures that play a critical role in biofilm formation. This study evaluated biofilm formation and curli production by *E. coli* O157:H7 isolated from the feces of beef cattle across western Canada (sampled monthly from May 2013 to May 2015). Biofilm formation by 205 *E. coli* O157:H7 isolates was evaluated (72 h at 22°C) on polystyrene surfaces using the Crystal Violet (CV) staining assay and data was analyzed using cut-off OD₅₉₅ values defined as 3 standard deviations above the mean of the blank OD₅₉₅ (n=8). The experiment was repeated twice. The Congo red binding assay was used to determine curli expression with curli-producing and curli non-producing isolates forming red and colourless colonies, respectively. Biofilm formation occurred in 109 (53.2%) of isolates and 19% (39/205) were strong, 10% (21/205) were moderate and 24% (49/205) were weak biofilm producers. Of the strong, moderate and weak biofilm producers, 15, 8 and 3 isolates were curli-producers, respectively with no direct correlation between biofilm production and curli production among the strong and moderate biofilm producers. The high potential for biofilm formation by *E. coli* O157:H7 strains may contribute to bacterial colonization of food contact surfaces resulting in endproduct contamination. Understanding the mechanism of biofilm formation will aid in the development of intervention strategies to prevent outbreaks of foodborne illness.

P1: 59

Bioinspired nanostructured aluminium oxide surfaces with antiadhesive properties

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Biofouling poses a high risk for industrial processes and human health as well. Therefore anti-biofouling surfaces are needed in areas, such as in food industry, shipping, engineering and public health. Many such surfaces exist in nature, and artificial systems that mimic or are inspired by these natural systems could potentially be valuable technical surfaces. Recent studies on the interactions of bacterial cells with cicada wings revealed that rather than being effective at repelling bacteria, the wing surface prevents bacterial attachment by binding to and disrupting bacterial cell walls. Inspired by these structures, SmartMembranes GmbH used electrochemical precision etching to produce porous anodized aluminum oxide (AAO) surfaces with structurally well-defined surface nanopatterns having pore diameters of 200-300 nm. The porosity, topography, and hydrophobicity of the resulting surfaces suggested that they should exhibit strong anti-adhesive activity. We investigated these AAO surfaces to assess their potential to prevent biofouling, using *Escherichia coli* as a model microbe. Bacterial adhesion tests were conducted using a modular flow cell system that is designed to enable on-line observation of biofilm formation in continuous flow on opaque surfaces using a fluorescence microscope. The system was adapted to permit testing of multiple samples with various dimensions and material properties simultaneously. Bacterial adhesion tests showed that AAO surfaces with pore diameters of 300 nm exhibit 99 % less biofilm growth than electropolished stainless steel, which is widely used in the construction of industrial equipment.

P1: 60

C-di-GMP regulates the expression of *lapA* and *bcs* operons via FleQ in *Pseudomonas putida* KT2440

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Cyclic diguanylate (c-di-GMP) positively modulates the production of biofilm matrix components at the transcriptional and allosteric level in a variety of bacterial species. However, the mechanisms by which it regulates these components in *Pseudomonas putida* KT2440 remain unclear. Here we showed that c-di-GMP regulated the adhesin LapA, LapF and polysaccharides Bcs, Pea at the transcriptional level. Transcriptional regulator FleQ is required for the modulation of *lapA* and *bcs* expression by c-di-GMP, and has a minor influence on that of *lapF* and *pea*. We also found the *fleQ* mutant of *P. putida* was defective in biofilm formation and had smooth colony morphology. The transcription assay indicates the regulation pattern of FleQ to *lapA* and *bcs* operons is different. The expression from the *lapA* promoter decreased in the *fleQ* mutant, the opposite was true for *bcs* promoter. *In vitro* experiments show that FleQ binds to *lapA* and *bcs* promoter DNA. The binding to *lapA* promoter was likely to be slightly promoted by c-di-GMP, while c-di-GMP showed an undetectable influence on the binding of FleQ to *bcs* promoter. Our results show that c-di-GMP regulates the expression of *lapA* and *bcs* operons via FleQ in *P. putida*.

P1: 61

Differences in virulence and resistance between biofilm-producing *Enterococcus faecalis*, *Enterococcus faecium* and other *Enterococcus* clinical isolates

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Enterococcus spp. are one of the major nosocomial pathogens with the ability to form biofilm. The aim of this study was to compare the antibiotic resistance and the presence of selected virulence genes between biofilm-producing *E. faecalis*, *E. faecium* and other *Enterococcus* strains. Biofilm forming ability of 64 isolates (30 *E. faecalis*, 20 *E. faecium* and 14 other: 5 *E. avium*, 3 *E. casseliflavus*, 3 *E. gallinarum*, 3 *E. durans*) was determined by the tube method and Congo Red agar method. Then, biofilm-positive strains were chosen for further study. Antibiotic susceptibility was examined with Etests. Virulence genes (*esp*-surface protein, *ace*-collagen adhesin, *hyl*-hyaluronidase, *as*-aggregation substance, *gelE*-gelatinase, *cyl*-cytolysin) were tested by PCR followed by gel electrophoresis and sequencing. The ability to form biofilm occurred in 13.3% *E. faecalis*, 90.0% *E. faecium* and 57.1% rarely isolated strains ($p=0.026$). All *E. faecalis* strains were susceptible to β -lactams; while 37.5% of other strains and all *E. faecium* isolates were resistant. Resistance to gentamicin was detected in 75.0% *E. faecalis*, 55.5% *E. faecium* and 25.0% other strains; to streptomycin – in 25.0%, 83.3% and 50.0%, respectively. All isolates were susceptible to linezolid and tigecycline. Analysis of the virulence revealed that *esp* gene was found in all *E. faecium*, 75.0% *E. faecalis* and 37.5% other strains; *ace* in 100%, 25.0% and 37.5%; *hyl* in 83.3%, 0% and 37.5%, respectively. *as*, *gelE* and *cyl* genes were detected only in *E. faecalis* strains. This findings indicate that rarely isolated *Enterococcus* biofilm-producing strains have lower resistance and virulence potencies than *E. faecalis* and *E. faecium*.

P1: 62

Evidence of quorum sensing in the biofilm-forming soil and plant-associated *Pseudomonas fluorescens* SBW25

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The ability to form aggregates of different kinds, including biofilms, appears ubiquitous amongst bacteria. The sessile mode of bacterial communities is generally assumed to be regulated by quorum sensing (QS). Pseudomonads are not an exception: for many strains, QS is key to the ability to colonize a wide variety of environments. However, in the soil and plant-associated *P. fluorescens* SBW25, mutations in diguanylate cyclase (DGC) increase c-di-GMP levels to induce cellulose biofilm-formation at the air-liquid interface of static microcosms, and no involvement of QS-dependent behaviour has been reported. However, azithromycin (AZM) which is used as a QS-inhibitor in *P. aeruginosa* appears to have the same effect in *Pf.* SBW25, inhibiting biofilm-formation in static cultures whilst planktonic cells remain AZM-resistant. This suggest a possible QS involvement in biofilm-formation by *Pf.* SBW25. Our bioinformatics analysis of the *Pf.* SBW25 genome has identified putative N-acyl homoserine lactone (AHL) and α -hydroxy ketone (AHK)-dependent QS pathways, including an AHL/AHK synthase-like protein belonging to the HdtS family and a CqsA-like protein. Furthermore, a putative link can be established between QS and the regulation of c-di-GMP levels in *Pf.* SBW25 based on the TpbA/TpbB system of *P. aeruginosa*. Although quorum compounds have yet to be identified, tests using exogenous dodecanoyl homoserine lactone suggest that in *Pf.* SBW25, biofilm structure, eDNA, and possibly siderophore production, may all be regulated by QS pathways. This work is the first to provide experimental proof that *Pf.* SBW25 is capable of responding to AHL/AHK quorum signals like many other pseudomonads.

P1: 63

Identification of genes involved in biofilm formation in *Bacillus cereus*

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Bacillus cereus can form robust biofilms on the surfaces of food processing equipment, where cells and spores that detach become a troublesome source of food contamination. Toxins produced by *B. cereus* cause diarrheal and emetic food poisoning which typically lasts for 24 unpleasant hours. Despite the medical and industrial significance of *B. cereus*, molecular studies of the genus *Bacillus* have focused on the more easily transformed *B. subtilis*. In order to identify novel genes involved in biofilm formation of *B. cereus*, we have transformed *B. cereus* 10987 with plasmid pBTn, which carries an unbiased mariner transposon, and created a library of 5000+ transposon mutants of this notoriously difficult to transform strain. Using high-throughput microtiter assays, this library was screened for deficiencies in formation of surface attached biofilms and pellicle biofilms. We identified several interesting mutants deficient in surface attachment, as well as more than 300 mutants deficient in pellicle formation. Mutants deficient in surface attachment have disruptions in genes potentially involved in biosynthesis of cyclic-di-GMP and D-alanyl-lipoteichoic acids, which in other bacterial species are known to be involved in biofilm formation and regulation of surface charge, respectively. Other attachment deficient mutants have disruptions in genes with functions which were previously unknown, but we now show that they are involved in cell division, motility, and/or adhesion and biofilm formation. The characterization of all our biofilm deficient mutants is ongoing, with the aim of identifying and characterizing novel genes involved in biofilm formation of *Bacillus cereus*.

P1: 64

Inhibition of *Campylobacter jejuni* adhesion quantified by PCR-based methods

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Bacterial adhesion is the first step in development of a complex microbial community and once a biofilm is formed, it is very difficult to treat microbial cells because microorganisms within the structure are more resistant to antimicrobial agents than planktonic cells. In our study, we aimed to develop a novel approach for quantification of *Campylobacter jejuni* cells adhered in microtiter plates by using PCR-based methods. DNA isolated from 10 mixed *C. jejuni* strains was quantified by digital PCR (dPCR) to create the standard curve. Further quantification of the *C. jejuni* adhered cells in microtiter plate was performed by quantitative real-time PCR. We evaluated the anti-adhesion effect of natural antimicrobials from *Juniper communis* in sub-inhibitory concentrations (1 mg/ml) prepared as Juniper crude ethanolic extract from berries, from waste material after distillation of "brinjevec"- traditional drink and essential oil, two isolated fractions and pure amentoflavone, characterized by HPLC/DAD/ESI/MS. Juniper crude ethanolic extract and essential oil showed the greatest anti-adhesive effect on *C. jejuni* cells with adhesion reduction of $94.2 \pm 5.7\%$ and $99.9 \pm 0.1\%$, respectively. Following investigations focused on factors that can modify adhesion: i) roughness of stainless steel discs, ii) matrix (medium) of *C. jejuni* adhesion and iii) co-culture of *C. jejuni* with *Listeria monocytogenes*. In all cases, Juniper crude ethanolic extract and essential oil reduced adhesion of *C. jejuni* comparing to control. To our knowledge, this is the first accurate, sensitive and specific quantification of *C. jejuni* adhesion assays in microtiter plate with PCR-based methods.

P1: 65

Modification of platelet bag surfaces for prevention of bacterial biofilm growth in platelet concentrates

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Bacterial contamination of platelet concentrates poses a large risk to platelet transfusion recipients, with approximately one in 2000 units being contaminated. The most common bacteria that lead to transfusion infections are a part of normal skin flora, such as *Staphylococcus epidermidis* or *Serratia marcescens*. Whilst a number of bacterial detection systems are on the market, once bacteria become retained on a surface or form biofilms they may become more difficult to detect. Further, bacteria in biofilms demonstrate increased virulence and proliferation. Therefore, it may be advantageous to the transfusion service to implement a method of inhibiting biofilm formation on the surface of the blood bag so that contaminating bacteria remain in a planktonic state and are therefore more readily detectable prior to transfusion. Samples of plasticised polyvinylchloride (pPVC) film cut from platelet bags were flattened between polished stainless steel plates in a hot press and/or treated using atmospheric pressure plasmas. Surface hydrophobicity was determined via contact angle and surface roughness measurements using an optical surface profiler. Microbial adhesion to hydrocarbon assays demonstrated the physicochemistry of the bacteria, and retention assays were used to determine their retention on different surfaces. The results demonstrated that planarised pPVC showed a reduction in retention compared to unmodified pPVC. The plasma treatment of flat coupons demonstrated a change in surface hydrophobicity, altering bacterial adhesion. These results may lead to understanding a route to which surface modification can reduce initial bacterial adhesion, and reduce biofilm formation.

P1: 66

Morphological, nano-mechanical and molecular bases of *Shewanella algae* biofilm formation

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Shewanella algae is a marine bacterium of environmental, biotechnological and clinical relevance. The species combines a remarkable respiratory versatility, e.g. being able to reduce uranium or plutonium in anaerobic respiration, with a pathogenic potential to humans, being responsible for up to 80% of all *Shewanella* infections. Often, these processes are linked to biofilm formation. *In vitro* biofilm models are by far the most widespread when it comes to biofilm analyses. Even if biofilm formation is strongly influenced by the culture conditions, there is a lack of systematic studies to quantitatively examine the effect of growth conditions (e.g. nutrient environment) on relevant biofilm properties such as architecture, development and nano-mechanics. Hence, the first objective of our work pursued the quantitative determination of *S. algae* biofilm morphology, thickness, roughness, surface coverage, elasticity and adhesion forces under different *in vitro* conditions by means of Confocal Laser Scanning Microscopy, Atomic Force Microscopy and image-based analyses. In addition, we have identified that *S. algae* responds to certain environmental stimuli by forming elevated amounts of biofilm. Analyzing this phenomenon in >20 isolates, it was found to be strain-dependent. Thus, our second objective pursued the molecular understanding of this behavior. We show here for the first time that cell motility and biofilm formation are regulated by cyclic diguanylate (c-di-GMP) in *S. algae*. Insights on the implications of these findings for the colonization of biotic and abiotic surfaces by *S. algae* are discussed.

P1: 67

New insight on antibacterial mechanism of immobilized antimicrobial peptides

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Antimicrobial peptides (AMPs) covalently immobilized on surface devices have been recognized as promising candidates for a new generation of antibacterial coatings. These protein-like compounds have been the focus of great interest in recent years owing to their fewer propensities for inducing pathogen resistance compared to conventional antibiotics, their broad-spectrum activity, their high efficacy at very low concentrations, their target specificity, and their synergistic action with classical antibiotics. These advantages, however, are challenged by concerns about AMPs tethering to solid supports: after immobilization, these compounds are indeed less free, less flexible and are often at weaker local concentrations than in solution, thereby limiting their efficiency. Whereas the antibacterial activity of grafted AMPs is largely reviewed, understanding their structure-function relationship in their tethered conformation remains largely unknown. We describe in this study the crossed contributions of AFM, fluorescence microscopy and microbiological methods to decipher the role of AMPs immobilization parameters on their activity against *Staphylococcus aureus* contamination. First, AMPs, e.g. magainin II and nisin Z, have been immobilized through different orientations on self-assembled monolayers. Other parameters have also been studied. Then physicochemical characterization and microbiological evaluation of the modified gold surfaces have been correlated to provide a better knowledge of the structure-function relationship of tethered AMPs. This is a mandatory step to improve peptide-tethering strategy according to the physico-chemical properties of the substrate's surface.

P1: 68

Oral biofilm and caries-infiltrant interactions on enamel

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This study aimed to analyze interactions between oral biofilms and a dental triethylene glycol dimethacrylate (TEGDMA)-based resin infiltration material on enamel. Demineralized enamel (14 days, acidic buffer, pH 5.0) was infiltrated with a commercial TEGDMA resin and subjected to a three-species biofilm (group 1), *Streptococcus mutans* OMZ 918, *Streptococcus oralis* OMZ 60 and *Actinomyces oris* OMZ 745. Two other groups were applied to either biofilm growth (2) or resin infiltration (3). A control group received no treatment (4). Biofilm formation and metabolic activity were measured for group (1) and (2) after 24 h using colony forming units (CFU) and a resazurin assay. Biodegradation, uncured resin, was measured for group (1) and (3) by high performance liquid chromatography (HPLC) coupled with mass spectrometry after 6 and 24 h incubation. Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) images were taken to study the biofilm and material's autofluorescence in groups (1-4) after 24 h. SEM and CLSM images showed reduced biofilm formation on resin-infiltrated specimens (group 1). CFU data (\log_{10} CFU per mL) showed significantly reduced bacterial numbers ($p < 0.05$) in resin-infiltrated specimens with biofilms (group 1) compared to mere biofilm-coated specimens (group 2). However, HPLC analysis of TEGDMA leakage after 6 h and 24 h revealed no differences between biofilm-covered resin-infiltrated specimens and bacteria-free resin-infiltrated specimens. The results of the current study indicate that freshly resin-infiltrated enamel surfaces show a biofilm reducing effect, while monomer leakage was not affected by bacterial presence.

P1: 69

Photosynthetic biofilm formation as a strategy to avoid microalgal harvesting: the effect of surface physicochemical properties and culture medium composition

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The increased interest in photosynthetic microorganisms for wastewater treatment processes has led to the demand for new biomass harvesting strategies. Biofilm systems have emerged as a good alternative to planktonic photosynthetic cultures. However, knowledge on the environmental aspects influencing microalgal/cyanobacterial biofilm formation is required. This study reports the influence of: (i) surface physicochemical properties of selected microorganisms (*Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*) and materials (copper - Cu; glass - G; poly(methyl methacrylate) - PMMA; polystyrene - PS; polyvinyl chloride-PVC; and AISI316 stainless steel - SS); and (ii) culture media composition (glucose-deficient and glucose-enriched media) on biofilm formation. The results have shown that the selected microorganisms presented a hydrophilic character. Similarly, PMMA and SS were hydrophilic, whereas Cu, G, PS and PVC were hydrophobic. With the exception of *P. subcapitata*, all microorganisms formed biofilms on the selected materials. Moreover, higher biofilm formation ability was observed when glucose-deficient medium was used. The overall results demonstrate that fine-tuning on photosynthetic biofilm formation can be obtained by optimizing the bulk fluid composition and the type of surface.

P1: 70

Staphylococcus aureus field strain and its surface-exposed proteins connected with biofilm development induced by disinfectants

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It has been reported that sub-MIC concentrations of some disinfectants can induce bacterial biofilm formation. This biofilm phase is much more resistant to environmental stresses in comparison with its planktonic counterpart, which suggests on different metabolic activity between these two bacterial life forms. *S. aureus* biofilm was cultivated 48h statically with sub-MICs concentrations of three chosen disinfectants using fluorescent nucleic acid stain Syto9. We chose commonly used disinfectants, ethanol as a member of alcohols, benzalkonium chloride as a member of quaternary ammonium compounds (QACs) and chloramine T as a member of chlorine-releasing compounds (CRAs). With the aim to describe the first step of biofilm formation under the treatment with sub-MIC concentrations of these disinfecting agents we prepared surface-exposed proteins by enzymatic “shaving” and quantified them using dimethyl labeling and LC-LTQ/Orbitrap mass spectrometry. We observed that some of the sub-MIC concentrations of disinfectants induce biofilm formation and during this induction were determined up-regulated adhesins, proteins of cell-wall synthesis and couple of proteins with unknown function.

P1: 71

The effect of surface physicochemical properties on flocs formation and sedimentation kinetics of selected microalgae and cyanobacteria

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Microalgae are photosynthetic microorganisms presenting a diversity of biotechnological applications. However, microalgal cultivation systems are not energetically and economically feasible. Possible strategies that can be used to improve the feasibility of microalgal production include (i) biofouling control in suspended cultivation systems; (ii) the use of attached growth systems; or (iii) bioflocculation (a low-cost harvesting process). Since these processes are ruled by surface physicochemical properties, the effect of these properties on cell-cell interaction, aggregates formation and sedimentation kinetics was evaluated for *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*. Additionally, mixed cultures of the selected microorganisms were performed to evaluate the effect of co-cultivation on microalgal settling. The results have shown that, except *S. salina*, all studied microorganisms presented a hydrophilic surface. Regarding zeta potential determinations, all studied suspensions presented a negatively charged surface (approximately -40.8 ± 4.4 mV). Sedimentation experiments have shown that the studied suspensions presented low recovery efficiencies. However, a negative linear relationship between recovery efficiencies and free energy of hydrophobic interaction was obtained. These results demonstrate the importance of surface physicochemical properties on microalgal cell-cell interaction and settling. However, the low recovery efficiencies achieved, as well as the high net zeta potential values determined, indicate that another factor to consider in microalgal settling is the ionic strength of the culture medium.

P1: 72

Virulence of *Salmonella enterica* Enteritidis biofilms after exposure to different disinfectants

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Salmonella Enteritidis is a pathogen responsible for numerous outbreaks worldwide. Although many disinfectants are available, bacteria can survive disinfection and then express resistance to antibiotics and changes in gene expression. However, little is known about these phenomena regarding biofilm cells. Hence, this study focused the effect of chemical disinfection in the resistance and virulence of *S. Enteritidis* biofilm cells. The minimum biofilm eradication concentration of three disinfectants commonly used was determined, and biofilms were periodically exposed to sub-lethal concentrations of the disinfectants. *S. Enteritidis* biofilm cells were then characterized in terms of biofilm formation ability, resistance to antibiotics, and expression of virulence genes. Results showed that benzalkonium chloride was the most effective against *S. Enteritidis* biofilm cells and, regarding antibiotic susceptibility, these cells were less susceptible than the planktonic ones. Moreover, exposure to disinfectants has slightly altered the susceptibility to antibiotics but no resistance was observed, except for ciprofloxacin (to which planktonic and biofilm cells, before and after exposure, were resistant). Exposure to sodium hypochlorite and peroxide hydrogen enhanced biofilm formation, and benzalkonium chloride was the disinfectant that most influenced the overexpression of *S. Enteritidis* virulence genes. This study shows that biofilm cells that survive disinfection may represent an increased public health risk, since they can have lower susceptibility to antibiotics, enhanced biofilm formation ability, and overexpression of virulence genes.

Poster Sessions

Session 2

Biofilms, industry and energy

Productive biofilms

Modelling and simulation

Biofilm detection and
characterization methods

P2: 1

Bacterial production of transparent exopolymer particles during static and laboratory-based cross-flow experiments

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Biofouling of seawater reverse osmosis (SWRO) membranes represents one of the leading causes of performance deterioration in the desalination industry. This work investigates the biofouling potential of microbial communities present in a reverse osmosis (RO) feed tank. As an example, water from the RO feed tank of the Penneshaw desalination plant (Kangaroo Island, South Australia) was used in a static biofilm formation experiment. Cultures of the indigenous biofilms formed during the static experiment showed that α -Proteobacteria and γ -Proteobacteria accounted for nearly 80% of the classes of bacteria present in the RO feed tank. *Pseudomonas* sp. was identified as the major species and isolated for testing in static and laboratory-based cross flow biofilm formation experiments. Results showed that the volume of TEPs generated by *Pseudomonas* sp. during the laboratory-based cross-flow experiment was 10 fold higher to that produced during the static experiment for the same time period, while both experiments were inoculated with cell concentrations of the same order of magnitude. The availability of nutrients was also shown to be a key driver in TEP production, particularly for the static experiments. This study provides insights into the phenomenon of biofouling by assessing the production of biofouling precursors from one of the main genera of biofilm-forming bacteria, namely *Pseudomonas* sp.

P2: 2

Electron transfer by carbon-immobilized *Shewanella oneidensis*: effect of polarization, geometry and encapsulation

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The ability of *Shewanella oneidensis* to perform direct and indirect electron transfers makes them promising candidates for the elaboration of bioelectrode for microbial fuel cells. However, this requires that electroactivity of *S. oneidensis* is preserved and controlled on or within a solid support. Among the many parameters that need to be optimized, polarization and oxygen quantity were suggested to be key parameters for bacteria organization and current production. In this context, this study proposes to determine electrochemical responses of *S. oneidensis* in various polarization conditions and by using different carbon-based electrodes. *S. oneidensis* was cultured in minimal media with lactate and fumarate as electron donor and acceptor. Cells resuspended in minimal media were inoculated in carbon felt, home-made carbon foam/fibers or encapsulated in a hybrid carbon-silica/polymer conductive matrix. Samples were polarized at -0.3 V, 0 V and 0.5 V. Electrochemical measurements were performed in lactate buffer. SEM and epifluorescence imaging were used to characterize the electrode and the cells before and after measurements. Polarization impacts the current on carbon electrode: higher current increase is observed for a positive polarization compared to an absence of polarization, with sigmoidal patterns from -0.3 to 0.8V/AgAgCl. However, negative potentials cause a slower increase. By contrast, cells encapsulated in carbon-percolated matrix by electrospinning and casting keep their ability to transfer electrons with a low influence of polarization. These results open prospects for innovating bioelectrodes.

P2: 3

Impact of ageing on projectile diameter, contact area and shear force

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The application of online Automatic Tube Cleaning System (ATCS), where sponge projectiles pass through the tubes of shell and tube heat exchangers in the mitigation of fouling is well documented. A world-wide commercially available ATCS specifies replacement times for the sponge projectiles at every 1000 hours of chiller operation based on application of 30-minute injections intervals. There is no known publication in the open literature to recommend the optimum projectile end-of-life replacement time that would best influence the cleaning action. In this experimental study, spherical-type projectiles of different sizes (15, 16 and 17 mm), were passed through a 16 mm I.D. test rig approximately 2000 cleaning cycles for each ball to simulate the standard replacement time. The present study endeavours to understand what the impact of physical ageing (number of cycles) has on the contact area, stiffness (shear stress) and the diameter of the projectiles. The experimental results show that the projectile diameter and contact area increased with the number of cycles, while the shear force can drop in magnitude by approximately half. The 17 mm projectile delivers the highest shear force, both before and after the testing procedure. Many investigations have established that fouling resistance reaches an asymptote with projectiles. This is a point where further growth of foulants is balanced by their elimination due to shear-induced forces between water and/or projectiles and the foulants inside the tube. The results of this study may have implications for the characterisation of the asymptotic phase deposition period and thus chiller efficiency over extended operating periods.

P2: 4

Microbial safety and biological stability: a practical approach to ensure safe drinking water

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Safe drinking water is a vital component for ensuring public health. It is therefore of paramount importance that the supplied drinking water is of a consistently high and reliable quality. An important approach to achieving this high quality drinking water is to focus on microbial safety. The reliability and high quality of non-chlorinated drinking water delivered by Dutch water companies is not obtained effortlessly but as a result of an integrated approach summarised by the following four key components: (1) Microbiological risk assessment, (2) Hygienic Risk Reduction within the water supply chain (source to tap), (3) Control of biological stability and prevention of microbial regrowth within distribution network and (4) Adequate monitoring and corrective measures. In recent years, Water supply company Oasen (Gouda, the Netherlands) has conducted research and implemented additional measures aimed to decrease microorganisms present in drinking water. This increased attention for microbial safety was motivated by research results from 2006 which indicated that the quantity of higher organisms and *Aeromonas* bacteria present in the distribution network was greater than perceived desirable. The focus Oasen has placed on bacteriology since 2006 has had a notable effect upon the quality of the drinking water produced. The long-term focus has enabled Oasen to obtain an in-depth understanding of water quality issues as well as implement extensive improvement measures throughout the water supply chain. In the article the various actions and their results are explained in more detail.

P2: 5

Revealing the effect of water quality and daily temperature cycle on biofouling inside drip irrigation devices by means of optical coherence tomography

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Drip irrigation is a worldwide used water-saving technology. Uniform dripping at a constant drip rate is achieved by an internal labyrinth structure of the drippers, which are integrated in drip pipes. In (semi-)arid areas (i.e., in Israel or the south of Europe), treated wastewater (TWW) is used for irrigation. This results in biofilm formation inside the drippers, which progressively leads to clogging and malfunction. To date, little is known about how biofilm forms and drippers clog. Our study innovatively used the inner structure of drippers in 3D printed microfluidic devices (MFDs), to assess the clogging behavior in detail. To mimic the temperature conditions in (semi-)arid areas, experiments were conducted in a temperature box, simulating the daily temperature cycle between 20 – 50 °C for 30 days. MFDs were either fed with TWW ($BOD_5 = 5 \text{ mg/l}$) or synthetic wastewater (SWW, $BOD_5 = 18 \text{ mg/l}$). The biofilm formation inside the dripper was monitored non-invasively and *in situ* by means of optical coherence tomography (OCT). OCT data sets (3D) illustrate that biofilm development was influenced by fluid dynamics. Total volumetric coverage (V_{MFD}) shows that the formation rate is $1.4 \%V_{MFD}/d$ with SWW and $0.1 \%V_{MFD}/d$ with TWW in ambient temperature in lab. The malfunction of drippers can be assigned to the biofouling in the labyrinth. Biofilm coverage in the labyrinth up to 50% did not reduced the flowrate, whereas further coverage to 80% reduced the flowrate by 60%. Moreover, there was a clear effect of the daily temperature cycle on the biofilm formation. Biofilm formation rate was inhibited to 0.1-0.2 $\%V_{MFD}/d$ in daily temperature cycle independent of cultivation medium used.

P2: 6

The effect of pH and buffer concentration on anode biofilms of *Thermincola ferriacetica*

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We assessed the effects of pH and buffer concentration on growth and current production (j) in biofilms of *Thermincola ferriacetica* – a thermophilic, Gram-positive, anode-respiring bacterium (ARB). The objective was to compare *T. ferriacetica* biofilms to mesophilic *Geobacter sulfurreducens* biofilms. Experiments were performed with biofilms grown on anodes poised at a potential of -0.06 V vs. SHE in H-type microbial electrolysis cells (MECs) at 60 °C with acetate as an electron donor. A potentiostat was used to perform chronoamperometry under varying pH and bicarbonate conditions. For all conditions, media was replaced via continuous flow and then MECs were switched to batch to monitor j . Increasing bulk pH from 5.2 to 8.3 in *T. ferriacetica* biofilms grown with 50 mM bicarbonate buffer resulted in ~80% increase in j – with a smaller ~14% increase from pH 7.0 to 8.3- suggesting j inhibition at low pH and that H⁺ diffusion limitations are less significant at high pH in thermophilic biofilms when compared to mesophilic biofilms. Increasing bicarbonate buffer concentrations from 10 mM to 100 mM resulted in an increase in j by 40 ± 6% from 6.8±1.1 A m⁻² to 11.2 ± 2.7 A m⁻². Confocal laser scanning microscopy indicated that higher bicarbonate buffer concentrations resulted in larger biofilm thicknesses (L_f) from 68±20 μm at 10 mM bicarbonate to >150 μm at 100 mM, suggesting that buffer diffusion rates have a strong influence on L_f . The faster transport rates at higher temperatures and the ability to grow at relatively lower pH allows *T. ferriacetica* to produce higher j with lower buffer concentrations, making it an attractive alternative for low alkalinity wastewater applications.

P2: 7

Biofilms of nitrile hydrolyzing bacteria on the fiber materials for the treatment of amide- and nitrile-containing wastes

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Wastewater treatment of nitriles and amides of carboxylic acids (acetonitrile, acrylonitrile, acrylamide, etc.) using nitrile hydrolyzing bacteria can be an alternative to the chemical method. Biofilms of nitrile-utilizing bacteria may be grown on the carrier and used as biofilter. Biofilms of *Rhodococcus ruber* gt1 (harboring nitrile hydratase), *R. erythropolis* 11-2 and *Alcaligenes faecalis* 2 (amidase) were grown on carbon and mineral fibers. Transformation of acrylonitrile and acrylamide was performed in a batch reactor and submerged packed-bed reactor with water recycling at the rate of 6 ml / min. The concentration of organic substances in the samples was determined by HPLC. Biofilm reactor was operated for one month and acrylonitrile (16 g/l for one portion) was added in 11-fold portions. All introduced acrylonitrile was utilized by mixed biofilm, but 4.3 g/l of acrylamide and 2.8 g/l of acrylic acid was accumulated to the end of reactor operation as a result of the bioconversion. Biofilms of *A. faecalis* 2 on 1 g of fiber material transformed 100 mM acrylamide to 762 mg/l of acrylic acid after 2 hours in the batch reactor. After 48 hours of operation it was only 10 mg/l, that indicating utilization of acrylic acid. For biofilms of *R. erythropolis* 11-2 the concentration of acrylic acid in the sample was increased to 48 hours and reached 568 mg/l. Biofilms of *A. faecalis* 2 can be used for the complete degradation of acrylamide, *R. erythropolis* 11-2 - for acrylamide transformation to acrylic acid. Mixed biofilms of *R. ruber* gt1 and *A. faecalis* 2 can be used for the transformation of acrylonitrile to acrylamide and acrylic acid.

P2: 8

Characterization of biofilms of isolates from meat retail facilities

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According to EFSA cross contamination is implicated in about 40% of reported foodborne outbreaks, as pathogens can be transferred from several sources including raw foods during food processing to hands, cloths and food contact surfaces. Specifically, during slaughtering meat can be contaminated with bacteria directly by fecal contamination and indirectly by inadequately disinfected surfaces. This is aggravated by the persistence of foodborne pathogens due to their ability to adhere and form biofilms in surfaces despite of disinfection procedures. Besides, there is also a concern that the use of biocides may contribute to the development of antibiotic-resistant strains. Accordingly, isolates were collected from meat processing surfaces of retail facilities, isolated by selective media, identified by 16S sequencing and phenotypically characterized in terms of biofilm formation ability, susceptibility to disinfectants and antibiotics, viability and acquisition of cross-resistance. Planktonic cells showed low susceptibility to the disinfectants tested (hydrogen peroxide and sodium hypochlorite) and to antibiotics (rifampicin and linezolid). Moreover, isolates presented good biofilm formation ability and, as expected, a lower susceptibility to disinfectants and to antibiotics compared to planktonic cells. After exposure of biofilms to hydrogen peroxide at concentrations higher than the recommended ones, biofilms were still able to survive to antibiotics at a 10 x MIC. These results showed that bacteria may be exposed to only sublethal concentrations and survive which may contribute to bacterial resistance to these compounds, as well as cross-resistance to antibiotics.

P2: 9

Development of an online detection method to monitor growth and detachment of biofilms

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An experimental setup was designed for the reproducible cultivation of biofilms under flow conditions. This method serves as a basis for the implementation, characterization and assessment of alternative cleaning and disinfection methods. The test rig consists of a flow channel connected with a bioreactor through a pump in order to circulate the cultivation broth. The flow channel with an optical access from the top is electrically heated from underneath, which allows to monitor the growth of biofilms via the deterioration of heat transfer, respectively the resulting fouling resistance. The online measurement of the fouling resistance at eight measuring points along the flow channel was used to show the local growth of *Pseudomonas fluorescens* and potable water biofilms. Compared to the optical detection of the microbial growth, the fouling resistance detected the state when colonies merge together to a film. The increase of the film thickness is very well reflected by the increase of the fouling resistance, as well as the removal. First results suggest that biofouling does not show the typical negative fouling resistance values in the induction period attributed to the increasing roughness of the fouling layer associated with an increase in turbulence. This leads to the assumption, that the microbial colonies do not influence the roughness significantly. Furthermore cleaning experiments can be monitored online. Purging the flow channel with sodium hypochlorite (100 ppm of free chlorine) shows a short increase of the fouling resistance, due to stagnant flow just before the purging, followed by a decrease, caused by the detachment of the biofilm.

P2: 10

Heavy metal tolerance of single and mixed-species biofilms

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A study was undertaken to examine the tolerance of single and mixed biofilms for yeast *Rhodotorula* sp. and bacteria *Escherichia coli* LM1 (isolated from environment) on the presence of heavy metals (Cd^{2+} , Ni^{2+} , Zn^{2+} , Pb^{2+} , Cu^{2+} and Hg^{2+}). Single and mixed biofilms were quantified by crystal violet assay. Minimal inhibitory (MIC) and minimum lethal concentration (MLC) for single and mixed biofilms were determined and results were confirmed by fluorescent microscopy. The largest difference in tolerance was observed in the presence of Cd^{2+} , Zn^{2+} , Ni^{2+} and Cu^{2+} . MIC for Cd^{2+} for biofilms (*E. coli* LM1, *Rhodotorula* sp. and mixed biofilm) was noticed at a concentration of 26 057 $\mu\text{g/ml}$, 52 115 $\mu\text{g/ml}$ and 208 462 $\mu\text{g/ml}$, respectively. MIC for Zn^{2+} for biofilms was noticed at a concentration of 80 625 $\mu\text{g/ml}$, 161 250 $\mu\text{g/ml}$ and 322 500 $\mu\text{g/ml}$, respectively. MIC for Ni^{2+} for biofilms was noticed at a concentration of 38 437 $\mu\text{g/ml}$, 76 875 $\mu\text{g/ml}$ and 307 500 $\mu\text{g/ml}$, respectively. MIC for Cu^{2+} for biofilms was noticed at a concentration of 4000 $\mu\text{g/ml}$, 8000 $\mu\text{g/ml}$ and 64 000 $\mu\text{g/ml}$, respectively. Difference in biofilms tolerance in the presence of Pb^{2+} (4000 $\mu\text{g/ml}$, 4000 $\mu\text{g/ml}$, 16 000 $\mu\text{g/ml}$) and Hg^{2+} (31.25 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$) was lower. The results of single biofilms were compared with each other and with the results obtained for mixed biofilm. Mixed biofilm tolerance to the presence of heavy metals was larger in comparison to single biofilms. Obtained results open up the possibility of potential use of mixed biofilms in bioremediation of waste water.

P2: 11

Production of desiccation induced biotechnological products using terrestrial cyanobacteria

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Cyanobacteria offer a great potential for the production of biotechnological products for pharmaceutical applications. They can be divided into two groups: terrestrial and aquatic cyanobacteria. Terrestrial cyanobacteria are embedded in extracellular polymeric substances (EPS) and show a pronounced surface-associated growth. The EPS of terrestrial cyanobacteria can obtain higher levels of pharmaceutically active substances than their aquatic counterparts. As a consequence of their surface-associated growth they have not been used for the production of biotechnological valuable substances. Microorganisms are still a potent source for novel pharmaceuticals. Within the group of cyanobacteria some antimicrobial compounds could be described like Noscomin and Nostoflan from *Nostoc commune*. The production of Nostoflan is dependent on the duration and the parameters of cultivation. This study focuses on the production of antibacterial substances obtained from the terrestrial cyanobacteria *Trichocoleus sociatus*. A new emers photobioreactor (ePBR) allows a surface-associated cultivation of phototrophic biofilms. For the first time a successfully EPS production in an emers (surface-associated) semi-continuous process could be shown. Compared to submerge cultivations in shaking flasks the EPS production per amount of media per day was ten times higher under emers conditions in the ePBR. Two different bioactivity assays were performed to test the EPS on antibacterial effect. An antibacterial substance could be found whose recovery is much easier under emers than under submerge conditions. Currently, the focus is to characterize the new antibacterial substance to determine the chemical structure.

P2: 12

Production of primary and secondary metabolites in biofilms: are the consortia influenced by structured substrata on the microscale?

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Bacterial adhesion and biofilm development depend on many different factors, which can be classified in the properties of the environment, organism and material. As the substratum surface properties are essential for initial cell attachment, growth and structure of biofilms, it cannot be excluded that productivity is influenced as well. The aim within this project, which is part of CRC 926 *Microscale Morphology of Component Surfaces*, is the investigation of productive biofilms on microstructured metallic surfaces. After the establishment of different cultivation and analysis systems for biofilm formation, dependences of growth on surface topography could be detected for biofilms of *Pseudomonas fluorescens*. Also the proteome of the lactic acid bacterium *Lactobacillus delbrueckii lactis* is influenced by the structured substratum. Here primary and secondary metabolite production on different microstructured metallic surfaces in continuous culture will be presented. As an energy metabolism coupled product, formation of lactic acid by *Lactobacillus delbrueckii lactis* is depended of the culture growth. In contrary, the production of bioactive compounds usually depends on different triggers and the metabolism can be altered by switching the mode of growth. Therefore, nisin production of *Lactococcus lactis* biofilms is characterized depending on the state of cultivation (suspension vs. biofilm). Nisin is a natural antimicrobial peptide that effectively inhibits Gram-positive and Gram-negative bacteria. The formation as well as the production of biofilms will furthermore be triggered by external stimuli like microbial lysates.

P2: 13

Cyanobacterial biofilms – Exploiting the potential of CO₂ as a non-fossil carbon source for the production of high value compounds

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Microbial biofilms are characterized by self-immobilization, continuous regeneration and an increased tolerance to toxic compounds, which implies a potential long-term stability enabling continuous cultivation. Due to this robustness heterotrophic biofilms have been applied as living catalysts since decades. While a lot of efforts have been invested to develop catalytic biofilms based on organic carbon consumption, knowledge of autotrophic biofilms consuming inorganic CO₂ from the atmosphere is nearly inexistent. In this study we evaluate cyanobacterial biofilms as possible biocatalysts. Cyanobacteria as prokaryotic oxygenic phototrophs utilize CO₂ and water as sole carbon and electron source, respectively, driven by energy from light. Successful proof-of-concept studies for a variety of compounds spanning the range from biofuels to bulk and fine chemicals have been reported. However, all examples are based on suspended cell cultures and suffer from low productivities and insufficient catalyst stability. In this study we evaluate the potential of *Synechocystis* species PCC6803 biofilms as possible biocatalysts. We focus on cultivation and growth characteristics of phototrophic biofilms and reproducibility of experimental data using a metabolite of the central carbon metabolism as a reference product. Furthermore, we evaluated different materials and experimental set-ups for the cultivation of phototrophic biofilms. During cultivation in a segmented flow system, *Syn.* sp. PCC6803 developed a stable biofilm in both, glass and polystyrene capillaries and allowed continuous cultivation for several weeks.

P2: 14

Reactor systems for investigation of productive biofilms

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In recent years many approaches have been made to expand the application of biofilms to biotechnological processes for the production of both low-cost and valuable chemical compounds. The benefits of using biofilms in biotechnology include a higher space-time yield resulting from higher cell densities compared to planktonic cultures, a high system variability and lower susceptibility of the cells to unfavorable growth conditions, allowing for continuous production processes. The probably simplest system to grow biofilms is the tube reactor, consisting of e.g. glass or silicone tubes. Especially with respect to defining and characterizing hydrodynamic conditions, this system represents many advantages. For applications where a selective barrier is needed, e.g. to separate liquid and gas phase, membrane biofilm reactors have been constructed, in which the biofilm is grown on the membrane surface. Different types of reactors have been developed, e.g. a flow cell with flat sheet membranes or, at a larger scale, a reactor with hollow fiber membranes. The analysis of productive biofilms does not only require different configurations of reactor systems for the various applications, but the bioreactors also have to allow the investigation of the biofilm formation and structure. In this study, we adapted the reactor design to the application of Optical Coherence Tomography (OCT), which proved to be a promising new technology in biofilm research. This non-invasive imaging tool can be applied *in-situ* during reactor operation without disturbing system or process and provides high resolution images to characterize the biofilm on the mesoscale (mm).

P2: 15

Recombinant *Synechocystis* biofilms for CO₂ based 1, 2 - propanediol production

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The application of photoautotrophic microbes for light driven and carbon dioxide fueled fermentation processes demands new reaction concepts. Low activities, low stabilities, and excess biomass formation are known challenges to overcome in order to develop efficient photoautotrophic fermentation processes. Here we report on a biofilm based reaction concept to realize a truly continuous bioprocess. In this study we investigate the ability of *Synechocystis* sp. PCC 6803 for biofilm formation in combination with different reactor concepts for controlled biofilm cultivation. Biofilm growth of *Synechocystis* was investigated by confocal laser scanning microscopy in a segmented flow capillary reactor. For the production of propylene glycol, a bulk chemical used in the food and pharmaceutical industry, a heterologous three step pathway leading from dihydroxyacetone phosphate to propylene glycol was introduced into *Synechocystis* sp PCC 6803. Propylene glycol was successfully produced with planktonically growing cells. The combination of pathway engineering and a biofilm based reactor concept will be investigated as continuous light driven fermentation process with the long-term goal to develop a biofilm catalyst able to produce value added compounds driven by sunlight and CO₂.

P2: 16

Evaluation of biofilm mechanical properties using optical coherence tomography (OCT) and fluid-structure interaction simulations

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Evaluation of material properties of biofilms is a challenging task and has been done either by mechanical testing or from image analysis methods. Mechanical testing is hard to conduct on intact biofilms. Techniques such as rotational rheometry allow the determination of average material properties, but destroy the biofilm structure during the measurement. On the other hand, image analysis from bright field microscopy or optical coherence tomography (OCT), allows to extract material properties from non-destructive structural deformation, but calculation depends on assumption of e.g. wall shear stress. In this study, we found that data of OCT can be used in computational fluid-structure interaction simulations to easily access biofilm mechanical properties, such as the Young's modulus E . Biofilm structures were extracted from OCT scans, following biofilm deformation caused by hydrodynamic loading. The 2D or 3D geometries of the undeformed biofilm structures were implemented in a computational fluid dynamics model using a finite element software (COMSOL Multiphysics). Simulations of the experimental conditions allowed to obtain an adequate estimation of the flow field and shear stress acting on the biofilm structure. To fit simulated deformation to real deformed biofilm, the Young's modulus was used as sensitive parameter. The study showed that under a range of different flow conditions, values for E were found between 70-325 Pa. The outcome of the study showed that the Young's modulus should not be assumed a constant parameter. Coupling non-destructive experimental techniques to appropriate computational modeling, is well suited for the study of biofilm material properties.

P2: 17

Influence of oxygen concentration on initial *Pseudomonas putida* biofilm growth

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It has been reported that oxygen concentration could affect aerobic bacterial growth rate. But how oxygen availability influences biofilm development was not well elaborated. In order to study the influence of oxygen on *Pseudomonas putida* OUS82 initial biofilm structure formation, experiments were conducted in a square biofilm growth chamber with one input or output channel on each side, which could form a controllable oxygen concentration distribution inside the chamber through proper configuration of the input and output conditions. Using confocal laser microscopy real time imaging method, initial biofilm formation for nearly 6 hours were recorded. Surface coverage was chosen as the characteristic parameter because only initial biofilm growth was considered. Results showed that under higher oxygen concentration, larger surface coverage data was obtained. Successful mathematical modeling using the individual based modeling software, iDynoMiCs, with the help of regression approach to find the suitable values for several growth related assumed parameters, was conducted, which showed very similar phenomena as the experimental observation. In conclusion, both experimental and simulation results confirmed that even though similar initial attachment patterns were formed, oxygen concentration could significantly influence *P. putida* biofilm initial growth and colonization on the surfaces.

P2: 18

Mass transfer and kinetics of aerobic granules degrading 4-chlorophenol using microrespirometric model calibration

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Aerobic granules are stable biofilms formed in sequencing batch reactors (SBRs), with short settling time. Aerobic granulation is considered a promising technology for the treatment of a wide range of pollutants, including highly toxic compounds. Mathematical modeling of aerobic granular sludge is a subject of growing attention, but is also a complex task, because it should account for the biodegradation kinetics in heterogeneous media, in the presence of oxygen and substrate gradients. The development of mathematical models for aerobic granules, requires high quality experimental data for model calibration, obtained under the actual conditions prevailing in the reactor. In this work, a microrespirometric method; i.e., pulse respirometry in microreactors, was developed and applied to characterize aerobic granules degrading 4-chlorophenol as sole carbon source. Intrinsic and apparent kinetic parameters were determined, including the substrate affinity constant and the maximum specific growth rate, among others. In addition, the method allowed to estimate the effectiveness factor, which is useful for study and design of biofilm processes. These parameters were used for calibration of a one-dimension model, considering oxygen and substrate uptake kinetics. Simulations indicate severe mass transfer limitations during substrate consumption, even for small size granules. Moreover, pyrosequencing was used to identify the bacterial community of the granules. It was concluded that the experimental approach and the model developed, are convenient tools for the study and modelling of aerobic granules.

P2: 19

Modeling and simulation of the dynamics of *Listeria monocytogenes* biofilms thickness

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The investigation of biofilm formation through either quantitative image analysis or mathematical modelling has received substantial attention. This work combines both approaches to study L1A *L. monocytogenes* biofilms. Confocal laser microscopy and quantitative image analysis were used to characterize L1A biofilms along the life cycle (1h to 120h). The two-dimensional analysis of the images revealed that L1A1 biofilms are rather flat. The thickness was computed for several sampling times and replicates were performed so as to assess variability. The mathematical model was formulated in terms of a quasilinear system of diffusion-reaction equations for biomass and nutrients concentrations. Model predictions depend on the initial and boundary conditions as well as a series of unknown parameters related to the diffusion of microorganisms and nutrients, microbial growth, nutrient consumption and microbial decay. Model was solved by means of a combination of a Crank-Nicolson finite differences scheme and a Newton algorithm. Unknown model parameters were estimated by means of an optimization based approach. The idea was to compute model parameters so as to minimize a measure of the distance between the experimental and the model predicted thickness. This measure was formulated in terms of the log-likelihood function, so as to explicitly consider experimental variability. For this purpose the AMIGO (Advanced Model Identification using Global Optimization) toolbox was used. The model is able to satisfactorily predict the experimental data. To the authors knowledge this is the first work that addresses the quantitative comparison among experimental data and model predictions.

P2: 20

Initial factors determine the formation of mushroom-shaped structures in *Pseudomonas aeruginosa* biofilms

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Pseudomonas aeruginosa often colonises immunocompromised patients and the lungs of cystic fibrosis patients. It exhibits resistance to many antibiotics by forming biofilms, which makes it hard to eliminate. *P. aeruginosa* biofilms form mushroom-shaped structures under certain circumstances. Bacterial motility and the environment affect the eventual mushroom morphology. This study provides an agent-based model for the bacterial dynamics and interactions influencing bacterial biofilm shape. Our simulations show colony formation by immotile cells. Motile cells escape from a single colony by nutrient chemotaxis and hence no mushroom shape develops. A high number density of non-motile colonies leads to migration of motile cells onto the top of the colonies and formation of mushroom-shaped structures. This model proposes that the formation of mushroom-shaped structures can be predicted by parameters at the time of bacteria inoculation.

P2: 21

An in vitro biofilm model of prosthetic joint infections

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Prosthetic joint infections (PJIs) carry a significant health and economic burden. PJIs occur in 2.2% of Australian patients who undergo a primary total knee or a total hip replacement. Severe PJI can leave a patient with a poorly functioning joint and on long term suppressive antibiotics. PJI management requires the use of conventional antibiotics, debridement surgery and staged reimplantation of prostheses. Current treatments are often ineffective as a prosthetic joint may be covered in biofilm that renders bacteria up to 1000 fold more resistant to antibiotics than planktonic bacteria. Typical drug delivery techniques have little effect on biofilms making it difficult to fully penetrate the biofilm matrix. Prevention of biofilm growth is the ultimate aim. There is a clear need to halt infection to minimise high cost interventions that cause pain, disability and stress for the patient. The project aim is to optimise biofilm growth protocols to develop a high through-put pathway to assess antibiotic therapy in the efficiency of preventing biofilm production. Protocols for biofilm growth are centred on *Pseudomonas* spp. and are limited regarding *Staphylococcus* spp. Clinically relevant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* will be tested using the Minimum Biofilm Eradication (MBEC) assay, optimised to best produce biofilms for standardised testing. Biomass and cell viability will be measured. It is anticipated that a standardised biofilm model for *S. aureus* and *S. epidermidis* will allow for high through-put testing of current and novel treatments against biofilms on PJIs, helping to identify more effective treatments.

P2: 22

Comparing the impact of spacer-biofouling in forward osmosis and membrane distillation by lumped parameter modeling

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Reclamation of highly contaminated water has prompted the need for novel membrane-based technologies with low biofouling propensity such as forward osmosis (FO) and membrane distillation (MD). Permeate flux in FO is driven by an osmotic pressure gradient across semi-permeable membranes, whereas in MD water vapor permeates across hydrophobic membranes due to a thermally induced vapor pressure gradient. The role of spacers in membrane modules consists in maintaining a flow path between adjacent membranes as well as inducing turbulence. Turbulent flow enhances flux in FO by promoting mass transfer, while in MD flux is increased by augmenting mass and heat transfer. Spacers also impact biofouling development, a critical hurdle in membrane-based reclamation of wastewater with high fouling potential. The goal of this study is to develop a spatially lumped parameter model describing the combined effects of spacer-biofouling on heat and mass transfer and consequently on permeate flux in FO and MD systems. Biofilm properties such as porosity, tortuosity and thickness are applied to compute transfer coefficients for heat and mass transfer equations, influencing concentration and temperature polarization. Comparison of spacer-biofouling impact in FO and MD systems was done by modeling different scenarios in which biofilm and spacers were absent or present. The simulations indicate that the distinct driving forces cause a variety of spacer-biofouling effects that lead to differing impacts on permeate flux of the two systems. Our results shed new light on the application of spacers to enhance the suitability of FO and MD systems for reclamation of highly impaired water streams.

P2: 23

Film structure of class II hydrophobins HFBI and HFBII from experiment and computation: possible mechanism for accelerated film formation?

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Hydrophobin proteins, released by filamentous fungi, are the means through which they effect their environment in order to promote growth through interfaces. Their mechanism of action is to form a film at the interface that alters its properties in a fashion that promotes the growth of mycelia through it. In addition to their biological relevance hydrophobin proteins have also found many industrial applications. There are two classes of hydrophobins, delineated by the nature of film they create: class I hydrophobins form highly characteristic mesoscale structures, known as rodlets, while class II hydrophobins are amphiphilic and form a 2D crystalline film at the air water interface. Our study involves a combination of protein docking, molecular dynamics simulation, and electron cryo-microscopy to determine the structure of films composed of two class II hydrophobins: HFBI and HFBII produced by *Trichoderma reesei*. Combined, our results indicate a unit cell composed of six proteins; however, our computational results suggest P6 symmetry, while our experimental results show P3 symmetry, in both cases with a unit cell size of 56 Å. Our computational results indicate the possibility of an alternate ordering with a three protein unit cell with P3 symmetry and a smaller unit cell size, and we have made use of a Monte Carlo simulation of a spin model representing the hydrophobin film to present a possible mechanism through which the alternate metastable structure may play a role in increasing the rate of surface coverage by hydrophobin films, possibly indicating a mechanism of more general significance to both biology and nanotechnology.

P2: 24

Modeling and validation of diurnal carbon partitioning in microalgae biofilm

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Microalgal biofilms are gaining more attention as an alternative to traditional suspension cultures as they can reduce downstream processing costs [1]. For profitable large-scale microalgae production, sunlight should be employed. However, the sun imposes a daily cycle of light and dark on microalgae which affects its biochemical composition [2]. To understand the influence of diurnal light fluctuations on microalgae, biofilm growth can be mathematically simulated and modeled. In this sense, this work intends to develop and validate a biofilm growth model that takes diurnal carbon partitioning into account to predict microalgae biofilm growth under outdoor conditions. *Chlorella sorokiniana* was used as the model microalgae. The designed model differentiates between a functional pool and an energy storage (sugar) pool to describe diurnal carbon partitioning. Biofilm day/night (D/N) cycle experiments were performed to assess biofilm growth and validate the model. Four light schemes (24/0 h, 16/8 h sine and block, and 12/12 h block D/N cycles) were investigated. In general, biofilm productivity is slowed down by day/night cycling. This is a result of a lower light supply, as well as total biomass lost overnight. Similar biomass yields were obtained for all the light regimes. As so, total biomass lost overnight is likely due to sugar consumption for new functional biomass production and maintenance related respiration. Therefore, this work reinforces the importance of studying microalgae biofilm growth under natural light conditions and the developed model can be a useful tool for the design of industrial biofilm reactors.

P2: 25

Modeling of a multi-component biofilm in a simplified pore-network

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Biofilms allow microorganisms to live in a comfortable environment by improving their immediate physical and chemical conditions. Moreover, the amount, characteristics and distribution of such biofilms govern the capacity of soils to let water through, to transport nutrients and contaminants, and the nature of the reactions taking place. The aim of this work is to bring some light on this issue by modeling the proliferation of biofilm. Emphasis is laid on the distribution of biofilms within the porous medium, and the dynamics of its composition. In this work we present a multi-component model where biofilm is made up of four compartments: active cells, inactive biomass, and bound and soluble microbial products. The model is tested in a pore-network consisting of a number of cylindrical capillary tubes of different diameters on which biofilms grow attached. Such a simplified scheme is used as a proxy of the diverse local physicochemical environments found in real soils. Simulations computed with LiveLink™ for MATLAB® provide of valuable information about the distribution and the dynamics of biofilms at pore-scale. Finally, results allow us to upscale changes on the macroscopic properties for different soil textural classes.

P2: 26

Population level modeling of genetic regulation of biofilms

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Microbial biofilm can be defined as a large heterogeneous collection of cells working as a close-knit community as a microbial biomass and located on a suitable substrate and held together within a matrix of polymers and other molecules that have been secreted by this heterogeneous collection. The cells in this collection have varied genetic expressions accounting for division into two fundamentally different states – Planktonic and Biofilm. A novel quantitative approach to analyze this multiple genetic expression levels is the method of a Matrix Of Multiple Solutions (MOMS) built over the known Flux Based Analysis (FBA) method supported by an Ordinary Differential Equation (ODE) regulatory network specific to the biofilm associated gene pool of about 100 non-essential genes characterizing the genetic expression for the Flagella and Curli modes that are mutually exclusive and initiate, sustain and stabilize biofilm. The model is built using the known genetic annotation of the ecoli strain –ijO1366 and the additional 110 genes connected with biofilm. The MOMS solution set shows the distribution of the solutions that encompass a large set of gene expressions profiles. Specific matrix vector elements of this MOMS solution set identified by the regulatory ODE solutions corresponds to possible genetic outcomes towards formation of biofilm and modulation of the Flagella and Curli pathways. The energy and redox factors in these possible regulatory states provide a predictive method and a "what-if" analysis from simulation results to understand and look at possible drugs targets to manage and prevent biofilm formation.

P2: 27

The modelling of the polymicrobial communities using the species-specific biofilm inhibition by furanones

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Many investigations confirm that opportunistic bacteria like *Staphylococcus*, *Micrococcus*, *Pseudomonas* form so called mixed biofilms, where the cells of two or more bacterial species are co-embedded in matrix. We asked whether pathogenic bacteria are able to embed into the pre-formed biofilm, forming polymicrobial biofilm and avoid the antimicrobials and immune system. We found that the derivatives of 2(5H)-furanone exhibit different activity to various bacteria with MBIC varying from 2.5 to 50 mg per l. Using these compounds, we produce the mixed biofilm consisted of either *S.aureus* with *P. aeruginosa*, *M. luteus* with *B. subtilis* or *S. epidermidis* with *B. subtilis*, where the biofilm formation by the first bacterial species is inhibited by furanone, consequently, the biofilm matrix is produced only by the second bacteria. Using these systems, we show explicitly that *S. aureus* and *P. aeruginosa* which antagonize in the absence of antimicrobials, produce the polymicrobial biofilm and successfully survive when the biofilm formation by *S. aureus* was inhibited. Moreover, being in the biofilm, *S. aureus* became less sensitive to the ciprofloxacin and ampicillin.

P2: 28

A novel microfluidic gradient-generation flow cell for biofilms study

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Biofilm, featuring for its robust nature in terms of highly structural stability and high tolerance to unfavorable physical chemical environments, is considered as an important workhorse for industrial applications, biocatalysis and waste water treatment as examples. However, biofilm-based bioprocesses are difficult to predict and control, due to the highly heterogeneity. The complex development and performances of biofilms are governed by intrinsic heterogeneity, interactions with neighbor microenvironment, and signaling systems. In particular, sensing and responding to environmental cues and signals have been reported to play critical roles in biofilm formation and in regulating biofilm-mediated bioprocesses. Hence, quantitative elucidation of the relationship between biofilm activity and environmental cues and signals is required for controllable and predictable biofilm. In our study, we developed a novel fluidic biofilm reactor by integrating biofilm chamber with microfluidic gradient generator, in which biofilms could be grown under defined chemical gradients. This device is also compatible with imaging system, allowing direct monitoring on the dynamics of biofilm development. The chemical gradients generated were simulated and validated experimentally. Using two strains in *Comamonas testosteroni* as model organisms, we investigated the environmental controls on the metabolic pathways in nitrogen metabolism, and respective impacts on co-organism biofilms. Our results demonstrated that the novel fluidic biofilm reactor is a promising tool to understand biofilm development and bacterial responses to environmental cues, as well as environmental control of biofilm activity.

P2: 29

Application of AFM for morphological, mechanical and adhesive characterisation of microbial biofilms

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Last three decades have established the atomic force microscope (AFM) as an indispensable high-resolution tool for analysis of morphology, mechanics, as well as adhesion profiles of specimens ranging from single molecules to complex biological systems, in combination to commercially available optical microscopy. A number of important research issues in the field of biomedicine, are directly related to the increased antimicrobial resistance of various biofilms to commonly prescribed drugs, as well as, understanding adhesion, as the leading factor for biofilm formation, colony progression, and pathogenesis of microbial agents. The very high sensitivity of novel AFM systems have led to the development and customisation of particular techniques, that enable the study of single-molecule forces and adhesion profiles at the cell/cell or cell/substrate interface. We will give examples of the application of a new force tool “Quantitative Imaging” (QI™) based on fast force mapping which offers nanotopographical resolution with the opportunity of obtaining mechanical properties from various bacterial strains with pN-resolution. The “entire force distance curve behind every pixel” philosophy enables a full-range user-customised topographic and force spectroscopy analysis, including contact point (“zero-force”) images, Young’s modulus maps, as well as recognition events. The nanoscale mechanical compliance of bacterial walls can therefore be directly correlated to existing/hypothetic models of their structure. We will also discuss the application of novel fast scanning AFMs for the characterisation of dynamic biofilms with high spatiotemporal resolution reaching seconds per frame.

P2: 30

Biomechanics in pellicles: internal force, detachment and visco-elastoplastic behavior

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Progress in developing new anti-biofilm therapies and in understanding the resilience of biofilm infection requires a better understanding of biofilm mechanical properties. If biofilms mechanically respond to any external small load like a visco-elastic film, little is known about the internal mechanical force within a biofilm, what is the role played by this force on the biofilm resistance and proliferation, how they impact the mechanical behavior of biofilms at small and large deformations. To this end, we performed mechanical experiments in which *Bacillus subtilis* pellicles, or biofilms at the air-liquid interface, were subjected to elongational deformations using a custom built apparatus while simultaneously tracking the force response and macroscopic structural changes. We observed that pellicles behaved viscoelastically when we applied small deformations, such that the internal compressive force was still present, and viscoplastically at large deformations, to the point where the pellicles were under tension before the detachment. In addition, by using particle imaging velocimetry we found that the pellicle deformations were non-affine, indicating heterogeneous mechanical properties with the pellicle being more pliable near attachment surfaces.

P2: 31

Brevibacillus thermoruber B93: a remarkable biofilm producing strain for further industrial applications

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The biofilms of many thermophilic bacilli are still unclear. Hence, *B. thermoruber* B93 isolate was evaluated to detail the characteristics of its biofilm. The biofilm of B93 was examined on the basis of its motility, colony morphology on Congo Red Agar, the presence of cellulose and pellicle. The biofilm production of B93 was tested on polystyrene and stainless steel surfaces. For the determination of biofilm production conditions, the crystal violet assay was applied on the biofilms incubated at different temperatures (37-50°C), pH (4.0-11.0) and salinities (0.0-5.0 %). B93, having peritrichous flagella and non-mucoid biofilm morphotype, was determined as a powerful biofilm producer. It produced cellulose and a rigid pellicle swiftly. B93 also formed strong biofilms on polystyrene (OD: 1.47) and stainless steel surfaces (1.2×10^5 CFU/cm²). The optimum temperature for biofilm production was 50°C. When NaCl concentration increased, biofilm production of B93 was dramatically decreased. The isolate effectively produced biofilm between pH 6.0-9.5. Optimum conditions were applied for stainless steel application and the B93 formed stable biofilms on these surfaces. This is the first report for the biofilm characteristics and biofilm forming capabilities of thermophilic *B. thermoruber* under different conditions. The biofilm of B93 was significantly influenced by pH, NaCl concentration and temperature. In conclusion, *B. thermoruber* B93 have some features like fast productivity and proved to be a very promising model microorganism for extracellular polymeric substance production having industrial importance.

P2: 32

Calcium as an indicator of enamel demineralization caused by cariogenic biofilm

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Streptococcus mutans biofilms exposed to sugars are used to simulate cariogenic biofilms, and the quantification of calcium in culture medium would favor to understand the demineralization process over time. Therefore, this study evaluated the calcium released during enamel demineralization using *S. mutans* biofilms formed in the presence of sucrose or its constituent monosaccharides, glucose and fructose. Bovine enamel slabs (4 x 7 x 1 mm) were used. Saliva-coated slabs were immersed in LMW medium with 1% glucose containing bacterial inoculum and incubated for 8 h (37°C, 10% CO₂). *S. mutans* biofilms were formed during 5 days in LMW and exposed 8 x/day to two different conditions (n = 12): 10% sucrose or 5.25% glucose + 5.25% fructose. At the beginning and at the end of each day, the slabs were placed in fresh LMW. The medium was used to evaluate the pH and the calcium concentration. Calcium concentration was measured by colorimetric method using Arsenazo III. The enamel surface hardness was measured before and after the experiment and the percentage of hardness loss (% SHL) was calculated. The Student t-test ($\alpha = 5\%$) was used. The calcium concentration in the medium increased progressively after the cariogenic challenges, reaching a maximum value of 1.46 mM in the biofilm exposed to sucrose. The % SHL for sucrose group (35 %) was higher than glucose and fructose group (12%). A significant positive correlation between the % SHL and the total calcium amount was also observed ($r=0,82$). It can be concluded that the method was appropriate to evaluate the calcium released from enamel over time, and sucrose was the carbohydrate that induced more enamel demineralization.

P2: 33

Development of the *Galleria mellonella* animal model for studies of in-vivo biofilm colonization, virulence, and evolution in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a commensal human pathogen that causes significant morbidity and mortality within human populations. It is known that the biofilm lifestyle can play a role in both the carriage and disease states of this organism; however, the mechanisms by which biofilms may contribute to the transition from carriage to disease are not yet understood. Host colonization and biofilm formation by *S. pneumoniae* are impacted by a wide range of factors, including the ability of the organism to adapt, evolve and survive under *in-vivo* conditions within the host. *S. pneumoniae* biofilms rapidly undergo genetic and phenotypic variation during *in-vitro* biofilm growth. Small colony variants have been shown to display very different metabolic, proteomic, and biofilm formation characteristics. In order to best understand the importance of these variants, and their potential implications for survival within the host it is necessary to investigate biofilm formation *in-vivo* using an animal model. Here we use *Galleria mellonella* (wax moth) larvae as a convenient model to investigate biofilm formation and virulence characteristics in *S. pneumoniae in-vivo*. Through inoculation of the *G. mellonella* larvae with *S. pneumoniae* we observe decreases in the virulence of *S. pneumoniae* small colony variants relative to the parent strain calculated through the CFU required for a LD50. We have also utilised a range of imaging technologies to detect and quantify *S. pneumoniae* biofilm formation within *G. mellonella*.

P2: 34

Dynamics of biofilm development under well-controlled conditions: real-time imaging at high spatiotemporal resolution

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Biofilms are heterogeneous in nature. The development of biofilm is dynamic and closely correlated to their microenvironments. Imaging biofilm behavior at high spatiotemporal resolution combined with robust environmental controls are all essential for studying biofilm development in order to unravel the physics of life in microscales. We developed a robust precise engineering approach for (a) establishing well-defined microenvironments by employing our novel flow cell system and protocols; (b) enabling long-term, high-content, highly reproducible live imaging of biofilm development at single micrometer and single minute resolutions. Using this newly-developed method, *Pseudomonas putida* biofilm development was observed and quantified using confocal laser scanning microscopy. We present here for the first time unpredicted dynamic nature of *P. putida* biofilm formation followed by total dispersal under different conditions. Key biofilm development parameters, including bio-volume, cluster distribution, biofilm growth and removal rates, and doubling time were quantified. The biofilm exhibited three main behavioural differences, which clearly correlate to flow conditions, including biofilm clustering pattern, dispersal dynamic and doubling time. This correlation of biofilm behaviour to flow could only be observed with the spatiotemporal resolution made possible by our precise approach. Thus, we demonstrated that appropriate spatiotemporal resolutions are essential for unravelling the mechanisms underpinning the development of microbial biofilms.

P2: 35

Impact of *Actinobacillus pleuropneumoniae* biofilm mode of growth on the lipid A structures and stimulation of immune cells

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Actinobacillus pleuropneumoniae, the etiologic agent of porcine pleuropneumonia, forms biofilms on both biotic and abiotic surfaces. *A. pleuropneumoniae* biofilms are significantly more resistant to antibiotics than their planktonic counterparts. To our knowledge, no studies have examined the role of *A. pleuropneumoniae* biofilm in immune evasion and infection persistence. The objectives of this study were: (i) to investigate biofilm-associated lipopolysaccharide modifications occurring during the switch to biofilm mode of growth, (ii) to characterize pro-inflammatory cytokines expression in porcine pulmonary alveolar macrophages (PAMs) and proliferation of porcine peripheral blood mononuclear cells (PBMCs) challenged with planktonic or biofilm *A. pleuropneumoniae* cells. Extracted lipid A samples from biofilm and planktonic cultures were analyzed by high performance liquid chromatography-high resolution accurate mass spectrometry. Biofilm cells displayed significant changes in lipid A profiles when compared to their planktonic counterparts. Furthermore, *in vitro* experiments were conducted to examine the inflammatory response of PAMs (3D4/21 cells) exposed to UV-inactivated *A. pleuropneumoniae* serotype 1 grown in biofilm or in suspension. Using a qRT-PCR approach, relative mRNA expression of pro-inflammatory genes IL-1, IL-6, IL-8 and MCP-1 decreased in PAMs when exposed to biofilm cells compared to planktonic cells ($p < 0.05$). Additionally, the biofilm state reduced PBMCs proliferation. Taken together, *A. pleuropneumoniae* biofilm cells show a weaker ability to stimulate innate immune cells which could be due, in part, to lipid A structure modifications.

P2: 36

Investigating the relationship between antimicrobial agents mechanisms of action and anti-biofilm activity using confocal microscopy techniques

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Bacterial biofilms consist of densely packed communities that grow attached to surfaces and are responsible for severe infections in the human body. A major concern associated to biofilm-related infections is their high resistance to antimicrobial agents. As a result biofilms are hard to eradicate and there is an urgent need to better understand the mechanism of action of *conventional antibiotics* to develop new and effective antimicrobial agents. In this study, we use vancomycin that is known for its activity against gram-positive bacterial biofilm-forming strains in order to develop and optimize methods for studying the activity of antimicrobial agents against bacterial biofilms. To this end, viability and biomass were quantified using i) resazurin and crystal violet staining and ii) Live/Dead kit (Invitrogen) staining together with confocal microscopy z-stacks images. Data showed that vancomycin cannot eradicate completely mature biofilms and possible this is related with its diffusion within the biofilm structure. To further investigate this, the rate of biofilm penetration by fluorescently labeled vancomycin was studied using fluorescence intensity imaging techniques. The latter was quantified using an image-based FRAP (Fluorescence Recovery After Photobleaching) methodology. The results showed that the presence of a matrix of extracellular polysaccharide is not a barrier for vancomycin diffusion within biofilms. However, diffusion and penetration of vancomycin varies significantly through biofilm structure. Moreover, our results also support the idea that bacteria in biofilm form can produce compensatory mechanism in response to certain antibiotics.

P2: 37

Linking biofilm structural and mechanical properties: a case study of *Pseudomonas fluorescens* biofilms formed under different environmental conditions

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Biofouling occurs as a direct consequence of bacterial adhesion and colonization on surfaces. This phenomenon is generally undesirable on clean or technical surfaces such as medical devices or water filtration membranes. Despite the extensive work carried out to study and characterize biofilms in their environments, the link between biofilm structural and mechanical properties still remains poorly understood. The objective of this study was to determine whether the changes in biofilm structure and mechanical properties can be affected by environmental cues such as calcium ion or shear stress levels. In this investigation, static and dynamic biofilms of *Pseudomonas fluorescens* were grown in centrifuge tubes and flow cell devices at different concentrations of CaCl_2 and shear rate conditions. Biofilm mechanical properties were determined using AFM-based indentation and retraction analyses. In addition to this, biofilm structural properties were characterised using confocal microscopy and scanning electron microscopy. Initial results showed that biofilm structural and mechanical properties were affected by CaCl_2 levels in the environment, which determined the level of extracellular polymeric substances produced by the biofilm [1]. Current work now focuses on the biofilm structural and mechanical properties following development under different shear rate conditions in flow cells.

[1] A. Safari, O. Habimana, A. Allen, E. Casey, The significance of calcium ions on *Pseudomonas fluorescens* biofilms – a structural and mechanical study, *Biofouling*, 30 (2014) 859-869.

P2: 38

Mathematical methods to assess biofilm thickness in biofilters

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Experimental measurements of pressure drops through two biofilters (7.6 L in volume) filled with expanded schist for H₂S degradation were used to determine the change in the porosity of packed beds over time, and to calculate the thickness of biofilm covering the material. Three mathematical models characterizing the fluid flow through porous media were used for this purpose, i.e. (i) the modified Ergun model, (ii) the model of Comiti & Renaud and (iii) the granular RUC model. In order to highlight the influence of inoculation on biofilm development, one biofilter was inoculated with activated sludge from a wastewater treatment plant, whereas the other biofilter was not inoculated. Porosity values calculated by the models were compared to the experimental porosity measurements carried out in biofilters. Results showed that the model of Comiti Renaud cannot be used to predict the porosity of the packed bed, whereas the RUC model slightly underestimates the porosity values (discrepancy values from - 0.7 to - 21.2%). Conversely, the agreement between the experimental values and the values calculated by the modified Ergun model is remarkable for both biofilters. From porosity values obtained by this model, it was calculated that biofilm grew to reach average thicknesses up to 140 micrometers for the biofilter inoculated with activated sludge. Moreover, the development of biofilm slightly decreased the specific surface area of the packed bed (- 7% in comparison with the initial value) without modifying the tortuosity. For the non-inoculated biofilter, the development of a biofilm (thickness of up to 50 micrometers) was also deduced from pressure drop analysis.

P2: 39

Monitoring of fouling agents and fouling formation using 2D fluorescence spectroscopy

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In membrane processes involving biological compounds, membrane fouling is a major concern. Thus, the ability to monitor such complex compounds can significantly improve process operation, increase knowledge about these systems and help in process optimisation and control. 2D fluorescence spectroscopy is a highly sensitive and non-invasive technique suitable to monitor complex biological systems *in situ* and on-line using an optical probe. Fluorescence spectra are large matrices of data (EEMs) that may be regarded as system fingerprints. To extract and deconvolute the complex information contained in the fluorescence EEMs, mathematical tools are required, such as principal components analysis (PCA) and projection to latent structures (PLS). Fluorescence EEMs acquired from membrane bioreactors for domestic wastewater treatment were used successfully to develop multivariate statistically-based models able to predict several parameters: Cellular concentration and organic load (Galinha et al, *Water Research* 2012); potential fouling agents (polysaccharides in permeate, and bound and soluble extracellular polymeric substances). Additionally, 2D fluorescence spectroscopy was also used successfully to scan different membrane surfaces to evaluate fouling formation in two distinct processes: reverse osmosis (RO) and reverse electrodialysis (RED). Biofilm formation was evaluated at RO membrane surfaces covered with different biocides and used to treat brackish waters contaminated biologically. Fouling development was studied in a RED process operated during 30 days with real surface and sea waters (Pawlowski et al, *Water Research* 2016).

P2: 40

Multispectral mapping of microfouling development on colored coatings for ship hulls

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Biofilm fouling substantially increases frictional drag of marine vessels, with a global total fuel penalty exceeding \$25billion p.a. Biofilm characterization methods are key in quantifying marine fouling control coating performance. Multispectral vegetation indices hold promise as objective, macro-scale photosynthetic biomass mapping methods. Here multispectral indices are used to map developing biomass on six colors of the same non-toxic fouling control coating to test the hypothesis that coating color will affect the amount of biofilm due to absorption or reflection of specific wavelengths which in turn will influence photosynthetic biofilm biota. A panel of six colored coatings arranged in a 6x6 Latin square design was immersed in a marina in July 2016 and imaged on weeks 2, 4, 6, 8, 10, 12, 16 with a multispectral digital camera. Biofilm samples were collected on week 16, extracted in 90% acetone and chlorophyll concentrations determined by spectrophotometry. Nine reflectance index images were built and mean pixel values for the sampled areas were correlated to measured chlorophyll a densities. Best fits differed between colors (R^2 ranging from 0.14 for black to 0.85 for red) and combined green, red, and near infrared reflectance. At week 16, photosynthetic biomass in these fouling marine biofilms is not significantly related to coating color as measured by extracted chlorophyll density (ANOVA). Results from the reflectance indices time series will be further discussed. Relative to time intensive field sampling, mapping photosynthetic biomass by multispectral imaging is fast, non-destructive and potentially a useful quantitative monitoring and mapping tool.

P2: 41

Numerical characterization of biofilm structures formed by three *L. monocytogenes* isolates

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This work presents the numerical characterization of the life cycle of biofilms formed by three *L. monocytogenes* strains (L1A1, CECT5873 and CECT4032) in different surfaces (polystyrene and stainless steel). For this purpose, we combined several tools: epifluorescence and confocal laser scanning microscopy and 2D and 3D quantitative image analyses as well as a live/dead biofilm viability kit. Results show that all three strains follow a common sequence of patterns during their life cycle (up to 120 h): independent clusters, clusters interconnections, honeycomb like structures, flat pattern and finally decay and detachment. However, large differences regarding the magnitude of the structures and the duration of patterns were observed among strains and surfaces. Remarkably L1A1 forms the most stable biofilms, with denser long lasting structures. CECT5873 and CECT4032 result in thinner structures with faster life cycles. The origin of such differences can be: a) the differences in adherence due to the roughness of the surfaces considered; b) differences in motility among the species, L1A1 being the less motile and 3) the early appearance of dead cells for CECT5873 and CECT4032. This work exemplifies the potential of combining quantitative 2D and 3D image analysis to characterize the structure of biofilms throughout their life cycle. 3D parameters, i.e. the biovolume or the maximum height, facilitate the comparison of structures over time while 2D parameters are more informative in regards to structural features such as the presence and location of clusters, channels, etc. as well as the location and appearance of dead cells.

P2: 42

Sampling and identification of the dominant microbial population of presumptive biofilms in the food processing industry

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Detection and characterisation of biofilms in the food industry is fundamental. The aim of this study was to evaluate different surface sampling methods to assess the presence of biofilms in different food processing companies and to identify the microbial population of these presumptive biofilms. In 8 companies surfaces were sampled after cleaning and disinfection using 2 methods. The first method was scraping with a cell scraper followed by swabbing with a floqswab, the second method swabbing using a spongystick. Different microbiological and chemical analyses were performed on both types of samples. The dominant bacteria were identified using (GTG)₅ clustering followed by 16S rRNA gene sequencing. For total aerobic plate count (TAC) and *Pseudomonas* spp. slightly more points were found to be contaminated using the spongystick. Beside, average count for TAC on contaminated areas was 2.04 and 2.53 log CFU/100cm² for scraper and spongystick respectively. The same trend is observed for *Pseudomonas* spp. On the other hand, research showed that spongystick gives interference in the chemical analysis of the extracellular polymeric substance of the biofilm, making this method less suitable for chemical analysis of biofilms. In a meat processing company the microbial population in presumptive biofilms was dominated by Gram-negative bacteria mainly *Pseudomonas* spp. Also *Psychrobacter* spp., *Rahnella* spp., *Klebsiella* spp. and *Stenotrophomonas* spp. were identified. In a sauce producing company again Gram-negative bacteria dominated: mostly *Pseudomonas* spp. but also *Acinetobacter* spp., *Shewanella* spp., *Enterobacter* spp., *Lelliottia* spp., *Stenotrophomonas* spp and *Citrobacter* spp.

P2: 43

Simultaneous measurement of biofilm growth and flow using optical coherence tomography

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Biofouling of industrial water treatment systems is initiated by the attachment and growth of bacterial biofilms. These biofilms cause operational problems, such as an increase in operational pressure drop. This increase in frictional resistance is related to the morphology of the growing biofilms, which in turn is coupled to the local flow conditions. For better operational strategies and designs, an understanding of the growth dynamics under different flow conditions is crucial. Here we make use of Optical Coherence Tomography (OCT) to simultaneously measure/visualize the biofilm morphology and the surrounding velocity profile. This approach allows for detailed hydrodynamic analysis of multiple velocity components over the heterogeneous structure. A microfluidic platform is used as an experimental set-up. Channels with different geometries are fabricated from PDMS, typically between 200-400 micron wide and 400 micron deep. To measure the velocities, 200nm PS-PEG coated particles were used as tracers. From the OCT Doppler shift we measure a longitudinal velocity (parallel to the beam direction), and from the intensity signal we measure a transverse speed (perpendicular to the beam direction) [1]. The measured velocities are compared to numerical estimates from a 3D Comsol model. In the model, the Navier-Stokes equations are solved over an imported biofilm geometry obtained from the OCT signal. The results show good agreement between the measurements and simulations.

[1] N. Weiss, T. G. van Leeuwen, and J. Kalkman, "Localized measurement of longitudinal and transverse flow velocities in colloidal suspensions using optical coherence tomography," Phys. Rev. E88, 042312 (2013)

P2: 44

Solubilizing the hydrogel matrix of aerobic granular sludge is crucial to extract structural extracellular polymeric substances

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Within various kinds of biofilms, (aerobic) granular sludge is considered as a special case of biofilm, shaped to spherical structure. Granular sludge is a collection of cells, self-immobilized in extracellular polymeric substances (EPS) without any involvement of carrier material. EPS consists of polysaccharides, proteins, nucleic acids, lipids and humic substances, forming a dense and compact tertiary network matrix. At present, the idea of using one single method to extract all EPS components at the same time and under mild conditions is recommended for EPS extraction. However, mild extraction methods do not work well for aerobic granular sludge. Therefore, more harsh extraction methods targeting for specific EPS have to be established. The current research aimed to setup a methodology to extract EPS that contributes to the formation of the hydrogel matrix of aerobic granular sludge. Physical, chemical and physical in combination with chemical extraction techniques were compared. In addition, reactions of the extracted EPS with calcium ions were examined to test the gel-forming property of the EPS. It was concluded that, although different kinds of gel-forming EPS (may) exist in different types of aerobic granular sludge, solubilizing the hydrogel matrix of aerobic granular sludge (or biofilms) under harsh conditions is the prerequisite and crucial step to extract structural EPS.

P2: 45

Staphylococcus epidermidis, commensal to pathogenic?

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The commensal *Staphylococcus epidermidis*, is a leading cause of nosocomial implant-associated infections, and successfully evade the host by forming biofilms. It has been hypothesised that *S. epidermidis* colonising the skin and those causing infections differ significantly in the prevalence of factors involved in adhesion and biofilm formation. Here we took phenotyping and genomics approach to investigate if pathogenic *S. epidermidis* form biofilms better than commensals; and if this is related to genomic elements such as those involved in adhesion and biofilm formation. Genomic DNA from pathogenic and commensal *S. epidermidis* were sequenced, *de novo* assembled and archived in the Staphylococcal Bacterial Isolate Genome Sequence database. Phylogenetic trees were constructed and implemented in CLONALFRAME. For biofilm phenotype analysis, *S. epidermidis* were cultured, stained and visualised with a Confocal Laser Scanning Microscope (CLSM). Biofilm thickness, biovolume and roughness coefficient (Ra) were determined from the CLSM z-stacks using COMSTAT. The presence/absence of genes involved in adhesion and biofilm formation were examined. Genotypic classification randomly distributed the isolates into three clades, with the isolates being diverse in biofilm thickness, biovolume, and Ra. Statistically, only biofilm thickness had a significant association with clade distribution. The only significant association between the presence/absence of adhesion/biofilm formation genes and phenotype was between *aap*, *sesE*, and biofilm thickness. Thus *S. epidermidis* are from diverse lineages, and form different biofilm structures due to variations in presence of key adhesion/ biofilm genes.

P2: 46

Study of the biofilm characteristics of a single chamber microbial fuel cell (SCMFC) in fed-batch operation

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Microbial Fuel Cells (MFC) are a promising technology with potential application in electricity generation and in wastewater treatment. Electricity generation in MFC is accomplished by microbial catabolism, electron transfer from microbes to the anode, reduction of electron acceptors at the cathode and proton transfer from the anode to the cathode. Since MFC are complex systems involving biological and electrochemical processes, charge, mass and energy transfer, a better understanding of their fundamental phenomena is essential to achieve energy densities needed for real applications. Among the different phenomena, a better understanding of the transfer mechanism of electrons and protons between the biofilm and the anode is particularly important. Therefore, this study aims to evaluate the effect of different operating conditions (inoculation, yeast extract concentration, hydrodynamic stress and chemical mediator) on the biofilm growth on the anode of a SCMFC operated in fed-batch mode, using *Lactobacillus pentosus* and a synthetic dairy wastewater. The SCMFC has a cubic anodic chamber with 1L of working volume, a Nafion 212 membrane with an active area of 25 cm² and an open air cathode. The anode was a carbon fiber graphite brush and the cathode a plain carbon cloth coated with 1 mg.cm⁻² of platinum black. The electrical efficiency of the cell was evaluated by polarization and power density curves and impedance tests. The biological treatment was confirmed by estimating the chemical oxygen demand and the total organic carbon. The biofilm was extracted and characterized considering the *L. pentosus* active cells, its biomass dry weight and polysaccharides and proteins content.

P2: 47

The chemical structure of the biofilm matrices of *Candida glabrata* induce resistance to antifungal drugs

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Candida infections are often associated to biofilms and consequently to high resistance to the most common drugs. These resistance mechanisms are not only associated with the biofilm yeast physiology, but also with the presence of a barrier imposed by the biofilm matrix. However, the biochemical role of the biofilm components remains very unclear. Therefore, this work intends to further enlighten the effect of antifungal agents on *C. glabrata* biofilm resistance. As a good biofilm former, *Candida glabrata* ATCC 2001 was selected to this study. Several antifungal drugs, belonging to different groups, were used in this work, namely fluconazole, voriconazole, amphotericin B, caspofungin and micafungin and their effect on biofilm matrix was assessed. Biofilm matrix chemical composition and structure was evaluated by analytical methods, specifically by HPLC and mass spectroscopy. As expected, *C. glabrata* biofilms were resistant to the antifungals used in an agent-dependent manner. The results showed significant differences in polysaccharides and proteins contents, in the matrix of biofilms formed in the absence and in the presence of the drugs. Moreover, the diffusion through the matrix was evaluated demonstrating that different agents, even belonging to the same group, present very different diffusion profiles, explaining the different tolerance registered. So, with this study we confirmed that *C. glabrata* biofilm's resistance to antifungal drugs is a very complex mechanism, where the matrix plays a major role.

P2: 48

The MagPI device or how to determine biofilm adhesion in fine sediments?

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The ETDC (Erosion, Transport, Deposition, Consolidation) cycle of fine sediments is crucial for the ecological and commercial health of aquatic habitats. It is now commonly accepted that the organisms inhabiting natural sediments mediate their erosive response. This is especially true for microbes that form biofilms to glue fine sediment particles together. Since long, engineers aim to determine sediment stability as such and the biofilm-induced changes in particular; however, all methods established so far require bed failure to occur. MagPI (Magnetic Particle Induction) is a highly sensitive and non-destructive method with a small footprint that allows high resolution measurements - both in time and space. Ferromagnetic particles are spread onto the biofilm surface and the force needed to retrieve those particles by an overlying electromagnet is a direct measure of the adhesive forces of a surface biofilm and a good proxy for sediment stability. Still, the method has been taken further in the last years to better understand the working principle and the forces acting, as well as to further automatize application and data evaluation. The presentation will give an overview on the main steps in the development of the MagPI system as well as the status quo of research and application while highlighting significant results on biofilm-induced sediment stability in freshwater systems.

P2: 49

Using new scanning electron microscopy (SEM) approaches to elucidate bacterial biofilm architecture.

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Healthcare-associated infections (HAI) are a major public health problem being *Klebsiella pneumoniae* and nontuberculous mycobacteria, both with high antibiotic resistance rates, among their etiological agent. Since biofilm assembly is pointed as one of the mechanisms involved in emergence of antibiotic resistance understanding bacteria organization within the biofilm and the identification of differences between planktonic and sessile forms of bacteria will be a step forward to fight HAI. In the present work we used SEM as a tool to characterize the internal structure of biofilm assembled on different surfaces. For SEM analysis, biofilms were allowed to form either on six-well cell culture plates, silicon or metallic disks placed inside the wells for different incubation periods at 37 °C. The biofilm assembled on the cell culture dish was for both secondary and backscattered electron analysis as described before. Biofilms assembled on silicon disks instead of being sectioned were prepared as metallographic samples, by grinding with grit SIC paper and polishing with diamond particles. Samples were cleaned (70% ethanol), dried with hot air, further coated and analysed. A preliminary study using FIB-SEM has been performed to access the ultrastructure of biofilms assembled on metallic surfaces. The results obtained showed that the same bacteria assembled biofilms with different ratios of biomass and extracellular matrix depending on the surface. SEM performed on thin sections of biofilms is a powerful tool to elucidate biofilm structure allowing the quantification of the major components. FIB-SEM is also a promising tool in this field.

P2: 50

X-ray microtomography imaging of biofilms in porous media

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Biofilms developing in porous media can induce significant changes in transport properties (porosity, permeability, flow) with implications in many engineering and medical applications (biofilters, soil remediation, MEOR¹, implants and orthopaedic² infections). Various processes controlling the growth of biofilms in porous media (e.g. the impact of environmental constraints on spatial distributions of microorganisms and the relationship between the various scales involved) are still poorly understood. This is mostly due to the limits of imaging methods currently used. CLSM allows for imaging on a flat surface with micrometer accuracy up to several millimetres but its application to a porous (and therefore opaque) structure is extremely limited. MRI-based methods in development do not currently provide sufficient isotropic spatial resolution. Approaches using X-ray microtomography are also being developed as, in theory, the technique can be used for imaging large volumes of porous media with submicron resolution. However, absorption coefficients for an aqueous phase and the biofilm are very similar and, therefore, contrast agents must be used to differentiate the two phases. Methods using contrast agents such as silver-coated microspheres³, C₁₀H₇Cl⁴ and BaSO₄⁵ have been elaborated. We will present an improvement over previous work by Davit *et al.* 2011⁵ using BaSO₄ which provides a sharp contrast between the different phases of the medium. This approach has been validated against two-photon microscopy of biofilm growth in glass capillaries. We will also demonstrate how it provides for both biofilm distribution in pore space and biofilm morphology in 3D-printed porous media.

P2: 51

A novel polysaccharide synthesized by a KPC-producing *Klebsiella pneumoniae* clinical isolate. Investigation of the *cps* gene cluster differences.

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Polysaccharide production is a well-recognized virulence factor of *Klebsiella pneumoniae* (KP), being involved both in escape from phagocytosis and in biofilm formation. Strains well adapted to the hospital environment are often good biofilm producers. On the contrary, *K. pneumoniae* producing KPC-type carbapenemase (KP-KPC), of great concern for their multidrug resistance phenotype and for high morbidity and mortality, usually do not form massive biofilm. Our attention was caught by KpMn7, a KP-KPC isolate collected from a urinary infection, because it displayed a very good biofilm forming ability compared to other KP-KPC strains. Therefore, the polysaccharide produced by KpMn7 in biofilm was isolated and its primary structure determined. Composition and linkage analysis together with NMR spectroscopy revealed that the polysaccharide repeating unit has the structure: [2)-L-Rhap-(α 1-2)-L-Rhap-(α 1-2)-L-Rhap-(α 1-3)-D-Galp-(β 1-3)-[L-Rhap-(α 1-4)]-D-Galp-(α 1-)]_n. This structure is slightly different from that one reported for the KP-KPC strain KP-3264 capsular polysaccharide, having a galactose residue in place of a galacturonic acid. The *cps* gene cluster coding for the KP-3264 capsular polysaccharide (*cps*_{BO-4}) was recently described (D'Andrea et al., 2014, PLoS ONE 9(5): e96827). These findings prompted the search for differences between the known *cps*_{BO-4} and the *cps* cluster of KpMn7, by PCR amplification and sequencing. In particular, on the basis of the polysaccharides structural diversity, the comparison of the 2000 bp region corresponding to *ugd* and *uge-1* genes in the *cps*_{BO-4} cluster with the same region in the KpMn7 *cps* cluster, might reveal interesting genetic differences.

P2: 52

Biofilm formation by *Escherichia coli* isolated from hospitalized and community acquired urinary tract infections

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The uropathogenic *Escherichia coli* strains (UPEC) cause urinary tract infections (UTI) worldwide; colonize the urinary tract, form biofilms and favoring the bacterial survival and persistence long time, regardless the effect of antibiotics and immune system of the host, leading to recurrent infections and serious sequels as renal scarring. To determine the relationship between the ability to form biofilms the strain characteristics and patients, 74 *E. coli* isolates from urinary tract infections were studied based on their genotypic and phenotypic characteristics. Thus, in this study we analyzed biofilm production, antimicrobial resistance patterns, virulence factors presence and phylogenetic groups in a set of 74 UPEC isolated from hospital- and community-acquired urinary tract infections. Curli, cellulose, motility and haemolysin production were also assessed. The results obtained shows that all UPEC form biofilm, around 90% of them were strong and moderate biofilm producers. They presented higher levels of resistance than the weak biofilm producing strains. Children were more susceptible to have an UTI. Isolates from hospital-acquired infections were more virulent and multi-drug resistant as compared with community-acquired infections. Most of the biofilm producer strains belonged to phylogenetic group D. Overall, the presence of *fimH* and *papC* coincided with *in vitro* biofilm formation in the uropathogenic *E. coli* isolates, nonetheless, non-significant association was found between these genes. Our findings may provide new insights into the relationships between pathogenesis; patient characteristics and resistance of *E. coli* from urinary tract infections.

P2: 53

Biofilms of *Candida* spp. formed on the four types of catheter' surface

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Infections due to *Candida* species are the fourth cause of morbidity in hospitalized patients and are associated to indwelling of various catheters, mainly central venous catheter (CVC). Other frequent risk factors as broad-spectrum antibacterial, hemodialysis, granulocytopenia and hematologic malignancies have increased the incidence of infections caused by this genus. The characteristics of catheter' surface are variable; someone as roughness can promote the formation of biofilms. The aim of this study was to demonstrate the relationship between the roughness degree (Ra) of surface of four different catheters (CVC, FLY, MHK, TNK) and the quantity of biofilm formed by four *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*. The study included 46 *Candida* spp clinical isolates and four strains of the ATCC collection. The Ra for each catheter was determined by atomic force microscopy (AFM). Biofilm formation was induced in 3-4 mm² catheter fragments and incubated for different times (0, 24, 48 and 72 h). The metabolic activity was determined by tetrazolium reduction (OD) and by triplicate. The biofilm morphology was followed by fluorescence and scanning microscopy. The correlation between Ra and metabolic activity for each *Candida* species was established. From out four catheter types, FLY showed the highest Ra (0.245 µm) and TNK the lowest Ra (0.0345 nm). *C. albicans*, *C. tropicalis* and *C. parapsilosis* showed a high metabolic activity on FLY catheter. In addition, *C. tropicalis* and *C. albicans* formed the biofilm with highest OD on the surface of four catheters. In this work we demonstrated that to higher catheter' roughness the higher formation of biofilm.

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P2: 54

Characterization of *Desulfovibrio desulfuricans* biofilm on high-alloyed stainless steel: XPS| and electrochemical studies

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MIC behaviour of high-alloyed grades has received less attention from researchers and the mechanism of bacteria action in a respect to these alloys is different compared to low alloyed grades. Unexpected failures of stainless steels where the environment is regarded to be completely harmless are usually connected with microbial activity and sulphate-reducing bacteria (SRB). In the present work, the effect of pure culture of SRB on the biofilm formation on austenitic-ferritic (duplex) and superaustenitic stainless steels was studied. Biofilm development was evaluated with reference to their metabolic activity, expressed as specific activity of hydrogenase and total protein concentration. Surface characterization including the structure, configuration and chemical composition of SRB biofilms were carried out using SEM and XPS analysis. The OCP and EIS measurements were used to monitor the attachment activity of bacteria on the steel surface. It was proved that investigated steels are rapidly colonized by SRB and the process of biofilm growth had selective character. The presence of SRB biofilm caused extended "enoblement" of the steel surface, which result in an increase of corrosion potential. XPS results verified the formation of biofilm containing extracellular polymer on all the samples exposed to bacteria. Significant sulphidization of passive films was revealed. Sputter results indicated that some metallic ions were preferentially accumulated within the biofilm. SEM studies of the surface after biofilm removal has revealed selective dissolution of phases in DSS were revealed, what confirmed the role of chemical composition in SRB activity.

P2: 55

Complex interplay between FleQ, FleN and c-di-GMP controls transcription initiation at flagellar and biofilm-related *Pseudomonas putida* promoters

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The alternation between a free-swimming planktonic lifestyle and the formation of structured highly cooperative polymer-encased sessile communities, named biofilms, is a staple of bacterial life in the environment. Our previous work with the root-associated bacterium *Pseudomonas putida* showed that FleQ, an activator of σ^{54} -dependent promoters, and the second messenger c-di-GMP participate in the coordinate regulation of multiple functions related to motility and surface colonization. We have recently shown that FleN, a regulator of flagellar number, is also required for correct regulation of a number of flagellar and biofilm-related target promoters. To extend our understanding of this regulatory network which controls the switch between different lifestyles, we screened a library containing promoters potentially involved in motility and biofilm development fused to *gfp* and *lacZ* and measured expression in different mutant backgrounds, including a $\Delta fleQ$ and $\Delta fleN$ mutants. In addition, we carried out *in-vitro* assays such as electrophoretic mobility shift assays (EMSA) or DNase footprint assays in order to assess the interaction between FleQ, FleN and c-di-GMP on these promoter regions. Our results show that FleQ ability to bind DNA at its target promoters is modulated by interaction with FleN, which acts as an accessory protein to FleQ regulation. This interaction is differentially modulated by c-di-GMP in each promoter. Possible mechanisms by which FleN interacts with FleQ and how c-di-GMP changes affect FleQ-FleN affinity will be discussed.

P2: 56

Coordinate regulation of flagellar motility and adhesion by flagellar protein FleN in *Pseudomonas putida*

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The switch from a planktonic lifestyle to biofilm formation in *Pseudomonas putida* is regulated by FleQ and the intracellular levels of c-di-GMP. We have isolated insertion mutants defective in biofilm formation mapping in the flagellar gene *flhF*. FlhF encodes a GTPase required for correct flagellar placement, and is located upstream from *fleN*, which is involved in the regulation of flagellar number, and *fliA*, encoding a flagellum-specific σ factor. We have investigated the possible involvement of some of these elements in the regulation of motility and biofilm development. To this end, we have characterized null mutants in *flhF* and *fleN*. We have performed planktonic and biofilm growth curves, adhesion and swimming motility assays. To analyse expression of biofilm and flagellum related promoters, β -galactosidase assays of *lacZ* transcriptional fusions were performed in all mutants. Furthermore, RT-PCR was performed to study the transcriptional organization of the *flhF* and *fleN* genes. Results indicate that *flhA*, *flhF*, *fleN* and *fliA* are organized in a single operon. Both FleN and FlhF are involved in swimming motility, but only FleN is required for adhesion and biofilm formation. Expression analysis indicates that FleN is involved in regulation of biofilm- and motility-related genes. In addition, FlhF appears to be implicated in the regulation of at least one of the *P. putida* flagellar promoters. Taken together, our results suggest that FleN is a major player in the coordinate regulation of flagellar motility and adhesion.

P2: 57

Desiccation of *Listeria monocytogenes* biofilms

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Listeria monocytogenes is a foodborne pathogen able to adhere and form biofilms on various types of surfaces. Associated with a high mortality rate, it is one of the major biological concerns in food hygiene. Since few years, industries attempt to reduce the environmental impact of hygiene operations in the workshops of refrigerated food processing, through optimized use of dehumidification after cleaning disinfection treatments. This study propose a standard protocol optimized for cells growth in biofilms and for applying a desiccation stress mimicking the conditions encountered in food industry. Several experimental conditions were tested to obtain sufficient biomass of *L. monocytogenes* EGD-e sessile cells on stainless steel and a device for submitting mature biofilms to a desiccation stress (Relative Humidity decreasing to 75%) was developed. The structure of biofilms obtained and the viability and / or culturability of cells were evaluated by confocal microscopy and conventional methods of enumeration, respectively. To complete the structural analysis of biofilms and have a view on the cell integrity after desiccation stress, biofilms were observed by SEM. This work led to validate a methodology allowing (i) to obtain enough biofilm biomass for further molecular analyses and (ii) to expose to a desiccation stress mimicking conditions encountered in food plants. Plate counts showed that the application of a desiccation stress at a rate of 75% RH affected cells viability with a 75% decrease in the number of viable culturable bacteria in the first 3 h. Microscopy analysis showed that biofilms obtained were unaffected either in their morphology or in their cellular integrity.

P2: 58

Detection and characterization of *Lactobacillus reuteri* DSM 17938 Membrane Vesicles (MVs) from biofilm and planktonic phenotypes

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Membrane vesicles (MVs) are bilayer structures containing several macromolecules which bleb from bacteria. However, little is known about the MVs produced by the Gram positive commensal-derived probiotic *Lactobacillus reuteri*, particularly in the biofilm phenotype. The aim of this study was to detect and physical-chemical characterize the MVs produced from the biofilm (bMVs) and planktonic (pMVs) phenotypes of *L. reuteri* DSM 17938. MVs structure was first evaluated by Transmission Electron Microscope analysis. pMVs and bMVs were then isolated by ultracentrifugation and characterized for size distribution and surface charge using dynamic light scattering (DLS) analysis. A DNaseI, ProteinaseK and PhospholipaseC enzymatic treatment was performed to determine the MVs composition. eDNA was detected and quantified using the Quant-iT™ PicoGreensDNA assay and NanoDropUV-VIS spectrophotometer. PicoGreen showed that eDNA was associated with both pMVs and bMVs. However, DNaseI treatment showed no MVs structural modification, suggesting the eDNA-MVs complex was protective. ProteinaseK and PhospholipaseC treatments showed MVs structural changes. The DLS demonstrated that *L. reuteri* generates MVs with sizes in the nanometer scales and a broad size distribution. We conclude that: *L. reuteri* produces MVs in the both biofilm and planktonic phenotypes; lipids and proteins are important structural components; eDNA is also associated to both pMVs and bMVs. The biological activity and composition of MVs may be important for better understanding the role of MVs in the intestinal microbiota and for the development of anti-biofilm pharmacological systems.

P2: 59

Dynamics of mono- and dual- species biofilm formation and interaction between *Staphylococcus aureus* and Gram-negative bacteria

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Microorganisms are found in a wide range of diverse ecosystems as highly structured, multi-species communities termed biofilms. Interactions among bacterial species can have a profound influence on the structure and physiology of microbial communities in dual- or polymicrobial biofilms. Dynamics of mono- and dual- species biofilms formed by food-borne pathogens *Staphylococcus aureus* and *Salmonella enterica* and non-pathogenic *Escherichia coli* and *Raoultella planticola* were studied. Quantification of total biomass was assessed using crystal violet assay and viable cells were determined by enumeration of colony forming units (CFU). Fluorescent protein- labeled bacteria were constructed and visualisation of biofilms was performed using confocal laser scanning microscopy (CLSM). Scanning electron microscopy (SEM) was used to obtain high resolution three-dimensional images of dual- species biofilms. A significant reduction in CFU of *S. aureus* was noted when co-cultured with *S. enterica* ($P < 0.05$) and with *E. coli* and *R. planticola* ($P < 0.01$). Conversely, there is no influence on biofilm formation of Gram- negative bacteria when co-cultured with *S. aureus*. Specific spacial arrangement such as grape-like structures of *S. aureus* and monolayer of Gram-negative bacteria on the abiotic surface was observed by CLSM. SEM images revealed adherence of Gram-negative bacteria at the the bottom of wells and *S. aureus* overlaying them and forming clustered structures. The combined use of quantitative methods and imaging techniques enable detailed, high-quality study of formation, development and architecture of biofilms.

P2: 60

Effect of bacteriophage infections on bacterial biofilm structure

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Biofilms are dynamic structures which follow a development-detachment cycle after they have reached the mature state. Physical environmental condition act on biofilms developed in riverbed surfaces to cause the collapse of biofilm fragments. Biofilms are also exposed to biological factors that may interfere with biofilm development. However, the biological factors, including the infection of intrinsic cell by viruses, are not well understood. We studied the effect of bacteriophage infection on biofilm development, and compared the susceptibility of live and dead host cells in biofilm structure to bacteriophage infection. *Escherichia coli* ATCC 15597 was grown in F medium, a minimal medium which does not exhibit fluorescence. The culture was transferred to grass bottom dishes and stationary cultured for 3 d to develop *E. coli* biofilm on bottom surface of the dishes. Thereafter, culture solutions in the dishes were exchanged with MS2 bacteriophage solutions, and the culture was continued for 3 d. Biofilms were stained with AO (live cells) and PI (dead cells) and examined by a confocal laser microscope. At the same time, the fluid layers were tested for bacteriophage titer. Addition of bacteriophage solution caused detachment of bacterial cells in the biofilm layers. Dead cell decreased faster than live cells. However, there were no significant increase in bacteriophage titer in the liquid layer. Bacteriophage may accelerate detachment of host cells in biofilm, but bacteriophage particles were not released to the water column. Bacteriophage infection may affect biofilms to cause collapse in the structure.

P2: 61

Effects of introducing *P. aeruginosa* into *S. aureus* biofilms.

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Bacteria are commonly found in various environmental niches where they grow mainly as biofilms. These biofilms are composed of several bacterial species such as the ones found in wounds or in lung infections. Conventionally, it is thought that although *S. aureus* is the initial colonizer, upon the introduction of *P. aeruginosa*, *S. aureus* is outcompeted to the point of eradication. This idea first originated from research in which elimination of *S. aureus* was observed in dual species biofilms allowed to develop simultaneously. However, this does not mimic the progression of a biofilm *in vivo*. We therefore developed a model where *S. aureus* biofilms are allowed to establish prior to the introduction of *P. aeruginosa*. This enables *S. aureus* to reach a state more phenotypically similar to the one found *in vivo*. Biofilm development was assessed by viable cell determination and fluorescent image analysis together with determination of QS related gene expression. Introduction of *P. aeruginosa* into preexisting *S. aureus* biofilms, resulted in an increase of *lasI*, *lasR*, *rhII*, *rlhR*, and *pqsH* transcription, which then decreased to expression levels of single species *P. aeruginosa* biofilms. In *S. aureus*, the introduction of *P. aeruginosa* led to an increase of *agrB* transcription and a decrease of *sarA*, *sigB*, and *icaR* compared to *S. aureus* alone. Thus, introduction of *P. aeruginosa*, *S. aureus* viability decreased and QS gene expression increased, followed by an equilibrium, allowing for the establishment and maintenance of the dual-species biofilm infections. These data provide further evidence that dual-species biofilms are the product of cooperation between the 2 bacterial species.

P2: 62

Examination of biofilms on explanted medical devices: development of methods to maintain biofilm structure and identify bacterial pathogens

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Implanted medical devices are increasingly being utilised in modern medicine and are responsible for at least half of all nosocomial infections. Medical devices infections are often linked to the development of microbial biofilms on the device surfaces. These device-related biofilm infections are often difficult and costly to treat and consequences include life-threatening systemic infection, device failure and device explantation. Both clinicians and medical device manufacturers are interested in identifying the microorganisms responsible for device infections and also in determining the locations where the biofilms form on the devices as this data may be used to inform future treatment and prevention strategies. In this study we have optimised a fixation protocol that maintains biofilm structure while the explanted devices are shipped internationally from the surgical clinics to our analysis laboratory. We have also optimised protocols for the visualisation of evidence of microbial biofilms on explanted medical devices using 16S rRNA fluorescence *in situ* hybridisation (FISH) coupled with fluorescence microscopy. Very importantly, our fixation protocol is compatible with our FISH procedure that enables determination of the presence and location of Gram negative bacteria, Gram positive bacteria and specifically identifies two of the most common infectious pathogens of implanted medical devices - *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In conclusion, the development of these methods is likely to be generally applicable for the detection and analysis of biofilms on implantable medical devices.

P2: 63

Exopolysaccharide produced by a *Klebsiella pneumoniae* clinical isolate in biofilms and flocs. Primary structure and interaction with antimicrobial peptides.

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The genus *Klebsiella* comprises opportunistic pathogens which cause bacteremia, pneumonia and urinary tract infections in humans. Although these microorganisms do form biofilms, little is known about the polysaccharides present in their matrix. *K. pneumoniae* strain Kp113 was isolated from a patient with urinary tract infection. Kp113 was grown as biofilm on cellulose membranes deposited on agar plates, in order to recover enough matrix for polysaccharide extraction and its structural determination. Established chemical derivatization methods followed by GC-MS, and NMR spectroscopy, lead to the definition of the repeating unit structure of the polysaccharide produced by Kp113 (Kp113 Epol) which is identical to that of *Klebsiella* capsular polysaccharide K24: [2)-[D-Manp(β 1-4)]-D-GlcpA-(α 1-3)-D-Manp-(α 1-2)-D-Manp-(α 1-3)-D-Glcp-(β 1-)]_n. Kp113 was also grown in liquid medium, where it formed flocs. NMR analysis of the polysaccharide extracted from flocs revealed an identical chemistry, thus suggesting a structural role in the biofilm matrix for the Kp113 Epol. The protective effect of the polysaccharide towards bovine cathelicidin antimicrobial peptides BMAP-27 and Bac7(1-35), which have distinct modes of action, and towards colistin was assessed. Interaction of the polysaccharide with BMAP-27 was demonstrated by circular dichroism spectroscopy, thus explaining the protective function. The present investigation shows that the polysaccharide produced by Kp113 is not only part of the biofilm architecture but it also possesses specific biological functions.

P2: 64

Fluorescent *in vivo* hybridization as a tool for unraveling the human microbiome

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The human body is densely populated by bacterial communities that shape health and disease. The current understanding of host-bacteria interactions is hampered by the lack of specific and sensitive methods for the visualization of biofilms that are part of the microbiome. Fluorescent *in situ* hybridization (FISH) is a technique commonly used in biofilms, but the high hybridization temperatures and toxic reagents limit its *in vivo* potential. In addition, the robustness of the hybridization and consequent fluorescence signal may be affected by the *in vivo* environment at the infection site. We previously studied the applicability of fluorescence *in vivo* hybridization (FIVH), using the gastric pathogen *Helicobacter pylori* as a model for a biofilm-forming bacteria causing human infections. We tested nucleic acid mimics (NAMs) to not only perform FISH at human body temperature and gastric pH, but also overcome the gastric mucus layer associated with the infection site. In here, we evaluated the applicability of liposomes to deliver the NAMs in untreated bacteria, under non-toxic/noxious conditions. DOTAP/DOPE liposomes were shown to be suitable as they were stable in simulated gastric juice for more than one day, they efficiently interacted with *H. pylori* membrane and complexed the NAMs. This resulted in improved NAMs hybridization and twice stronger fluorescent signal, suppressing the need of fixation/permeabilization. This proof of concept indicates that in the future FIVH employing liposomes could provide *in vivo* visualization of the host-microbiome interactions, helping to decipher the biogeography of the microbiome.

P2: 65

Identification of biofilm signature metabolites via metabolomic profiling

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Chronic infections caused by bacterial biofilms are difficult to diagnose in the clinic. Biofilm bacteria have slow growth rate and distinct metabolism compared to their free-living counterparts. Biofilm formation is regulated by intracellular content of the second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) in a wide range of bacteria. Variations in the intracellular levels of c-di-GMP due to catalytic enzymes may change cellular metabolic profile and lead to expression of specific biomarkers of the biofilm mode of growth. Identification of biomarkers regulated by c-di-GMP might provide alternative methods to diagnose biofilm-associated chronic infections. Three pathogens, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Burkholderia cenocepacia*, were genetically modified to express different intracellular levels of c-di-GMP to mimic either acute or chronic mode of growth. Their metabolomic profiles generated by LC-MS were statistically analysed and visualized to identify common signature metabolites under each state of growth. Among three high c-di-GMP containing strains, we have found 2 common signature metabolites between *P. aeruginosa* and *K. pneumonia*, while 15 were found between *P. aeruginosa* and *B. cenocepacia*. Among three low c-di-GMP containing strains, 1 common feature were found among all the strains, while 19 were found between *P. aeruginosa* and *K. pneumonia*, and 7 were found between *P. aeruginosa* and *B. cenocepacia*. Our study provides evidence for the possibility of detecting and diagnosing biofilm infections in clinical prospective using c-di-GMP regulated metabolites.

P2: 66

Influence of electrolytes in mediating *Pseudomonas aeruginosa* PAO1 biofilm composition and virulence factors

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Environmental factors, such as medium composition and electrolyte concentration affect biofilm formation. Human blood, rich in nutrients and electrolytes, offers the necessary ingredients for the growth of biofilm forming bacteria capable of circumventing the immune system in an immune compromised individual. *Pseudomonas aeruginosa* sp, a nosocomial pathogen establishes biofilms on implants surrounded by freely circulating blood and other bodily fluids. Typically, bodily fluids contain an abundance of salts such as magnesium, chloride ions, sulphate ions, sodium, potassium and phosphate ions that are transported in plasma to different parts of the body and provide the suitable environment for biofilm formation. The aim of this study was to investigate the production of extracellular polymeric substances (EPS) and virulence factors displayed by *P. aeruginosa* PAO1 in presence of salts commonly found in human blood. Concentration of EPS of *P. aeruginosa* PAO1 in LB broth, a nutrient rich medium, in the presence of Mg^{2+} , SO_4^{2-} and Cl^- was quantified by biochemical assays after the separation from their cells. The difference in biofilm structure was pictorially documented. Biochemical quantification of the EPS produced with SO_4^{2-} resulted in an estimable reduction in EPS whereas Cl^- resulted in increase in EPS in comparison with the control. Three visually distinct biofilms with differing structural morphology were observed and documented. These studies will help develop procedures to minimise/eradicate biofilm formation and assist the immune system of the body to combat microbial invading diseases.

P2: 67

Interactions between *S. mutans* and other oral bacteria in biofilms

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One major culprit in dental carries is the formation of *Streptococcus mutans* (*S. mutans*) biofilms on teeth. The human oral cavity is home to 100s of different bacteria that form multi-species biofilms. It is also known that cariogenic biofilms are multi-species. However the relationships between interspecies interaction and biofilm structure remains unclear. We have developed a system to examine multi-species oral biofilms on a hydroxyapatite (HA) substrate using confocal reflection microscopy (CRM) (Inaba *et al.*, 2013). CRM uses reflected light to create 3D images, thus permitting visualization without fluorescent probes. In the present study, to visualize the location of *S. mutans* within *multi*-species biofilms, we constructed a *S. mutans* strain tagged with an anaerobic fluorescent protein gene. *S. mutans* and other oral bacteria, such as *Streptococcus mitis* and *Aggregatibacter actinomycetemcomitans* (*Aa*) were co-cultured on a HA substrate, generating two-species biofilms. We visualized the localization of *S. mutans* in the mixed-species biofilms, using a combination of CRM and confocal laser scanning microscopy and found that the co-culture of *S. mutans* and *Aa* form more robust biofilms than either mono- or other mixed-species biofilms. Within these co-culture biofilms, *S. mutans* are distributed uniformly. We also observe pit formation in the HA substrate caused by biofilm development. These results suggest that *S. mutans* and *Aa*, which is a known factor in periodontal disease, act synergistically in mixed-species biofilms leading to tooth decay and aggressive periodontal disease.

P2: 68

Monitoring whole-biofilm CO₂ evolution rates as a proxy for biofilm establishment and recovery rates

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The availability of laboratory systems or methods for the non-destructive, real-time quantitation of biofilm metabolic activity under diverse environmental conditions remains limited. Since carbon dioxide (CO₂) is a universal product of microbial respiration and fermentation processes, it is an attractive target for the metabolic activity monitoring. To this end, a real-time measurement system was developed and validated to quantify whole-biofilm CO₂ production rates under continuous-flow conditions. Here we describe an extension of the demonstrated utility of this system, namely the potential of using CO₂ production rates as a proxy for biofilm biomass accumulation rates during primary surface colonization, in addition to biomass recovery after a physical or chemical insult. Single species biofilms of *Pseudomonas aeruginosa* PAO1 and *Pseudomonas* sp. strain CT07 were established under a range of nutrient concentrations (0.3 to 3.0 g.L⁻¹ Tryptone soy broth, TSB) while logging CO₂ production rates at 1-minute intervals. The maximum specific rate of CO₂ production during biofilm establishment was determined for each nutrient concentration using custom scripts and the CellGrowth package in R, followed by ANCOVA analysis. In analogy to maximum planktonic growth rates, an increase in nutrient concentration from 0.3 to 1.5 g.L⁻¹ TSB likewise resulted in faster biofilm growth rates, whereas a further increase from 1.5 to 3.0 g.L⁻¹ TSB did not enhance the tempo of surface colonization. In addition to evaluating the effect of nutrient concentration on biofilm establishment rates, attached biomass recovery rates after physical and chemical insults were quantified.

P2: 69

Orientation of the fim-switch which controls expression of type 1 fimbriae in *Klebsiella pneumoniae* upon cultivation of biofilms in tryptic soy broth (TSB) and artificial urine medium (AUM).

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Klebsiella pneumoniae is an important opportunistic pathogen that forms biofilms. Previous studies have suggested that type 1 fimbriae (T1F) and type 3 fimbriae (T3F) play a complementary role in *K. pneumoniae* biofilm formation and contribute to bacterial adherence in a murine catheter-associated urinary tract infection (UTI) model. A major subunit of T1F is FimA. The *fimA* gene is regulated by an invertible DNA element; fim-switch (FS). The orientation of FS is controlled by the FimB and FimE recombinases. ON and OFF orientation results in a fimbriated and nonfimbriated cells respectively. The capacity of two *K. pneumoniae* isolates (Kpneu_1 from blood and C3091 from a UTI) to form biofilms in microtitre plates was determined using crystal violet. We found that both strains formed similar amounts of biofilm in TSB. However, C3091 formed more biofilm than Kpneu_1 in AUM. We investigated whether FS orientation might account for this difference. DNA was extracted from planktonic and biofilm cells cultivated in each medium, and used as a template for PCR to amplify the FS region. FS orientation was found to be OFF in planktonic and ON and OFF in biofilm cells cultivated in TSB. However, the FS in both strains was completely OFF in planktonic and biofilm cells grown in AUM. Thus the difference in biofilm formation by the two strains in AUM cannot be accounted for by FS orientation. Further studies are aimed at investigating the factors responsible for the differential FS genotype and biofilm formation of the two strains in AUM and TSB to give a better understanding of how the environment influences biofilm formation.

P2: 70

Physical stress induces phase variations in *Filobacterium rodentium*, rodent pneumonic bacterium

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Filobacterium rodentium gen. nov., sp. nov. (Int J Syst Evol Microbiol 2016, 66:150), formerly known as the cilia-associated respiratory bacillus ('CAR bacillus'), is a Gram negative, filamentous bacterium without flagella. *F. rodentium* has gliding activity and induces chronic respiratory diseases (CRD) in rodents. We reported that 'CAR bacillus' developed biofilms at Biofilms5, 2012. In this study, we investigated a phase variation of *F. rodentium*. [Materials and Methods] Strain SMR-C^T (JCM 19453^T) of *F. rodentium* originally isolated from rat CRD was cultured in Vero E6 conditioned medium (culture supernatants of Vero E6 cells cultivated in Iscove's Modified Dulbecco's Medium supplemented with 10 % fetal bovine serum). *F. rodentium* cultures were carried out in 95% CO₂-5% air at 37 °C in a humidified chamber. Morphological changes were monitored under phase contrast microscopy. [Results and Discussion] In normal cell culture flasks, SMR-C^T grew in sessile state and made net-like structure on flask surface. In ultra-low attachment flasks (attachment surface unavailable), the organisms grew in planktonic; floating bacteria gradually formed aggregates and finally constructed classic type biofilms. When methylcellulose (0.3–0.5%) was added to suppress movements, SMR-C^T formed ropey, cord-like structure (CLS) 4 weeks after addition. CLS-state SMR-C^T stopped proliferation but regrew 3 weeks after medium change. Collectively, SMR-C^T showed phase variations according to physical stresses. We have already found gliding related genes in genome of SMR-C^T. We think *F. rodentium* uses its unique structures to survive in fields and infect mammals by responding to physical stresses.

P2: 71

Regulation of gene expression from primary to chronic infection in *Achromobacter* biofilms

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Achromobacter has the capacity to form biofilm, which is crucial to persisting lung infections in CF patients, as biofilms are less susceptible to treatment. Upon transition from primary to chronic infection, the bacteria undergo evolutionary adaptations to the environment in the CF lung. The bacteria adapts to a biofilm lifestyle, where it is protected from antibiotics and the host immune system by the biofilm matrix and low cellular metabolism. In early stages of infection, the bacteria express virulence genes involved in an acute response and biofilm initiation. Once a biofilm is established, the infection has become chronic and extremely difficult to eradicate. The aim of this project is to study gene expression as an adaptation to a specific niche; the CF lung. Whole-genome transcriptome analysis will be performed on RNA extracted from planktonic cultures and biofilms from clinical isolates of *Achromobacter xylosoxidans* and *Achromobacter insuavis*. Expression levels of selected genes will be investigated by qPCR in sequential isolates collected from the same patient over several years. We hypothesize that we will be able to identify genes specifically regulated in *Achromobacter* biofilms as opposed to planktonic cultures in stationary growth-phase and that possible changes in isolates from primary and chronic infections will be caused by altered expression of genes related to biofilm formation rather than loss or gain of new genetic material. Gained knowledge on genes specifically involved in biofilm formation will provide the foundation for research on mechanisms specific for biofilm formation and how these may change in the transition from primary to chronic infection

P2: 72

Structural and biochemical characterization of the exopolysaccharide of *Staphylococcus epidermidis* 1457

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Coagulase-negative staphylococci like *Staphylococcus epidermidis* are one of the main causes of implant associated infections. *S. epidermidis* produces biofilms on prosthetic devices like e.g. catheters or shunts. The main component of the biofilm is an exopolysaccharide, a poly β -1,6-linked N-acetyl-glucosamine (PNAG), which to a certain degree can be N-deacetylated and 3O/4O-succinylated. The backbone structure of PNAG is well known, but the amount of modifications fairly differs. There is a huge uncertainty about the molecular mass of PNAG, which is considered to be either a molecule of ~28 kDa or bigger than 200 kDa. Our objective is to isolate and characterize PNAG from *S. epidermidis* 1457, starting with molar mass over the number and distribution of different charges by the different modifications up to the agglomeration behavior of PNAG. We aim to receive the full modification pattern of PNAG after detailed structural characterization. PNAG is isolated from cells by sonification. After concentration PNAG is purified by SEC and AEC. Molecular mass is determined by GPC-DALS and MALDI-MS. Oligomers are received after hydrolization with DspB. Structural analysis of oligomers by LC-MS and NMR. Biofilm formation is observed in 96-well-plate biofilm assays. Quantification of PNAG via hexosamine assay. Biofilm integrity, stability and the condition of PNAG heavily depends on pH value and accordingly on its state of charge. PNAG is a molecule that can be separated in cationic and neutral/weak anionic entities and is a molecule that tends to agglomerate. Ratio of cationic to anionic PNAG is about 7:1. PNAG appears to be of an apparent molecular mass of ~23-28 kDa.

Poster Sessions

Session 3

Biofilm prevention and
control strategies

P3: 1

A non-antibiotic approach to combat *S. aureus* biofilms using deferiprone and gallium-protoporphyrin

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Staphylococcus aureus biofilms are clinically relevant playing a major role in various infectious diseases, including osteomyelitis, endocarditis, chronic wounds and chronic sinusitis. As standard medical care frequently fails to eradicate biofilms, novel treatment approaches are urgently needed. Adding to this problem is the universal threat of antibiotic resistance, looming with the overuse of antibiotics. This study proposes a new anti-microbial strategy with a distinctively different mechanism of action to antibiotics. *S. aureus* colony biofilms were grown on membrane filters and treated with a novel drug-delivery-system combining the iron-chelator deferiprone (Def, 20 mM) and the haem-analogue gallium-protoporphyrin (GaPP, 250 µg/ml). After 5 days the treatment efficacy was assessed by CFU counting. Cross-sections of colony biofilms were visualised by correlative microscopy using live/dead staining. The formulations incorporating GaPP and Def-GaPP demonstrated anti-biofilm properties (3.7-fold and 3.8-fold Log₁₀ reduction in CFU/ml, respectively) and were significantly more effective in the elimination of *S. aureus* biofilms than formulations loaded with the antibiotic ciprofloxacin (CIP, 5 µg/ml, 1.3-fold Log₁₀ reduction). Microscopy images supported the efficacy of Def and GaPP. In conclusion, this study revealed promising anti-biofilm properties of a novel non-antibiotic treatment against *S. aureus* biofilms. By interfering with the bacterial iron metabolism, the compounds Def and GaPP exceeded the efficacy of CIP *in vitro*. The potential for future clinical applications needs to be evaluated.

P3: 2

Anti-biofilm efficacy of clinically relevant antibiotics for preventing *Staphylococcus aureus* biofilm formation in two different models

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Biofilm formation leads to the failure of antibiotic therapy and contributes to the chronicity and persistence of infections, especially those associated with the use of medical devices. Thus, biofilm prevention is a desirable goal of antimicrobial research. In this work, two distinct biofilm models; microtiter well plates (MWP) and drip flow reactors (DFR) were used in parallel to measure the efficacy of conventional antibiotics in preventing biofilms. Bacteria were exposed to antibiotics for one hour followed by further incubation (MWP) or continuous flow phase (DFR) for 24 hours. Biofilms were quantified by performing viable plate counts and the efficacy was assessed based upon the log reduction (LR). Selection of the antibiotics was performed on the basis of susceptibility testing in microtiter well plates, in which a panel of 27 antibiotics of various mechanistic classes was tested and the minimum inhibitory concentrations (MIC) were determined using resazurin and crystal violet staining assays. Overall, LRs obtained from DFR were significantly lower than those from MWP. Anti-biofilm efficacy could not be connected to a particular class of antibiotics, since it varied between the systems. No statistically significant difference in cell density of control biofilms (8.054 ± 0.147 CFU/cm² and 7.596 ± 0.412 CFU/cm² for MWP and DFR, respectively) was observed, and the calculated surface area to volume (SA/V)-ratios were found to be in the same order of magnitude (7.961 cm⁻¹ and 2.708 cm⁻¹ for MWP and DFR, respectively). Hence, it seemed that the reduced susceptibility of DFR grown biofilms can only be attributed to the presence of continuous nutrient flow.

P3: 3

Biofilm disruption using rhamnolipids produced by *Burkholderia thailandensis*

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A wide range of biosurfactant molecules are known to be produced by microorganisms and many of these have potential applications in commercial products as partial or complete replacements for existing chemical surfactants. Two groups of microbial biosurfactants, the rhamnolipids produced by the bacterium *Pseudomonas aeruginosa* and the sophorolipids produced by yeasts of the genus *Candida* have already been extensively investigated by various research groups. To our knowledge, the area of biofilms and role of biosurfactants within are becoming an increasingly important topic of research, in this work, we would like to examine biofilm characteristics of clinical isolates, and the effect and efficiency of rhamnolipids produced by a non-pathogenic bacteria, as dispersal or inhibition agents of biofilms. In turn, this will move the current treatments from synthetic molecules to biological ones for a better effect on infection pathologies.

P3: 4

Biofilms in pig production facilities: characterization of microbial communities and insights for control strategies

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Antibiotics have been widely used to reduce microbial infection in pig production since the early 1950s and until their ban in Europe in 2006. Antibiotics provide benefits to both animal's growth performance and health by reducing the development of harmful organisms among which *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus* (MRSA) are prominent in the pork sector. Although little is known about the mechanisms contributing to the persistence of these agents in the herds, one cause for their high prevalence rates is their ability to exist as biofilms. The aim of this study was to investigate the presence of microbial biofilms in pig farming equipment's and to analyse their population compositions. Samples of biofilms were collected in ten Breton herds from drinking water pipelines and also from the water distribution systems of a pilot plant devoted to the optimization of the fermentation process of liquid feeding. Sessile bacterial and fungal communities were compared with the profiles of the planktonic populations. Changes within the microbial communities were monitored in conjunction with zootechnical performances and health records after two treatments *i*) a mix of essential oils (Pigfast™ from Dinastim) and *ii*) a zootechnical feed additive (Bactocell® from Lallemand). The results indicate that, in a steady-state microbial ecosystem, the biofilm taxa are representative of the planktonic community which profile can be shifted subsequently to one of the above treatments. This strongly suggests that the plankton-to-sessile transition of some taxa can be controlled in order to favour the formation of a positive biofilm.

P3: 6

Calcium-chelating alizarin and other anthraquinones inhibit biofilm formation and the hemolytic activity of *Staphylococcus aureus*

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Staphylococcal biofilms are problematic and play a critical role in the persistence of chronic infections because of their abilities to tolerate antimicrobial agents. Thus, the inhibitions of biofilm formation and/or toxin production are viewed as alternative means of controlling *Staphylococcus aureus* infections. Here, the antibiofilm activities of 560 purified phytochemicals were examined. Alizarin at 10 µg/ml was found to efficiently inhibit biofilm formation by three *S. aureus* strains and a *Staphylococcus epidermidis* strain. In addition, two other anthraquinones purpurin and quinalizarin were found to have antibiofilm activity. Binding of Ca²⁺ by alizarin decreased *S. aureus* biofilm formation and a calcium-specific chelating agent suppressed the effect of calcium. These three anthraquinones also markedly inhibited the hemolytic activity of *S. aureus*, and in-line with their antibiofilm activities, increased cell aggregation. A chemical structure-activity relationship study revealed that two hydroxyl units at the C-1 and C-2 positions of anthraquinone play important roles in antibiofilm and anti-hemolytic activities. Transcriptional analyses showed that alizarin repressed the α-hemolysin *hla* gene, biofilm-related genes (*psmA*, *rbf*, and *spa*), and modulated the expressions of *cid/lrg* genes (the holin/antiholin system). These findings suggest anthraquinones, especially alizarin, are potentially useful for controlling biofilm formation and the virulence of *S. aureus*.

P3: 7

Candida tropicalis biofilm is highly influenced by the environmental human pH

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In the last decades, the increase of candidiasis has been accompanied by an intensification of infections caused by *Candida tropicalis*. Indeed, *C. tropicalis* has been described as able to colonize and infect several anatomically distinct sites, including the skin, gastrointestinal, genitourinary and respiratory tracts. Adaptation to diverse pH that exists in each human niche has been shown to be critical for virulence in many commensal pathogens, but there are no reports concerning *C. tropicalis*. Biofilm formation ability is one of the most important virulence factors that have important clinical repercussions due to its increased resistance to antifungal therapy. Thus, the aim of the current study was to characterize the influence of pH on *C. tropicalis* biofilm formation, structure and composition. The effect of pH (3, 4, 7 and 8) on *C. tropicalis* biofilms was evaluated by enumeration of culturable cells, total biomass quantification and matrix composition. Biofilm structure and the morphology of its cells were analysed through scanning electron microscopic and confocal laser microscopy. The results revealed an intensification of *C. tropicalis* capacity to form biofilm at neutral and alkaline conditions, with an increased number of culturable cells and total biomass and in its structural complexity, comparatively to acid conditions. For the first time, we have demonstrated that *C. tropicalis* biofilm formation is highly influenced by the environmental human pH, which has an important clinical impact, which may partly explain the increase incidence of candidiasis.

P3: 8

Characterization of interactions at the interfaces between bacteria and abiotic surfaces to limit the bacterial adhesion and biofilm formation

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The ability of pathogenic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* to adhere and to form biofilms on abiotic surfaces represents a major safety problem in food industries. In addition, it is known that bacteria embedded in biofilms are more resistant to sanitizing agents than bacteria growing under planktonic state. Thus, a greater understanding of bacterial adhesion and biofilm formation on surfaces is of great interest in order to setup efficient cleaning and sanitizing strategies. In addition, such investigation will help to setup new materials with controlled surface properties which decrease or inhibit bacterial adhesion. In the present work, a cellular approach was used to study the major parameters involved in the bacterial adhesion and the biofilm formation. These investigations were carried out through the characterization of the surface properties of both planktonic and sessile bacteria. The results underlined that the temperature and the surface type have a significant effect on the charge, the hydrophobicity and the virulence of bacterial cells. These environmental conditions have also influenced the resistance of sessile and planktonic cells to disinfectant agents. Our work also investigated the effects of the substrate surface properties, such as its hydrophobic character and roughness, on the bacterial adhesion. A cold plasma assisted deposition of a nano thick organosilicon coating was used to monitor the surface hydrophobic character. The results gave a clear evidence of a decrease of the bacterial adherence on the modified stainless steel surfaces inhibiting the biofilm formation.

P3: 9

Colistin heteroresistance in *Klebsiella pneumoniae* and its association with slow-growing sub-populations within biofilms

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The emergence of multidrug-resistant strains of *Klebsiella pneumoniae* is a growing clinical concern that is leading to the re-introduction of the old and toxic colistin as a salvage therapy. However, several cases of heteroresistance to this antimicrobial have been recently reported in planktonic studies. Therefore, the understanding of the conditions that trigger heteroresistance is attracting considerable research interest. In this scope, this work aimed to more comprehensively study the response of *K. pneumoniae* biofilms to colistin and to inspect the occurrence of heteroresistance in biofilm-cells. *K. pneumoniae* presented susceptibility to colistin in its planktonic form, though biofilms presented an enhanced resistance. The population analysis profiles pointed out the existence of a slow-growing sub-population resistant to colistin within a *K. pneumoniae* strain that seemed to be exclusively associated with biofilms. This resistant sub-population is characterized by a small colony morphology (diameter around 5 mm), which remains unchangeable, and a completely different response to colistin compared to the observed in the wild-type morphotype. Colistin was ineffective in this small colony variant since it was never achieved any reduction in biofilm-cells viability. These findings suggest that heteroresistance is linked to biofilm formation and to a morphological distinct sub-population. Moreover, this is the first evidence that biofilm formation can trigger the emergence of heteroresistance from an apparently susceptible strain.

P3: 10

Comparative efficacy of chlorine-based disinfectants and UV-light to control *Escherichia coli* biofilms

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Sodium hypochlorite (SH) is the most common antimicrobial used in food industry, but its interaction with organic matter produces unhealthy and toxic by-products. Therefore, new chlorine-based strategies are emerging for the disinfection of surfaces, decontamination of fresh produce and water. The aim of the present work was to assess the effects of chlorine-based disinfectants (SH, neutralized electrolyzed oxidizing water - NEOW and chlorine dioxide - CD) and UV-light (used alone and combined with SH) on the control of *Escherichia coli* biofilms formed on stainless steel surfaces. The selected disinfectants have recognized antimicrobial activity. However, their potential to control biofilms is still unknown. The free chlorine concentration of the disinfectants was also assessed overtime at three different temperatures (5, 25 and 30 °C). NEOW was the most efficient disinfectant in the control of *E. coli* biofilms (3.46 log CFU.cm⁻² reduction) and SH was the least effective (2.78 log CFU.cm⁻² reduction). UV-light applied alone had reduced effectiveness (reduction <1 log CFU.cm⁻²), while its combination with SH slightly improved biofilm control (3.02 log CFU.cm⁻² reduction). In terms of free chlorine content in solution, CD has the fastest chlorine decay (lag time of 0 days) at every temperature. As in industry the processing of fresh produce is performed at 5 °C, tests on chlorine decay were performed at this temperature revealing that NEOW had the highest stability (lag time of 70 days) and the lowest chlorine loss rate (0.013 ppm.min⁻¹). In conclusion, NEOW can be considered an effective disinfectant and a relevant alternative to SH for the control of sessile cells.

P3: 11

Complex microbial ecology in the food processing industry: a competitive multiculture biofilm for meat processors.

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Even though decontamination process and strict hygiene control are used, it is well known that biofilms can appear in food industry. Most studies have been conducted in monoculture during early stage biofilm development (24-48hrs), a scenario rarely encountered in the real world. Therefore, a study on multispecies biofilm development which mimics conditions in meat processing facilities has been realised. CDC Biofilm Reactors were filled with meat slurry culture media for a period of 16h following a starvation period of 8h in water. These steps were repeated over 12 days except for a starvation period (dry environment) of 2 days simulating a weekend interlude. Polystyrene and stainless steel coupons were used as biofilm surfaces while *Pseudomonas fluorescens*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were selected to form multispecies biofilms. Our results show that biofilms attach more easily to polystyrene than stainless steel coupons. Mixed culture biofilms not only displayed higher levels of adherence for the bacterial constituents but also greater resistance to the nutritional (starvation) and desiccation. Of the three strains examined, *P. fluorescens* exhibited the greatest adhesion ability and was also the fastest to attach. Experiments using pioneer bacteria (i.e. inoculating one of the 3 strains 1 day before the others), did not have a significant impact on the microbial counts of the resulting biofilms. This study brings important new knowledge on complex biofilm ecology in the food industry. The 12 day / CDC model can serve as a basis for competition studies between foodborne pathogens and non-pathogenic bacteria during biofilm development.

P3: 12

Contribution of plant-derived phenolic compounds to combat *Candida* species biofilms

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Opportunistic fungal infections, namely involving *Candida* species, constitute a hot topic for scientific researchers. The present work aims to access antifungal potential of plant-derived phenolic extracts against planktonic cells and biofilms of *Candida* species. *Eucalyptus globulus* Labill. (blue gum), *Glycyrrhiza glabra* L. (licorice), *Juglans regia* L. (walnut) and *Salvia officinalis* L. (sage) evidenced to be the most effective *Candida* growth inhibitors, using disc diffusion assay. Minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations, and chemical composition of extracts by using HPLC-DAD-ESI/MS were also determined. Blue gum and walnut mainly exerted fungistatic potential, while sage exerted an interesting anti-*Candida* potential. However, the most prominent candidacidal potential was observed to licorice extract, being achieved the lowest MIC and MFC values. The candidacidal potential of these phenolic extracts was mainly attributed to their high abundance in flavonoids, mainly flavones: luteolin (sage) and apigenin derivatives (licorice), and flavanones: liquiritin derivatives (licorice). In order to deepen the knowledge on the most effective extract, its ability to inhibit biofilm formation was evaluated. Overall, a double concentration of MFC value was necessary to achieve similar results in biofilms. Flow cytometry assays were also carried out, and the obtained results revealed that primary lesion of cellular membrane appear to be most relevant mode of action. Thus, plant derived phenolic compounds evidence a promising potential to combat *Candida* species biofilms, both individually or combined with conventional therapy.

P3: 13

Cooperation or conflict? Impact of intraspecific diversity on *Escherichia coli* biofilms

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Intraspecific diversity in biofilm communities is associated with enhanced survival and growth of the individual biofilm populations. In here, we assess if this apparent cooperative behavior still holds as the number of different strains in a biofilm increases. Using *E. coli* as a model organism, the influence of intraspecific diversity in biofilm populations composed of up to six different *E. coli* strains, was assessed. Biofilm quantification was evaluated by crystal violet (CV) staining and colony forming units (CFU) counts. In general, with the increasing number of strains in a biofilm, an increase in cell counts and a decrease in matrix production was observed. This observation was confirmed by cluster analysis that indicated that after 24h of biofilm formation the best model, according to the Bayesian information criterion (BIC), consisted of three clusters that grouped together biofilms with an equal number of strains. It hence appears that increased genotypic diversity in a biofilm leads *E. coli* to maximize the production of its offspring, in detriment of the production of public goods (i.e. matrix components), that would be beneficial to all strains individually and the consortium as a whole. Apart from the ecological implications, these results can be explored in the area of clinical biofilms, as a decrease in matrix production might render these intraspecies biofilms more sensitive to antimicrobial agents.

P3: 14

Damages and re-growth after photocatalytic treatment of bacteria adhered on new TiO₂ nanomaterials-based coatings

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Photocatalysis based on TiO₂ nanomaterials (NPs) is an oxydation approach with high promising applications in the degradation of polluting organic compounds and microorganisms in air and water. When deposited or embedded to create coatings, TiO₂ NPs can confer efficient and durable antibacterial properties to materials by leading to partial or complete mineralization of bacteria, with therefore neither a resulting fouling of the surface by bacterial debris, nor an exhaustion or toxic effect of any biocide agent. Here, we developed new TiO₂ NPs with enhanced photocatalytic oxidative properties and we investigated the degradation and re-growth after UV-A illumination of bacteria adhered on TiO₂ NPs-coated materials. Quantity, viability, metabolic and respiratory activities resulting from the treatment were analysed for three different bacterial species by *in situ* fluorescent confocal microscopy in liquid media. Aside from a significant higher efficiency of materials coated with the new TiO₂ NPs compared to well refered TiO₂ NPs, we showed a large variability of the bacterial susceptibility and degradation with time according to species and dependent on the UV-A dose. Capacity of bacterial populations to regrow in fresh nutritive medium after the photocatalytic treatment was also shown to vary with UV-A dose and bacterial species. Besides, we highlighted the impact of the O₂ content in medium on the antibacterial efficiency. Finally, this work provides new evidence to support the potential of TiO₂ NPs-based coatings for fighting surface colonization by bacteria, as well as new specifications regarding their mechanisms of action and adequate conditions of use.

P3: 15

Destabilization of biofilms in a microfluidic environment

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Prevention and control of biofilm formation is a key focus in biofilm research today. Control of biofilms implements the surveillance of biofilm growth as well as biofilm manipulation strategies. Biofilm destabilization and inactivation are important parts of biofilm manipulation processes. To online characterize them in a fluidic environment for short-term and long-term effects, suitable sensors are necessary. Using an impedimetric and amperometric based sensors, which is able to detect biofilm formation by biomass and activity in real-time, different destabilization and control strategies for biofilms were tested. Combinations of killing and removal agents were used as well as enzymatic destabilization based on DNase I. Thereby, a focus was taken on the impacts on biofilms directly after treatment, but also on the long-term biofilm progression after re-incubation. eDNA was found to be a common EPS component of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* biofilm matrices. DNase I was tested as suitable destabilizer, especially in young biofilms. Regarding long-term effects, biofilms treated with DNase I showed an increased regrowth after treatment. Similar biofilm behavior was observed for biofilm removal with other agents. Also combinations of DNase I with antibiotics did not improve the situation. Developing novel biofilm control strategies, substances should not only be tested for their biofilm removal capacity, but also for their effect on the residual surviving biomass.

P3: 16

Detachment of oral biofilm by cysteine proteases

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Dental plaque is a potential reservoir for respiratory infection in the elderly. Therefore, there is a critical need for the development of effective methods to remove oral biofilm. We have shown the effects of tablets containing actinidin, a cysteine protease, on tongue coating removal. To find out more details of oral biofilm removal by cysteine proteases, we conducted *in vitro* biofilm assay using the oral initial colonizer, *Actinomyces* species. Solutions containing various concentrations of cysteine proteases, purified papain or partially purified actinidin from kiwifruit, were applied to the biofilm formed by *Actinomyces oris* strain MG-1. Purified trypsin was used as a control. After 10 minutes of incubation at 37°C, biofilm removal was observed only in solutions containing more than 1 mg/mL of papain. After further incubation, papain and trypsin detached the biofilm at the same concentration (1 mg/ml), and actinidin also detached the biofilm at high concentration. Since these enzyme solutions exerted no bactericidal effect, we are investigating the relationship between biofilm removal and proteolytic specificity for fimbriae.

P3: 17

Determination of biofilm characteristics and evaluation of the sanitation procedures on biofilms of thermophilic *Aeribacillus pallidus*

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In this study, we aimed to determine the properties of biofilms produced by *A. pallidus* E334 and the efficiency of procedures commonly used in industry for removing the biofilms. For this study, the biofilm production of E334 was tested on polystyrene plates and stainless steel surfaces. The extracellular matrix content was quantified and crystal violet binding assay was performed depending on various conditions such as temperature (50-65°C), pH (4.0-11.0) and salinity (0.0-5.0 %). The optimal conditions were applied for biofilm formation on other surfaces. The sanitation regimes tested depending on the matrix content, physiology of biofilm and the chemistry of agents. Plate counting assays and CLSM were performed to confirm the eradication of the biofilms. E334 formed strong biofilms on polystyrene (OD:1.64) and stainless steel (6.4×10^4 CFU/cm²). The optimum temperature, pH and salinity were 60°C, 7.5 and 1.5%, respectively. All experiments were carried out in optimum conditions. Polypropylene and glass were found to be ideal for biofilm adherence. The most effective sanitizers were alkaline protease and SDS (95.3%, and 86.9% removal of biomass, respectively). The results were also correlated by extracellular matrix, plate counting and CLSM analyses. This is the first report regarding with the characteristics and the physiology of *A. pallidus* E334 under various conditions. The most satisfactory results were obtained by alkaline protease and SDS to eliminate the biofilms. Therefore, we screened the effects of many sanitation regimes and successfully eradicate the thermophilic biofilms from the abiotic surfaces.

P3: 18

Development of a phage cocktail to prevent *Proteus mirabilis* biofilm formation in urinary catheters.

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Proteus mirabilis is an enterobacterium that causes catheter-associated urinary tract infections (CAUTIs) due to its ability to form crystalline biofilms on the surfaces. These CAUTIs are very difficult to treat, since the biofilm structures are extremely tolerant to high concentrations of antibiotics. Bacteriophages (phages) have been used widely to control and prevent a diversity of bacterial species, however a limited number of phages for *P. mirabilis* have been isolated and studied. Here we report the isolation of two novel virulent phages, the myovirus vB_PmiM_5460 and the podovirus vB_PmiP_5461 able to target respectively 57% and 100% of all *Proteus* strains tested in this study. Both phages have been characterized thoroughly and sequencing data revealed no traces of genes associated with lysogeny. To further evaluate the phages ability to prevent catheter colonization by *Proteus*, phages adherence to silicon surfaces was assessed. Both phages were able to adhere, but the extent of adhesion was found to be phage dependent. Further tests in phage-coated catheters using a dynamic biofilm model simulating CAUTIs, have shown a 90% significant reduction of *P. mirabilis* biofilm formation up to 168 h of catheterization. These results highlight the potential usefulness of the two isolated phages for the prevention of surface colonization by this bacterium.

P3: 19

E. coli biofilm dispersal: searching for mechanisms

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Dispersion is one of the key strategies for fighting biofilms. There are several cues that can trigger biofilm dispersal. Among these, the fatty acid *cis*-2-decenoic acid (CDA) seems to be promising by the extent of its action between species and life kingdoms. However, the mechanisms behind biofilm dispersal with CDA are not fully understood: Does CDA destabilize the extracellular polymeric substance and the interactions between cells or does it actually induce a change in gene expression and differentiation of the sessile cells, both leading to biofilm dispersal? To assess these questions, *Escherichia coli* (*E. coli*) biofilm dispersal was studied in a microfluidic system using confocal laser scanning microscopy (CLSM). Reporter strains were constructed by cloning selected promoters in front of the gene encoding for the green fluorescent protein (GFP). The results show that CDA has both surfactant and physiological effects on the biofilms. Indeed, the dispersion efficacy was more than two-fold higher in the CDA assays compared to control experiments. Differences in GFP intensity levels of one reporter strain between assays with and without CDA were also observed, suggesting a change in gene expression in the presence of CDA; these results are still under investigation. Moreover, CDA does not seem to have an effect on planktonic *E. coli* cells nor on the activity of the selected promoters in the planktonic lifestyle. This suggests that CDA effects are biofilm-dependent. Thus, results confirm that CDA triggers *E. coli* biofilm dispersal and preliminary data suggest that CDA is sensed by the sessile bacteria, inducing a biological response that leads to biofilm dispersal.

P3: 20

Establishing an in vitro haemodialysis tunnelled cuffed catheters-model system

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aemodialysis (HD) is the most widely renal replacement therapy used for patients with end-stage renal disease. A major complication of this technique is the catheter-related infections. Many microorganisms colonize the surface of long-term HD catheters forming biofilms. These biofilms are difficult to eradicate, compromising the function of the catheter and are often associated with recurrent bacteraemia. The aims of this study were to characterize the inner surface of tunnelled cuffed catheters (TCC) currently used in the clinical setting for long-term HD and to setup a continuous flow reactor system to evaluate biofilm formation. Polyurethane TCC was characterized regarding its wettability, surface charge, morphology and roughness. The inner surface of TCC revealed to be hydrophobic and negatively charged. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) analysis evidenced the TCC surface roughness, ranging from micro to nanoscale features. For the reactor setup, TCC were initially incubated with medium inoculated with *Staphylococcus epidermidis* RP62A for 18 h at a flow rate of 1.5 mL/min. Afterwards, fresh medium was added at a flow rate of 3.5 mL/min during 24 h. Biofilm was quantified by colony-forming units, resazurin assay and SEM. The reactor system led to reproducible results for the different measured parameters, including biofilm formation. Bacteria were spread on TCC surface, with several aggregates and extracellular matrix. The established reactor system is set to better understand the ability of clinical isolates to form biofilm on TCC surface and to relate it to the material properties.

P3: 21

Impact of variable oxygen environments on resistance to acute antibiotherapy by cystic fibrosis related bacteria

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The existence of steep oxygen gradients within the cystic fibrosis (CF) airways mucus is well known, with zones ranging from aerobic to completely anaerobic. Those environments, of heterogeneous availabilities of oxygen, contribute for the proliferation of a phylogenetically diverse ecosystem. This study aimed to inspect whether CF-related bacteria - *Staphylococcus aureus* and *Pseudomonas aeruginosa* and other emerging species *Acinetobacter baumannii*, *Dolosigranulum pigrum*, *Inquilinus limosus*, *Klebsiella pneumoniae* and *Stenotrophomonas maltophilia* – are able to develop *in vitro* biofilms and be tolerant towards ciprofloxacin, an in-use antibiotic in acute CF infections. Single biofilms were formed *in vitro*, under aerobic and anaerobic environments, and further evaluated in terms of biomass and CFU counting. The antibiotic resistance profiles were analysed by constructing time-kill-curves. All species were able to growth under environments with distinct oxygen availability, demonstrating a great biofilm-forming ability highlighted by higher amount of biofilm mass, particularly under aerobic atmospheres. Biofilm time-kill curves showed augmented antibiotic tolerance of the bacteria, which was independent of the oxygen availability, except for *D. pigrum* where total eradication of biofilm-cells was noticed. Data highlighted that CF-related bacteria could persist under atmospheres with restricted oxygen availability, and form biofilms resilient to ciprofloxacin. Therefore, a more detailed knowledge about the effect of CF environments on the ability of the bacteria to proliferate and resist to antibiotics might be crucial for the success of CF infection treatment.

P3: 22

In vitro investigation of single and combined metal ions against three medical pathogens

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There has been a dramatic increase in the number of antimicrobial resistant bacteria. Finding alternative antimicrobial actives may reduce the proliferation and transmission of such microorganisms. The antimicrobial efficiency of five metal ions (silver (Ag), copper (Cu), platinum (Pt), gold (Au) and palladium (Pd)) was tested individually and in combination against planktonic *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Enterococcus faecium*. The metals were tested individually using zone of inhibition assays (Zoi), minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). The antimicrobial efficacy for metal combinations were evaluated using Zoi, fractional inhibitory concentrations (FICs) and MBCs. The Zoi tests evaluated the inhibitory efficiency of metals at 50, 100, 500 and 1000 mg/L. The FICs tests for metal combinations efficacy were evaluated in 2:1, 1:1 and 1:2 ratios. Silver, Au, Pt and Pd demonstrated significant antimicrobial efficacy against the three tested pathogens. However, a considerable difference was noted in antimicrobial potency between the tested metals. Copper demonstrated minimal antimicrobial activity. Silver demonstrated the greatest antimicrobial activity against *E. faecium* at low concentrations. The metal combination results showed a greater antimicrobial efficacy than single metals, suggesting synergistic effects. The FICs results for metal combinations showed a significant difference in inhibition with the different with the different ratios. Gram-positive *E. faecium* was evaluated as the most resistant organism. These results demonstrate a potential use of metal ions as active antimicrobials.

P3: 23

Insights into *Pseudomonas aeruginosa* and *Candida albicans* interactions in ventilator-associated pneumonia - effect of combinational antimicrobial therapy

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Ventilator-associated pneumonia (VAP) is a frequent hospital-acquired infection occurring in mechanically ventilated patients, with a mortality of 20-70%. These infections are often caused by mixed populations of bacteria and yeast, which combined with the indiscriminate use of antimicrobials leads to ineffective treatment and contributes to the emergence of multidrug resistant pathogens. The understanding of antimicrobial resistance and the development of new antimicrobial strategies is attracting considerable research interest. In this scope, this work aimed to characterize single and dual species biofilms of *Pseudomonas aeruginosa* and *Candida albicans*, common in VAP infection, and to assess the effect of combinational antimicrobial therapy using amphotericin B (AmB) and polymyxin B (PolyB). Phenotypic analysis of single species biofilms revealed *C. albicans* biomass reduction after 48 h. Dual-species biofilms were dominated by *P. aeruginosa*. The activity of isolated antimicrobials was evaluated against planktonic cultures with AmB being most effective against *C. albicans* and PolyB against *P. aeruginosa*. Mixed planktonic cultures required equal or higher concentrations. In biofilms, only PolyB reduced the microbial population, affecting *P. aeruginosa* in both single and dual-species biofilms, but only at the highest doses. The antimicrobial combinations had a synergistic effect in dual-species planktonic and biofilm cultures, but biofilms were able to regrow. To conclude, combination of antimicrobials shows promising results in the treatment of VAP involving mixed populations, but further optimization of doses and timing of administration are required to avoid reinfection.

P3: 24

Looking for a green solution to surpass disinfectant drawbacks

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The nonsense use of biocides for general disinfection has contributed to the increase incidence of antimicrobial tolerant microorganism to current solutions. Therefore, new antimicrobial agents or biocide formulations are required to help in the control of microbial growth. The purpose of this study was to evaluate the potential of seven phytochemicals (tyrosol, caffeic acid, ferulic acid, cinnamaldehyde, coumaric acid, cinnamic acid and eugenol) to control microbial growth of planktonic and sessile cells of *Staphylococcus aureus* and *Escherichia coli*. The phytochemicals with the most promising antimicrobials properties were cinnamaldehyde and eugenol since they displayed low MIC (3-5 and 5-12 mM, respectively) and MBC (10-12 and 10-14 mM, respectively). Adhesion control potential was observed with cinnamaldehyde, coumaric, caffeic and ferulic acids (all at 10 mM) against *E. coli*. Curiously, it was also observed that cinnamic acid at 10 mM was able to completely inhibit *S. aureus* and *E. coli* adhesion. Furthermore, this activity was comparable to peracetic acid and sodium hypochlorite and was more effective than hydrogen peroxide (all at 10 mM). Assessment of bacterial surface properties showed that cinnamic acid affected bacterial surface decreasing its hydrophilic character and surface charge. The observed effectiveness of selected phytochemicals, against bacteria makes them an interestingly alternative and/or complement to commonly used disinfectants.

P3: 25

Microaerophilic growth characteristics of *Pseudomonas aeruginosa* importance to the successful treatment of chronic infections in cystic fibrosis lungs

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Patients with CF lung disease are susceptible to chronic infections by various pathogens, such as *Pseudomonas aeruginosa*. This pathogen is able to adapt to the environment in CF lungs, characterized by inflammatory defences, oxygen restriction, and poor nutrient availability. It is well established that, once *P. aeruginosa* infection is installed in the lungs, it is almost impossible to eradicate, due to sophisticated genotypic and phenotypic adaptation mechanisms that develop according to the stage of infection. Understanding those changes and the identification of specific characteristics that allow *P. aeruginosa* eradication before the onset of chronic infection is urgent. In this work, *P. aeruginosa* adaptation under microaerophilic and anaerobic conditions was assessed in terms of phenotypic characteristics, antibiotic susceptibility and expression of antibiotic resistance mechanisms (*mexAB* operon). Results showed, when compared with aerobic conditions, similar growth and emergence of intermediate resistance profiles for ciprofloxacin and imipenem, but no significant variation in operon *mexAB* expression. It was also observed an increase of colony morphotypes with the decrease of oxygen availability, mainly in 5% O₂. Nevertheless oxygen depletion has no significant effect on *P. aeruginosa* growth and *mexAB* expression, affecting, however, the phenotypic characteristics and antibiotic susceptibility profiles. Despite the overall observations, the microaerophilic environment with 5% O₂, seems to demonstrate a transient distinct behaviour which can be a point of evolution into chronic infection and as such a possible treatment target.

P3: 27

New insights of sophorolipids ability for inhibiting biofilm on silicone catheters

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Catheters are relevant life-saving medical devices but due to their colonisable surfaces, biofilms frequently take place and as a result healthcare-associated infection (HAI) can occur. New strategies to prevent catheters surface colonization are mandatory and coating catheters with substances that affect microorganism-adhesion or have bactericidal effects are certainly a valuable approach. Surface active agents can play that role and among those are biosurfactants (BS). In the present work biosynthesised sophorolipids (BS) were used as an innovative coating for silicone catheters and as a new strategy for preventing biofilm formation. Sophorolipids (SLs) were synthesised by *S. bombicola* (CBS 6009) and after purification, different concentrations of SLs aqueous solutions were tested to promote SLs adsorption on silicone rubber surface. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as biofilm producer strains. Biofilm inhibition was evaluated with crystal violet staining method and SEM analysis while biofilm viability was evaluated through the resazurin viability method and CFU quantification. A surprising reduction in *S. aureus* biofilm formation was observed with SLs adsorbed on silicone surface when the BS solution used to promote adsorption presented a concentration higher than 0.375 mg mL⁻¹. All performed assays corroborated those results and with the plate count a reduction higher than 10² was observed. A more modest biofilm reduction was observed with *E. coli*. With this work SLs have shown promising features in controlling catheters colonization since they presented good ability in inhibiting biofilm formation on silicone intended for these medical devices.

P3: 28

New quorum-quenching compound based on a brominated nitrovinylfuran to fight *Pseudomonas aeruginosa* pathogenicity and virulence

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Bacterial quorum sensing (QS) plays an essential role in virulence factor production, biofilm formation and antimicrobial resistance [1,2]. This system is considered as a promising target for antipathogenic/antivirulence therapies. Although the great potential that QS inhibition (QSI) has revealed for infection control, drug discovery processes based on QS inhibitors are still in early-stage and clinical application remains distant [3]. This study aims at the screening for new QS inhibitors from drugs already applied in humans for diverse therapeutic purposes. This was achieved by a high-throughput QSI screening based on the co-cultivation of *Pseudomonas aeruginosa* PA14 wild-type strain with the biosensor PA14-R3. The potential of the selected molecules to down-regulate the production of QS-controlled virulence factors and the study of their effects on biofilm formation was also assessed. From different molecules, Furvina[®] (2-bromo-5-(2-bromo-2-nitrovinyl)furan) a brominated nitrovinylfuran compound commonly used in the formulation of ointments marketed in Cuba showed ability to interfere with *N*-3-oxododecanoyl-homoserine lactone (3OC12-HSL) *P. aeruginosa* QS-dependent system. Moreover, inhibition of initial cell adhesion and biofilm formation was observed. Regarding the virulence factors, Furvina[®] demonstrated potential to decrease the levels of pyocyanin, proteases, gelatinase and siderophores production. Overall results demonstrate the significance of Furvina[®] as a therapeutic molecule to tackle *P. aeruginosa* biofilms.

P3: 29

Protease inhibition by PAI-2 in a multispecies biofilm visualized using CSLM

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Periodontitis is associated with an increased flow of protein-rich gingival crevicular fluid resulting from the inflammatory response of the host to biofilm accumulation. This ecological change favors bacteria with a proteolytic phenotype. The bacterial proteases and breakdown products contribute to and trigger the inflammatory reaction resulting in tissue breakdown. The PAI-2/Serpin B2 is an inhibitor of serine proteases and has been detected in very high concentrations in gingival fluid. The aim of the present study was thus to study possible interactions between PAI-2 and proteases produced by biofilm cells in a multispecies bacterial consortium. A multispecies biofilm comprising nine strains was grown for seven days in an Ibidi mini flow cell. The biofilm supernatant was then carefully removed and PAI-2 was added to the biofilm consortium as well as to the supernatant and incubated for 1 hour followed by the addition of FITC-labeled casein, fluorescent serine protease substrate or a substrate specific for *Porphyromonas gingivalis*. After incubation the fluorescence in the supernatant was read in a fluorometer and the biofilm cells were examined in a confocal scanning laser microscope. PAI-2 inhibited proteolytic activity of the bacterial biofilms, as measured by casein breakdown as well as breakdown of the serine protease substrate. Inhibition of extracellular proteases specific to *P. gingivalis* was also seen. To our knowledge, this is the first time that PAI-2 has been shown to inhibit bacterial proteases. Given the high concentration of PAI-2 in the gingival region, this might indicate that PAI-2 plays a role for the integrity of the epithelial barrier.

P3: 30

Pseudomonas aeruginosa modulates *Inquilinus limosus* tolerance to acute antibiotherapy under cystic fibrosis variable oxygen conditions

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Cystic Fibrosis (CF) airways disease involves a complex polymicrobial infection whereby different bacterial species can interact and influence each other. To gain insights into the role that *Pseudomonas aeruginosa* and *Inquilinus limosus* interactions may play during CF infection, the reciprocal effect during biofilm formation, as well as ciprofloxacin activity against mixed biofilms under *in vitro* atmospheres with different oxygen availabilities were evaluated. The kinetics of biofilm formation showed that *P. aeruginosa* negatively affected *I. limosus* growth, under both aerobic and anaerobic environments. On the other hand, under aerobic conditions, *I. limosus* led to a decrease in biofilm production by *P. aeruginosa*, although biofilm-cells viability of remains unaltered. Given the differences measured by the crystal violet [biofilm biomass, consisting of both extracellular polymeric substance (EPS) and cells] and the viable count (biofilm viability) assays, these results may indicate that in mixed biofilms the presence of *I. limosus*, under aerobic conditions, leads to a reduction in *P. aeruginosa* EPS. Interestingly, *P. aeruginosa* might be responsible for the protection of *I. limosus* against ciprofloxacin activity. The analysis of the viable count dynamics revealed that *I. limosus* is less susceptible to ciprofloxacin when co-cultured in mixed biofilms with *P. aeruginosa*. Taken together, the results suggest a reciprocal interference between different bacterial species in CF lung. Alterations of bacterial behaviour due to interspecies interactions may be important for disease progression in CF infection.

P3: 31

Screening assay platform using *Chromobacterium violaceum* biofilms identifies flavones as quorum sensing inhibitors

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Bacteria have developed several fine tuned sensory systems to respond correctly to environmental signals. Quorum sensing (QS) is probably the best known and most thoroughly studied communication mechanism. It is essential for all bacteria populations to exist, but it is not affecting the bacterial growth. This intercellular communication is especially essential to biofilm populations that use QS for controlling formation, virulence, tolerance and resistance. Here we have optimized a screening applicable assay to search for new quorum sensing inhibitors (QSI) in a screening compatible setting using 96-well plate format for violacein quantification. We have used *Chromobacterium violaceum*, which wild type (ATCC 31532) produces the purple pigment violacein when its QS-system is activated and also the mutant strain, CV026 that requires addition of an autoinducer molecule to produce violacein. Compounds that show activity on the mutant but no activity at all on the wild type we hypothesize to be quorum quenchers (QQ). When these two assays are used in parallel with a viability staining it is possible to distinguish between QSI-, QQ- and bactericidal activities. This platform was used for a screening campaign using a library of flavonoids (n=411). The most active compounds found were flavones and structurally similar. The most active compounds had IC₅₀s of 3.7 µM and 8.7 µM, respectively. These compounds do not show any bactericidal activity against other tested bacterial strains. Thus, by targeting this communication system it might be possible to find an effective new antimicrobial strategy without the immediate risk of resistance.

P3: 32

Silver nanoparticles obtained by green synthesis associated to the calcium glycerophosphate: characterization and antimicrobial efficacy

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The 'green' synthesis has shown great efficacy in the production of metallic nanoparticles, providing less toxic products and economically more viable. Calcium glycerophosphate (CaGP) has presented anti-caries properties having a major impact on the prevention of dental caries. The aim of this study was to synthesize and characterize a biomaterial containing silver nanoparticles (Ag) and CaGP (Ag/CaGP) using pomegranate extracts (*Punica granatum*) as reducing agent. Its antimicrobial activity was also evaluated. Synthesis was performed using silver nitrate, pomegranate extracts, nano-sized CaGP, ammonium salt of methacrylic poly acid polymer and deionized water as solvent. Ag/CaGP was characterized by UV-Vis absorption spectroscopy (UV-Vis), X-ray diffraction (XRD) and transmission electron microscopy (TEM). Ionic silver concentration and minimum inhibitory concentration (MIC) for *Streptococcus mutans* ATCC 25175 also were determined. UV-Vis absorption spectra showed all nanocomposites contained silver with nano-sized by the presence of an absorption peak, which occurs between 420 and 450 nm. XRD pattern demonstrated that all nanocomposites were composed of Ag and CaGP. TEM images showed nanoparticles with spherical form. Nanocomposites obtained by the extracts of the peels and sheets presented lower values of ionic silver. All nanocomposites led to effective antimicrobial activity for *Streptococcus mutans* with values of MIC between 0.15 and 0.62 mg/mL. Therefore, it was concluded that the 'green' synthesis using pomegranate extracts as reducing agent was effective to obtain silver nanoparticles associated with CaGP, incorporating them antibacterial effect.

P3: 33

The decreased efficacy of enzymes on the control of flow-generated biofilms

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Current strategies to remove biofilms from equipment surfaces are generic and thus inefficient. Enzymes are considered a new and environmentally friendly approach for biofilm control. Four enzymes (β -glucanase, protease, lipase, α -amylase) alone and in combination with cetyltrimethylammonium bromide (CTAB) were assessed on their potential to control flow-generated *Pseudomonas fluorescens* biofilms, in terms of mass and colony forming units (CFU). β -glucanase and α -amylase showed a synergistic potential to control the biofilms by removing biomass and reducing the number of CFU. Long-term and regrowth effects were observed during a 24-hours period monitored after the treatments. The effect of the enzymes and their combination with CTAB against planktonic cells was also evaluated using a respirometer. Protease and lipase displayed antimicrobial properties. The exception was α -amylase showing increased bacterial activity. Protease and α -amylase were observed to hinder the antimicrobial activity of CTAB. Results show a synergistic potential of β -glucanase with CTAB for *P. fluorescens* biofilm control, and demonstrate that a careful selection and application of enzymes must be considered since these molecules can quench the activity of antimicrobial agents.

P3: 34

The effect of conditioning films on the antimicrobial efficacy and retention capabilities of *Staphylococcus epidermidis* on titanium nitride silver coatings

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Bacterial colonisation of surfaces is a multi-factorial process. Before bacteria can attach to a surface, it will become coated with an initial deposition of an organic conditioning film. Surface parameters were quantified using Energy dispersive X-Ray (EDX) (chemistry), Atomic Force Microscopy (AFM) (topography) and water contact angles (wettability). Microbiological tests were used to assess the effect of bovine serum albumin (BSA) or horse blood (HB) conditioning films on the antimicrobial activity and microbial retention of a range of surfaces. Stainless steel was used as the underlying substrata and was coated with titanium nitride (TiN) or titanium nitride containing silver coatings (TiNAg at 14.94 and 19.04 % atomic silver). Zone of inhibition assays were used to assess the antimicrobial activity of the surfaces whilst retention assays were performed to quantify the retention of bacteria and the conditioning films on the surfaces using differential staining and epifluorescence microscopy. The results demonstrated that the conditioning films were retained in the greatest amounts to the stainless steel surface. The presence of BSA on the surfaces demonstrated an possible adjuvant effect on the antimicrobial efficacy of the silver. Further, the presence of the conditioning films on the surfaces significantly reduced the numbers of bacteria retained on the TiN and TiNAg surfaces. This work suggests that the presence of a conditioning film may impede bacterial retention.

P3: 35

The effects of chemical and mechanical stresses on the removal of biofilms formed by drinking water-isolated bacteria

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The presence of biofilms in drinking water distribution systems (DWDS) is a global concern. Those are responsible for aesthetic problems in drinking water (DW) such as taste, odor and color, and can also be responsible for public health problems when pathogens are biofilm colonizers. Therefore, it is of utmost importance to use effective control strategies. The aim of this work was to understand the effects of the combination of chemical (sodium hypochlorite – NaOCl) and mechanical (pipe flushing) treatments on the removal of single and dual species biofilms of two bacteria isolated from drinking water, *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia*. Those are common strategies used in DWDS. A rotating cylinder reactor was used for the first time as DWDS model for biofilm formation and control. The combination of chemical and mechanical treatments was not able to completely remove biofilms from polyvinyl chloride (PVC) surface. Chemical treatment did not improve the flushing efficiency of *A. calcoaceticus* biofilms and increased the recalcitrance of *S. maltophilia* biofilms to mechanical treatment, even when high shear stress was applied. Nevertheless, NaOCl improved the mechanical removal of dual species biofilm. The dual species biofilm remaining after treatment with NaOCl at 0.5 mg.l⁻¹ was 78%, while after being treated with the NaOCl at the minimum inhibitory concentration 12% of biofilm remained on PVC surface. The overall results demonstrate that chemical and mechanical treatments commonly used in DWDS are not effective in biofilm control. The colonizer strain strongly influences the biofilm phenotype and its susceptibility to control strategies.

P3: 36

The material test “BioMig” predicts the biofilm formation potential on plumbing material

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Enhanced biofilm formation in household plumbing installations can deteriorate drinking water quality regarding the water's color, odour and taste, and by providing a habitat for opportunistic pathogens like *Legionella pneumophila*. However, excessive biofilm formation can be prevented by choosing plumbing materials that leach only small amounts of organic carbon, which serve bacteria as a food source in the otherwise rather nutrient-limited drinking water. We developed a comprehensive test method called “BioMig”, which allows reproducible assessment of the carbon leachate over time and the biofilm formation potential of a material under standardised conditions. We tested more than 100 materials including plastic and metal pipes, sealing rings, coatings and concrete materials and detected considerable differences between materials within the same product class and also among products of the same material but from different suppliers. This suggests that the potential of a plumbing material cannot only be derived from the material itself, but other factors like differences in production have to be taken into account. Furthermore, the application of the “BioMig” package on a variety of materials enabled new insights into bioavailability and potential inhibitory effects of carbon compounds, temperature dependency of materials and revealed that microbial growth can enhance carbon leachate from some materials on a large scale. This, together with a test period of only two weeks compared to several months for other tests, allows industry to use “BioMig” as a predictor of a material's biofilm formation potential during the optimisation and/or screening of plumbing products.

P3: 37

Transcriptomic study of *Listeria monocytogenes* subjected to stresses encountered in food processing plants

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The ability of *Listeria monocytogenes* to adhere and to persist on surfaces for months or even years despite correct and frequent applications of hygiene operations may be responsible for the transmission of *L. monocytogenes* from contaminated surfaces to food products in food processing plants. During food manufacturing and sanitation procedures, *L. monocytogenes* frequently encounters combined stresses, such as acid, heat, cold or freezing temperatures, drying and osmotic pressure changes. However, one hypothesis to explain persistence is the capacity of bacteria to adapt and to survive to these various stresses (1). Survival under these extreme and rapidly changing conditions requires the appropriate expression and induction of stress genes and proteins in bacterial cells in order to respond to stimuli signaling these environmental conditions. The aim of this study was to determine the adaptive mechanisms used by *L. monocytogenes* adherent on surfaces before and after hydric stress (desiccation), chemical stress (cleaning & disinfection), cold stress and/or saline stress as encountered in smoked salmon factories. Thus, a transcriptomic approach was performed in order to identify the functions which vary between stressed and unstressed cells. From the comparison of expressed genes common to all the studied stresses, potential viability markers could be found to improve detection of adherent cells including the viable but non culturable cells.

[1] B. Carpentier et O. Cerf. *International Journal of Food Microbiology*. 145 (2011) 1-8.

P3: 38

3D morphological features and antibiotic resistance in *L. monocytogenes* – *Pseudomonas* sp. biofilms

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In the present study, the adhesion ability on stainless steel, the 3D biofilm morphology and the sensitivity to ampicillin, gentamycin and ciprofloxacin of a strain of *L. monocytogenes* grown as monospecies and dual-species biofilm with *Pseudomonas aeruginosa*, *Pseudomonas putida* or *Pseudomonas fluorescens* was assessed. A significant increase in the number of adhered *L. monocytogenes* of ~ 2 log CFU/cm² was observed in all of the dual-species biofilms compared with monospecies culture. Confocal laser scanning microscopy (CLSM) images showed how 3D morphologies were also significantly different. *L. monocytogenes* biofilm presented a thick cellular monolayer whereas all dual-species biofilms formed cellular aggregates (microcolonies) that varied both in diameter and thickness depending on the accompanying species. *L. monocytogenes* logarithmic reductions of adhered viable cells after antibiotic exposure were lowered in all cases where *Pseudomonas* sp. was present. Outcomes also showed the relationship between the presence of cellular aggregates conferring higher structural complexity to the biofilm and the reduced antibiotic sensitivity. In conclusion, the presence of *Pseudomonas* sp. clearly affected the biofilm formation ability of *L. monocytogenes* and that the structural features and the higher *L. monocytogenes* presence were related. The fact that *L. monocytogenes* was less sensitive to antibiotics in biofilms with cellular aggregates demonstrated that these structures can act as a shield to antibiotics lowering its antimicrobial effect which also justifies the need to establish better monitoring and control procedures in both industrial and medical environments.

P3: 39

A novel matrix-based regulation mechanisms of bacterial biofilm formation

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Pseudomonas aeruginosa, the principal pathogen of cystic fibrosis patients, is able to form antibiotic-resistant biofilms and promote chronic colonization of the airways. Recent work suggests that the *P. aeruginosa* exopolysaccharides could serve as regulators for expression of biofilm related genes. In the present work, we investigated the roles of a CdrA, an extracellular adhesin that anchors Psl exopolysaccharide to the cell surface, on regulating biofilm formation. Using RNA-sequencing based transcriptomic analysis, we identified that CdrA is able to regulate the synthesis CupA fimbriae, which have been shown to be required for *P. aeruginosa* pathogenesis and biofilm formation. Presence of CdrA is crucial for CupA fimbriae-dependent pellicle formation. Our study sheds light on a novel matrix-based regulation mechanisms of bacterial biofilm formation.

P3: 40

Activity of conventional antibiotic enhanced through synthetic analogues of hylin a1

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Most microorganisms lie embedded in communities called biofilms. Among these stand out *Pseudomonas aeruginosa* an opportunistic pathogen of animals frequently associated with nosocomial infections difficult to be eradicated due to the presence of biofilm. The fluoroquinolone antibiotics such as ciprofloxacin (CIP) are frequently used against this bacterium. However, that allow, overpressure, a development of mechanism of resistance for this microorganism. Thus, new strategies of microbial control have been the subject of numerous studies in order to replace or enhance conventional methods. To this aim, antimicrobial peptides (AMPs), obtained from various living organisms, represent an alternative to the development of new drugs. Synthetic forms analogous to Hylin a1 AMP (Hy-a1), isolated from species *Hypsiboas albopunctatus*, has shown excellent antimicrobial efficacy, among which stand out the peptide Lys-[Trp⁶]hy-a1 (Lys-a1). Thus, this work was to verify the influence of the peptide Lys-a1 in antimicrobial activity of CIP against *Pseudomonas aeruginosa* ATCC 9027. The data showed that CIP, as well as Lys-A1, are capable of inhibiting the growth of *P. aeruginosa* biofilm. However, the combination of both substances caused a decrease of concentration of CIP necessary to prevent biofilm development and enhanced the antibiotic action against a mature biofilm. Considering the results of this study, the synthetic peptide Lys-A1 appears as an adjuvant therapeutic for the treatment of infectious diseases caused by *P. aeruginosa* biofilm.

P3: 41

Solidago virgaurea extract inhibits morphological transition and biofilm formation by *Candida albicans*

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Previously, we have demonstrated the antifungal activity of a medicinal plant *Solidago virgaurea* (SV) against the major fungal pathogen *Candida albicans*. SV extract strongly inhibit yeast-hyphal transition, pre-formed biofilm (77.8%) and biofilm formation (98.4%) (Chevalier *et al.*, 2012). The extract of SV consists of several secondary metabolites (flavonoids, saponins...). Some of them have known to cause damage to cell membrane. The aim of this study was to investigate the mechanisms of inhibition of *C. albicans* morphological transition and biofilm formation by whole SV extract. *C. albicans* (ATCC 10231) and 33.3% of SV extract prepared in RPMI were used for subsequent analysis. Propidium iodide (PI) uptake assay was employed to find the impact of SV on cell membrane integrity. The resistance mechanisms efflux activity, heat shock proteins 90 (HSP90) activation and chitin synthesis were also assessed. The effect of SV treatment on the transcription of genes involved in adhesion (*ALS3*) and hyphal formation (*HWP1*) were investigated using real-time qPCR. Assessment of PI fluorescence showed no significant difference in PI uptake suggesting SV is not a membrane active compound. Sensitivity assays showed inhibition of chitin synthesis or efflux activity or HSP90 by their respective inhibitors not significantly enhance the SV activity. Expression of *HWP1* and *ALS3* were significantly downregulated by SV at 4h and 24h. These data shows that SV significantly affects *C. albicans* biofilm formation by its ability to prevent change in cellular morphology. Further studies are required to investigate more about its mechanism of activity and determine its applicability for clinical use.

P3: 42

Adaptation of chronic wound isolates to ionic silver in planktonic and biofilm growth modes

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Silver compounds are commonly used in wound dressings to control bacterial growth with generally good efficacy although sporadic cases of reduced silver susceptibility have been described in certain Gram-negative bacteria. In the current investigation, we have exposed wound isolates to silver nitrate using a validated system in order to assess the potential for adaptation. An agar diffusion system was used to expose sub-inhibitory concentrations of ionic silver to wound isolates over ten passages (P10) and again following ten additional passages in silver-free media (X10). Test bacteria were as follows: *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacteroides fragilis*. Susceptibility to silver in planktonic and biofilm growth modes was assessed using standard methods. Gram-positive bacteria exhibited a >4-fold decrease in susceptibility to ionic silver following passaging (P10). Reductions in susceptibility were also observed in *S. aureus* and *S. epidermidis* biofilms. In contrast, adaptations in Gram-negative wound isolates were limited to *P. aeruginosa* (P10) which when grown in sessile form exhibited a highly mucoid phenotype and a ≥ 16 -fold reduction in silver susceptibility. In the absence of silver exposure, full or partial reversion to baseline susceptibilities was observed in all bacteria with the exception of *S. aureus*. Repeated exposure to silver resulted in transient reductions in silver susceptibility in wound isolates. Studies are currently underway to understand the molecular basis of these changes.

P3: 43

Anti-biofilm effect: combining bacteriophages with Portuguese honey

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Nowadays, the rising of multi resistant bacteria due to the misuse of antibiotics, together with the lack in the development of new antimicrobial molecules, represents a serious threat to Medicine. Many of these multi resistances bacteria are pathogens responsible for nosocomial infections causing problem in Human health and care. This work addresses the problematic of treating chronic wounds, lacerations and infected burns. In these types of infections, bacteria colonize the tissues and form biofilms, conditioning the effectiveness of antibiotics. Based on this reality, the development of news approaches to treat multi resistance organisms is essential. A combination of two antimicrobial solutions, bacteriophages (phages) and honey, is suggested herein. Bacteriophages (phages) are natural bacteria predators that specifically recognize hosts, destroying it. Honey is also able to destroy bacteria, but also to degrade biofilms, to improve the healing process, promoting tissue regeneration, and to decrease the inflammation. Both are efficient against antibiotic resistant microorganisms, including those present in chronic wounds. To date there is no study about this combinatorial therapy. In this study, the anti-biofilm effect of the combination of a Portuguese honey together with well-studied lytic phages was enhanced, in *Escherichia coli* and *Pseudomonas aeruginosa* biofilms relatively to the same antimicrobials used individually. This result was confirmed by a decrease in the total biomass, and by a reduction, in average, of more than 3 log of cultivable cells.

P3: 44

Anti-microbial effect of CHX-loaded nanohydroxyapatite against *S. aureus* and *E. coli* dual-species biofilm

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Devices-related infections (DRIs) are caused by surface-adhering microorganisms persisting as biofilm resistant to host defence and antimicrobial agents. These infections have a huge impact in terms of morbidity, mortality and medical costs. New strategies are therefore needed to prevent the occurrence of DRIs, as the design of biomaterials with antimicrobial features. The objective of this study was to evaluate the anti-adhesive properties of chlorhexidine digluconate (CHX)-loaded nanohydroxyapatite materials against single- and dual-species biofilm of *S. aureus* and *E. coli*. *S. aureus* and *E. coli* (alone and in combination) were inoculated into cultures containing the biomaterials. The resulting biofilms were quantified by the enumeration of colony-forming units and examined by confocal microscopy using both Live/Dead staining and bacterial-specific fluorescent in situ hybridization (FISH). CHX-loaded nanohydroxyapatite revealed to be successful to minimize both single- and dual-species biofilm. Interesting competitions for two species community were observed, where *E. coli* was the out-competing species. In conclusion, CHX-loaded nanohydroxyapatite surfaces appear as a promising approach to the prevention of DRIs.

P3: 45

Anti-quorum sensing strategy against *Agrobacterium rhizogenes* to prevent biofilm formation and hairy-root disease

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Hairy-root disease caused by *Agrobacterium rhizogenes* is induced by the incorporation of a bacterial DNA segment into plant cell chromosome. This is strongly regulated by quorum sensing (QS) communication using signal molecules of homoserine lactone (HSL) type. QS is also likely involved in biofilm formation on abiotic surfaces (e.g. irrigation distribution systems) to serve as pathogen reservoir, and on root surfaces prior to infection. We aim to isolate rhizosphere bacteria able to protect tomato cultures by degrading QS signals, which should disturb both biofilm formation and the infection process. We showed that 10 *A. rhizogenes* strains all produce the same HSL molecule as the only or very majoritarian quorum sensing molecule. We set up the conditions to grow *in vitro* biofilms of *A. rhizogenes* strains in static condition. These biofilms were observed by confocal scanning laser microscopy, showing that the biofilm structures were strain-dependent. We then isolated about 1,600 bacterial strains from 10 tomato greenhouses (infected or not by *A. rhizogenes*) and we screened these strains for their ability to degrade HSLs, using *Chromobacterium violaceum* CVO26 as a biosensor. 54 strains were shown to degrade HSLs, and their ability to specifically degrade the *A. rhizogenes* HSL was examined. We are now examining their ability to inhibit biofilm formation by *A. rhizogenes* and virulence of the latter. This could lead to a new strategy to control dissemination of hairy-root disease.

P3: 46

Antibiofilm activity of Brazilian propolis extracts against *Staphylococcus aureus* producers isolated from goat and sheep mastitic milk

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Mastitis, inflammation of the mammary gland, causes reduction in milk yield and quality. The main etiologic agent is *Staphylococcus aureus*, usually very resistant to antimicrobials and often biofilm forming. Propolis is a resinous mass, rich in flavonoids, produced by honeybees *Apis mellifera*, which has earned the attention of many researchers due to its antimicrobial action. The aim of this study was to investigate the action of inhibiting *S. aureus* biofilm formation, and the ability to eliminate formed biofilm, by alcoholic extracts of green, red and brown propolis from Brazil. Ten isolates of *S. aureus* have been tested, 8 field isolates, 1 MRSA and 1 ATCC 25923, by microplate quantitative method. For the evaluation of inhibitory action, the isolates were inoculated, in triplicate, in Tryptic Soy Broth (TSB) 1% glucose in the presence of green (1), red (2) and brown (4) propolis extracts. Biofilm formation was evaluated by optical reading, compared to a negative control consisting of a mixture of TSB and extract. For biofilm elimination assay, extracts were added to plates with 24h cultures of the same isolates. Assays were repeated three times on three different days. Eight out of the 10 isolates produced less biofilm in the presence of the green propolis extracts, so the inhibitory effect is 80%. Brown propolis extracts inhibited the formation of biofilm in 10% to 70% of the isolates and the red extracts in 30% to 80%. Regarding the biofilm elimination activity, green propolis extract was positive for 9 out of the 10 isolates (90%), the brown propolis extracts were positive for 20% to 100% isolates and red extracts for 10% to 20% isolates. According to these results, green propolis extract showed to have the greater ability to prevent and disrupt biofilm produced by *S. aureus* and might be promising for mastitis control.

P3: 47

Antimicrobial activity of biomaterials containing glycerophosphate and silver nanoparticles

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Calcium glycerophosphate (CaGP) present anti-cariogenic properties, and in association with an antimicrobial nanomaterial could prevent this dental disease. The anti-biofilm properties of nanobiomaterials containing CaGP (commercial form or nanoparticulated by milling for 24 hours) and silver nanoparticles (Ag, 1 or 10%) produced by two reducing agents (sodium citrate or sodium borohydride) is illustrated in this study. The compounds were dried by incubation or by a lyophilization process, and characterized by SEM and EDX in 2D. AgCaGP were previously tested against *Candida albicans* (ATCC 10231) and *Streptococcus mutans* (ATCC 25175) by broth microdilution method, and the samples with better results were tested in their single and mixed biofilms. Biofilms were quantified by colony formation unity, total biomass and metabolic activity assays. SEM and EDX images demonstrated the Ag nanoparticles associated to the CaGP. The significant reduction in both single and mixed biofilms was reached at 40 µg/mL of AgCaGP-citrate and 200 µg/mL of AgCaGP-borohydride. For both drugs, there were about 2.5 log reduction in the *C. albicans* biofilm, but it became more resistant in the presence of *S. mutans*. In the contrary, the cells of *S. mutans* biofilm were more susceptible to AgCaGP-citrate when grown with *C. albicans*. AgCaGP-citrate drastically decreased the total biomass of *C. albicans*. In general, the metabolic activity was reduced by both AgCaGP regardless of the biofilm tested. AgCaGP seemed to be a promise anti-caries biomaterial, especially considering the low concentration of silver needed to fight against the biofilms when sodium citrate is used as reducing agent.

P3: 48

Antimicrobial potential of *Eucalyptus globulus* against biofilms of *Staphylococcus aureus* isolated from bovine mastitis

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Staphylococcus aureus are among the most common species isolated from bovine mastitis. The pathogenesis of this bacterium is facilitated by a number of virulence factors, including the ability to adhere to abiotic surfaces and/or host tissues often leading to biofilms' formation. From the clinical perspective, the most important feature of *Staphylococcus* species' biofilms is their high tolerance to the conventional antimicrobial therapy. So, the increasing number of bovine mastitis and the higher levels of *Staphylococcus* species resistance to traditional antimicrobial agents are considered an important alert for the necessity to focus the future research on identification and development of new strategies to combat *S. aureus* mastitis. Recently, the interest in natural alternatives based on plant extracts has been rising. In addition to their health benefits, their antimicrobial potential has been increasingly reported. Taking this into consideration, the evaluation of hydromethanolic extracts of *E. globulus* against *S. aureus* biofilms was tested and compared with penicillin, one of the antibiotics most often used in the treatment of cattle infections. All mastitis' isolates tested were good-biofilm producers. As expected penicillin has demonstrated poor activity against *S. aureus* biofilms (<1 log reduction). However, *E. globulus* Labill was bactericidal, promoting a biofilm cell reduction of 2-3 log. Therefore, the present work showed the potential antimicrobial activity of *E. globulus* against *S. aureus* from bovine mastitis, namely in biofilm mode of growth and drew attention to its promising use as an alternative to penicillin.

P3: 49

Bacillus subtilis biofilms for the bioprotection of cultivated button mushrooms

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Worldwide, food losses amount to about one third of food for human consumption. A large fraction of these losses is due to microbiological deterioration. Until recently, chemical pesticides were the most commonly used solution in agriculture because of their radical effectiveness. In France, the government aims by 2018 to reduce by 50% the use of such pesticides through for sustainable agriculture, which led to the intensification of biological control in agriculture. Biocontrol represents at the present time, 5% of the plant protection sector. In the sector of button mushrooms (*Agaricus bisporus*) in France, the biocontrol agent used by more than 80% of the sector and compatible with organic agriculture is *Bacillus subtilis* QST713. It shows a clear biofungicidal effect against *Trichoderma aggressivum*, the most prevalent compost flooding. As part of this work, we evaluate the impact of this biofungicidal on mushroom yield of a crop of *Agaricus bisporus* exposed or not to *T. aggressivum*. We also evaluate the spatial organization of the plurimicrobial microbial biofilms formed on the carpophore at the step of fructification of *Agaricus bisporus* by scanning electron microscopy. This study will help to increase knowledge on the button mushroom biocontrol mechanisms, including biofilm-specific processes, and may allow possible applications to other crops.

P3: 50

Bacteriophage depolymerases– novel polysaccharide degrading enzymes

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In nature, biofilms are the most common lifestyle of bacteria and are difficult to eradicate, partially due to the extracellular polymeric substances content of the slime in which biofilms are embedded, which act as a primary defence against disinfection. (Bacterio)phages are viruses that specifically infect bacteria and can represent an important strategy for biofilm control. These viruses have evolved specialized enzymes, called depolymerises, to degrade polymers present in the bacterial surface and slime (e.g. capsular and structural polysaccharides) to facilitate access to their hosts. We performed an *in silico* analysis of all available phage genomes infecting different bacteria and found 160 putative depolymerises with specialized activity (e.g. hyaluronidases, alginate and pectate lyases). This illustrates how well phages are equipped to degrade a diverse range of biofilm-associated polymers. We cloned and recombinantly expressed an *Acinetobacter* phage depolymerise and demonstrated its activity by the spot-on-lawn method. To further characterize the enzyme activity, the bacterial host genome was sequenced and a cluster (24.6 kb) of genes responsible for the capsular polysaccharide biosynthesis was identified. Using DNA recombination, mutations in either of the two transcribing strands were introduced to generate capsular polysaccharides deficient mutants. Results demonstrate that the depolymerise specifically degrades *Acinetobacter* capsular polysaccharides. Overall, *in silico* and experimental results suggest that phage represent a source of enzymes to degrade polymeric substances presence in bacterial slime, which can be further exploited for biofilm control.

P3: 51

Bioactive glass combined with bisphosphonates provides protection against biofilms formed by the periodontal pathogen *Aggregatibacter actinomycetemcomitans*

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Biofilms play a pivotal role in the progression of periodontitis and they can be treated with antiseptics (i.e. chlorhexidine) or antibiotics. However, these therapeutic alternatives are unable of ameliorating periodontal alveolar bone loss, which has been, on the other hand, successfully treated with bone-preserving agents. The improved bone formation achieved in animal models by the combination of two such agents: bioactive glass (BAG) and bisphosphonates has attracted the interest for further exploring dental applications. However, the antimicrobial effects that may result from combining them have not been yet investigated. Here, our aim was to explore the anti-biofilm effects that could result from combining BAG with bisphosphonates (alendronate, clodronate, etidronate, risedronate and zoledronate), particularly in a dental biofilm model. The experiments were performed with an oral cavity single-specie (*Aggregatibacter actinomycetemcomitans*) biofilm assay, which was optimized in this contribution. Risedronate displayed an intrinsic anti-biofilm effect, and all bisphosphonates, except clodronate, reduced biofilm formation when combined with BAG. In particular, the anti-biofilm activity of risedronate was significantly increased by the combination with BAG. Since it has been proposed that some of the antimicrobial effects of BAG are caused by local pH changes, studies of pH variations were performed to gain a mechanistic understanding. However, the observed anti-biofilm effects could not be explained with lowered pHs. Overall, these results do provide further support for the promising use of bisphosphonate-BAG combinations in dental applications.

P3: 52

Biocide coated microparticles as anti-biofilm agents

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Control of undesirable biofilms often includes the use of chemical products with antimicrobial properties which are associated with the formation of harmful disinfection by-products. This study proposes a technological approach to minimize the use of antimicrobial agents and their deleterious effects, based on the principle of drug-delivery systems whereby the biocidal chemicals are transported on microparticles (MP). The MP were prepared using a layer-by-layer (LBL) self-assembly technique by the deposition of benzyldimethyldodecyl ammonium chloride (BDMDAC) onto calcium carbonate (CaCO₃) core. MP were characterized by zeta potential and by scanning electron microscopy (SEM). The efficacy of the microparticles was assessed against *Pseudomonas fluorescens* planktonic cultures and 48-hours-biofilms developed in a bioreactor. The application of 50 mg/L MP against planktonic cells revealed a similar antimicrobial action (3 Log reduction) as free BDMDAC. MP revealed also high efficiency in reducing biofilm number of cells, after 2 hours of exposure. At the same BDMDAC concentration (500 mg/L), it was observed a 4 Log reduction for the free compound as for the MP. The overall results indicate that BDMDAC immobilization can be a promising strategy to control biofilms. Additionally, preliminary data propose that the biocidal MP may be reused, which would reduce the environmental risks associated with excessive use of chemical agents, thereby providing environmental and public health benefits.

P3: 53

Biofilm formation in the presence of sub-inhibitory concentrations of antibiotics

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Antimicrobials compounds exhibit high biological activity and their sub-inhibitory concentrations in the environment can induce the evolution of resistance. The ability of microbial populations shift to a biofilm phenotype is directly related with an increase of resistance. The object of this work was to contribute to improved knowledge of the effect of antimicrobial compounds. Planktonic and biofilm populations of Gram-positive bacteria *Rhodococcus erythropolis* and Gram negative bacteria *Pseudomonas fluorescens* were exposed to the sub-inhibitory concentrations of bacitracin, polymyxin B, erythromycin and chlorhexidine. The effects of these substances on hydrophobicity and the zeta potential of cell surface, the parameters that are closely related with cell adhesion, were determined. Simultaneously, the influence of these substances on adhesion of cells of mentioned microorganisms in the first 24 hours and subsequent development biofilm on hydrophobic and hydrophilic surfaces was determined. It was demonstrated that only polymyxin B significantly affects the hydrophobicity of the cell surfaces and inhibits the initial adhesion of both microorganisms. The other three substances created positive pressure on the colonization of surfaces, wherein the strongest biofilm inducer was chlorhexidine. This induction of biofilm formation and next development has been associated with a change in its morphology towards increase of part of large objects. It was confirmed that unappropriated application of antimicrobial compounds leading to distribution of these substances in sub-inhibitory concentrations for a longer time cannot be ignored.

P3: 54

Biofilm formation of *Pseudomonas aeruginosa* isolates of various origins and effect of divalent ions

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Pseudomonas aeruginosa (*Pa*) is an opportunistic pathogen associated with nosocomial infections and disease complications. A particular case is in the lungs of cystic fibrosis (CF) individuals, where biofilm growth plays a crucial role in the persistence of *Pa*. Some strains of *Pa* are especially well adapted to the CF lung microenvironment, showing distinguishable phenotypes linked to biofilm production when compared to other strains. Knowing this, we focused on investigating if biofilms of different *Pa* isolates react the same way to changes in growth conditions. We compared the biofilm formation of four different *Pa* isolates: PAO1, the reference strain isolated from a wound, LESB58 from chronically infected CF patients' lungs, PPF1 and Urg7, two environmental isolates from dental unit waterlines. Unattached biofilms were obtained by 48h cultures at 37°C in 24-well plates in various growth conditions and characterized by macroscopic observations and electron microscopy. When grown in agitated LB medium, no biofilm could be detected for PAO1 and Urg7. PPF1 biofilm was greatly mucilaginous; whereas LESB58 biofilm was dense, with almost no planktonic cell growth. The addition of 1 mM of Mg²⁺ to the medium was sufficient to inhibit the biofilm formation of LESB58, but not of PPF1. In contrast to what has been shown previously when using PAO1, the addition of Zn²⁺ did not have the same inhibitory effect on LESB58 and PPF1, which kept producing biofilm. These results demonstrate the great biofilm formation diversity in *Pa* and indicate that chemical inhibition of biofilm formation for a specific strain or isolate should not be generalized to other *Pa* strains.

P3: 56

Candida albicans vulvovaginal biofilm response to progesterone: genes involved

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Vulvovaginal candidiasis (VVC) caused by *Candida albicans* is a common infection that has been considered an important public health problem. The most important *C. albicans* virulence factor is the ability to form biofilms, which in the vaginal environment may be formed on epithelium and on intrauterine devices promoting VVC. Despite it has been shown that VVC has a hormonal dependency the effects of progesterone on biofilm formation by *C. albicans* are still poorly understood. Thus, this work aimed to deepen the knowledge in that field by studying how the presence of progesterone modulates the transcriptional response of *C. albicans* biofilms, a knowledge that is essential to identify possible targets to control VVC. The progesterone effects on *C. albicans* biofilms were evaluated in terms of total biomass, metabolic activity, structure and matrix composition, while the transcriptional response was assessed by using species-specific microarrays. The results obtained showed that progesterone was able to reduce *C. albicans* biofilm, decreasing its biomass, structural cohesion, matrix production and matrix carbohydrate content. Additionally, progesterone decreased the expression of several genes involved in the carbohydrate metabolism and biological adhesion including four genes known to be required for *C. albicans* ability to form biofilms (*TEC1*, *PBR1*, *AHR1* and *CR_01410C_A*). Considering that the vaginal tract is one of the main driveways for the development of *C. albicans* infections, the identification of genes that may determine the ability of this yeast to survive and form biofilm in the vaginal environment may contribute to the disclosure of new targets to treat/prevent VVC.

P3: 57

Carvacrol, a component of essential oils, present a strong antimicrobial activity against CoNS planktonic and biofilm cells

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Coagulase-negative staphylococci (CoNS) are a group of bacteria that inhabit healthy human skin and mucosae, and hence, have long been considered non-pathogenic. However CoNS are currently recognized as important etiological agents of healthcare-associated infections, being particularly associated with patients with indwelling medical devices, due to their ability to form biofilms on the surface of such devices. Along with the overuse of antibiotics that resulted in a dramatic increase in resistant bacteria, CoNS bacteria within biofilms are intrinsically more tolerant to antimicrobial agents than their planktonic counterparts. As a consequence, biofilms are frequently associated with the development of persistent infections. Hence, the search for new prophylactic and therapeutic strategies for the management of CoNS biofilm-associated infections is essential. Herein, we studied the susceptibility of CoNS planktonic and biofilms cells to carvacrol, one of the most common components of essential oils found in aromatic plants. Carvacrol showed a significant effect on the viability of both planktonic and biofilm cells even after a short period of interaction. Low concentrations of carvacrol, such as 2 μM , were sufficient to reduce up to 3 Log_{10} of the initial challenge. Importantly, the antimicrobial effect of carvacrol on CoNS planktonic cells was greater than the one observed with vancomycin, one of the most frequently used antibiotics to treat staphylococcal infections. Overall, these results showed that carvacrol is a potential antimicrobial agent, which may be used in the future for the prevention and/or treatment of CoNS-associated infections.

P3: 58

Cometary plasma discharge with metallic grid as antibacterial and anti-biofilm agent

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Increasing rate of antibiotic resistance of biofilm communities brings necessity to find new solutions. The biofilm-cells are almost 1000 times more resistant to commonly used antibiotics than suspension cells what represents serious complication in medicine. Non-thermal plasma is a promising tool for the treatment of variety of human biofilm-associated infections. The cometary corona discharge has the jet pointing to the space which delivers the active species to the surface. Therefore, plasma beam is absolutely independent on exposed sample. Metallic grid embedded between plasma jet and the treated surface helps to increase the affected area. The aim of this study was monitoring of antimicrobial and anti-biofilm properties of cometary plasma discharge aperture involving metallic grid for the amplification of the inhibition effect on representative strains of opportunistic pathogenic bacteria *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The second aim was to evaluate possible synergistic effect of non-thermal plasma and antibiotics on selected bacteria. The antibacterial activity of non-thermal plasma was expressed as the size of inhibition zone of cell population on Petri dishes. The anti-biofilm effect on biofilms growing on titan steel was determined by crystal violet assay and MTT method. We have confirmed that non-thermal plasma has significant antibacterial activity and also high potential for the biofilm disruption. Indeed, by combination of non-thermal plasma and antibiotic it is possible to completely eradicate pre-formed biofilms.

P3: 59

Design of an online-detection system for the removal of biofilms in food industry

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Adhesion of bacteria and the development of biofilms can cause serious problems in food industry, such as contamination, causing food spoilage or food-borne illnesses, increasing energy consumption and corrosion. Repeated exposure to sanitizers can even lead to an enhanced resistance of bacteria in biofilms. Hence it is evident that biofilms have to be removed entirely from food contact surfaces. Even though cleaning and disinfection protocols exist in food industry, biofilms are still present. This might be due to the fact that the design of most cleaning protocols is still semi-empirical or based on personal knowledge. Thus the aim of this work was to establish an optical laboratory method for an online detection of biofilms during the cleaning process in order to understand and optimize the removing behaviour and cleaning time of biofilms on surfaces used in food industry. Therefore we used a colour CCD camera system which is able to record the cleaning process of a biofilm temporally and spatially at a millimeter scale. As subsequent processing steps like staining the biofilms might alter the results, we used GFP-tagged *Escherichia (E.) coli* SM 2029 expressing a green fluorescent protein and *E. coli* TOP 10 pTurbo RFP-B expressing a red fluorescent protein. Optical filters, lightning and detection mode were varied and optimized towards the fluorescence spectrum of each microorganism. With the established experimental setup we were able to visualize biofilm-forming *E. coli* which allows us to investigate their removing behaviour and thus optimize cleaning processes in industry in order to ensure food safety.

P3: 60

Ecological shifts in multi-species oral biofilms

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Periodontitis (gum disease) is a common biofilm-mediated infection; with 7-10% of adults aged 50 having extensive tissue destruction which may cause tooth loss. Recently, a link between chronic periodontitis and cardiovascular disease has been described. In periodontitis, the immune response to biofilm accumulation on the tooth increases the flow of protein-rich gingival exudate and growth of proteolytic, Gram-negative anaerobes e.g. *Porphyromonas gingivalis* and *Fusobacterium nucleatum* is seen. Since proteolytic activity from the biofilm can contribute to disease progression, we are investigating changes in this activity during ecological shifts in multi-species biofilms. A six-species consortium containing bacteria found in periodontitis, as well as the individual component species, was grown in the presence (10% human serum) or absence (nutrient broth) of serum for 2-6 days under anaerobic conditions. Species composition was studied by culturing and the proteolytic activity in the supernatant was determined by zymography or fluorometry after incubation with FITC-labelled casein. In the absence of serum, Gram-positive streptococci dominated the consortium and the Gram-negative anaerobes were lost. However, 10% serum favoured growth of the whole multi-species consortium. Proteolytic activity was found in supernatants from the consortium growing in 10% serum but was low in the absence of serum. Zymography indicated secretion of multiple proteases from *F. nucleatum* and *P. gingivalis*. Thus exposure to protein-rich exudate favoured growth of Gram-negative anaerobic bacterial species capable of secreting proteases that could contribute to disease progression in periodontitis.

P3: 61

Effect of new derivative of 2(5H)-furanone (F105) on biofilm formation by *Staphylococcus aureus* **Irshad Sharafutdinov¹, Airat Kayumov¹, Elena Trizna¹, Almira Kurbangalieva¹, Oliwia Makarewicz²**

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Nosocomial infections by methicillin-resistant *Staphylococcus aureus* (MRSA), an opportunistic biofilm-forming pathogen causing a variety of diseases including osteomyelitis, endocarditis, infections of indwelling devices and sepsis are still serious problem, because bacteria organized in biofilms become resistant to antibiotic treatment and immune system of the host leading to difficult to treat and recurrent chronic infections. Here we show the effect of new derivative of 2(5H)-furanone (compound F105), synthesized in Kazan Federal University, on biofilms formed by MRSA strain. Biofilms were grown in Muller-Hinton broth and minimal inhibitory concentration (MIC), minimal biofilm inhibitory concentration (MBIC) and minimal biofilm eradication concentration (MBEC) of F105 were determined. MIC was identified by broth microdilution assay by cultivating of the MRSA in presence of the furanone F105 for 48 hours and was found to be 40 mg/l. The MBIC was studied by cultivating biofilms in eight-well slides. After 48 hours biofilms were analyzed by live/dead staining with Syto9 and propidium iodide on confocal microscope LSM 780 (Zeiss, Germany). At the concentration of 80 mg/l, F105 completely inhibited the biofilm formation, and the cell growth was suppressed by two orders of magnitude. Finally, the MBEC was defined to be also 80 mg/l. In summary, furanone F105 seems to be a promising compound in treatment of MRSA biofilms, and further studies on other Gram-positive biofilm-forming species are in preparation.

P3: 62

Effect of α - amino acid on dispersal of *Pseudomonas aeruginosa* PAO1 biofilm.

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Pseudomonas aeruginosa, an opportunistic pathogen that is responsible for diseases such as cystic fibrosis (CF) in immunocompromised individuals. Outcome of CF is abnormally thickened mucus that causes problems in patient's respiratory system. This condition affects 2.5 million people in the UK alone with a high rate of mortality by the age of 40. Various methods of treatment to manage CF by dispersion and disassembly of biofilm have been intensively researched. Amongst the current methods of controlling CF, biofilm dissociation by the use of a combination of an amino acid and an antibiotic has been investigated in this research. During this study, biofilm formation by *P.aeruginosa* PAO1 was observed under aerobic conditions in Luria Broth and M63 minimal media at 37°C for a period of 24 hours. The cultures were treated with two isomeric forms of tryptophan at different concentrations (1mM, 4mM, and 8mM). Dispersal and dissociation of the cells in all cultures were investigated and compared with the control after 24 hours. The D isomer of tryptophan at concentrations of 4 mM and 8 mM showed higher rate of dispersion in comparison to the L isomeric form and the control. The effect of tryptophan varied with the medium that was used for biofilm growth. Extracellular polymeric substances (EPS) were extracted from the treated and untreated biofilm and quantified for their main components. Biofilm treated with tryptophan and erythromycin resulted in nearly 70% loss of EPS components in comparison with the control after 5 days of growth.

P3: 63

Emulsion encapsulation of isoeugenol to combat food-related biofilms

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Growth of biofilms in foods or on food-contact surfaces can potentially lead to food-borne illnesses or spoilage. Essential oils are aromatic compounds extracted from plants and they have attracted attention as possible candidates for natural food preservation. However, their use can be limited by their effects on the organoleptic properties of the food and by the high concentrations that are often needed to kill microorganisms. In this study we investigate emulsion encapsulation of the essential oil isoeugenol as a means of enhancing the antibacterial efficacy. Emulsions were prepared with the emulsifier n-OSA starch and some were coated with the positively charged polymer chitosan. We compared the antibacterial efficacy of emulsion encapsulated isoeugenol with unencapsulated isoeugenol against biofilms of *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas fluorescens* and *Leuconostoc mesenteroides* in media and carrot juice at 6°C and 25°C. Emulsions were characterized to give information about size, zeta potential, morphology, loading capacity and release profiles. The antibacterial efficacies were measured with Minimum Biofilm Eradication Concentration (MBEC) Assays and visualized with confocal laser scanning microscopy. We found that emulsion encapsulation enhanced the antibacterial properties of isoeugenol against *S. aureus*, *L. monocytogenes* and *L. mesenteroides*, but not against *P. fluorescens* biofilms in media at most conditions. In carrot juice we observed no effect of emulsion encapsulation. In the future it should be evaluated how much isoeugenol affects the organoleptic properties of the food and if encapsulation can circumvent possible adverse effects.

P3: 64

Environmental *Bacillus* spp. produce compounds capable of disrupting *Streptococcus mutans* biofilms

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Oral diseases, including dental caries, affect the majority of the world's population. The primary cause of caries is demineralisation of the tooth enamel by acid. This acid is produced by oral bacteria through the fermentation of dietary sugars. *Streptococcus mutans* is one species of oral bacteria which causes caries and also forms a biofilm (dental plaque). The disruption of *S. mutans* biofilms is therefore a potential treatment for caries. In this study, 89 bacterial isolates from a variety of environmental samples were screened for anti-biofilm activity against *S. mutans* using a microtitre plate biofilm assay. Cell free supernatants were screened for anti-biofilm activity by using a crystal violet biofilm assay. The results show that 7 *Bacillus* spp. produced extracellular compounds capable of disrupting *S. mutans* biofilms. If added at the start of the biofilm assay the supernatants either caused detachment of the biofilms or prevented biofilm formation without affecting the growth of *S. mutans*. These results show 7 environmental bacteria produce compounds exhibiting at least two anti-biofilm activities against *S. mutans*.

P3: 65

Evaluation of SICAN and SICON® surfaces for biofouling mitigation in the food industry

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Biological fouling in food industry leads increased maintenance costs, decreases operational efficiencies and promotes food contamination leading to economic losses and the dissemination of foodborne pathogens. In order to maintain production efficiency and hygienic standards, cleaning in place (CIP) procedures are required. However, the existence of critical zones shielded from the main flow carrying the CIP disinfectants requires new strategies for reducing biofilm buildup and/or easier to clean surfaces. The present work evaluates the performance of two Diamond-Like Carbon (DLC) coatings containing silicon: (a-C:H:Si or SICAN) and SICON® (a-C:H:Si:O). These surfaces were compared with stainless steel (316L) regarding bacterial adhesion, biofilm formation and cleanability. Assays included the natural flora present in industrial water from a salad washing line and *Escherichia coli*, one of the most persistent foodborne microorganisms. Results show that bacterial adhesion on SICAN and SICON was similar to stainless steel and therefore surface modification was not able to prevent biological fouling development. It was also shown that after performing a cleaning protocol with chlorine, reduction of bacterial counts was much higher in SICAN (3.3 Log reduction) and SICON® (3.5 Log reduction) when compared to stainless steel (about 1.7 Log reduction). Results suggest that these surfaces can be useful particularly in food processes with frequent cleaning schedules and may be applied to critical areas, such as dead zones, crevices, corners, valves and joints where bacterial attachment is more likely to occur and where cleaning is particularly difficult.

P3: 66

Fabricating N-halamine nanoparticles for antimicrobial and antifouling applications

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Biofilm formation is a serious problem in medical and industrial settings due to the increased resistance of these communities to killing compared to free-living bacteria. This has prompted the search for agents that can inhibit both bacterial growth and biofilm formation. In this study, N-halamine rechargeable nanoparticles (NPs) were synthesized by copolymerization of the monomer methacrylamide and the cross-linker monomer N,N-methylenebisacrylamide, and were subsequently loaded with Cl^+ , using bleach. The chlorinated NPs demonstrated remarkable stability and durability to organic reagents and to repetitive bacterial loading cycles. The antibacterial mechanism of the P(MAA-MBAA)-Cl NPs involved generation of reactive oxygen species (ROS) only upon exposure to organic media, but not upon suspension in water, revealing that the mode of action is target-specific. Further, a unique and specific interaction of the chlorinated NPs with *Staphylococcus aureus* bacteria but not with human cells was discovered, whereby these microorganisms were all specifically targeted and marked for destruction. Finally, in collaboration with Netafim Ltd. irrigation drippers containing the P(MAA-MBAA)-Cl were incubated in the field and were shown to prevent fouling on them for 5 months compared with the control, hence providing the drippers with 'self-cleaning' and 'self-sterilizing' properties. Further, the NPs offer recharging to the surface, thus providing long-lasting protection that does not exist in the products available today. In summary, our findings underscore the potential of developing sustainable P(MAA-MBAA)-Cl NPs-based devices for inhibiting bacterial colonization and growth.

P3: 67

Hamamelitannin analogs as potentiators for antibiotics in the treatment of MRSA biofilm infections

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The quorum sensing (QS) modulator hamamelitannin (HAM) was recently shown to affect *Staphylococcus aureus* biofilm susceptibility through the TraP receptor by affecting cell wall synthesis and eDNA release. However, from a medicinal chemistry perspective the structure of HAM has several disadvantages and it is envisioned that more potent and druglike HAM analogs could be synthesized. Over 150 HAM analogs were synthesized by applying three modifications: replacement of the ester groups, modification of the galloyl moiety and removal of the anomeric hydroxyl group. The mechanism of action (MOA) was investigated using *S. aureus* QS mutant strains and different phenotypic assays. The most active analogs were evaluated in a vertebrate murine model of mastitis infection. We identified analogs with 100-fold increased activity compared to HAM. These analogs potentiated the effect of different classes of antibiotics against *S. aureus* strains with a functional *traP* gene confirming the role of TraP. They also affected susceptibility towards lysostaphin and affected eDNA production of *S. aureus* biofilm cells, indicating a MOA similar to that of the native HAM molecule. Finally, the most potent analog increased susceptibility towards antibiotics in a murine mastitis model. We determined the SAR of >150 HAM analogs and identified analogs with improved activity and physico-chemical properties. The analogs affect cell-wall thickness and eDNA release, both leading to the increased susceptibility of *S. aureus* biofilm cells towards antibiotics. Our results finally indicate that HAM-analogs also increase susceptibility *in vivo* and that this *in vivo* effect is superior to that of HAM.

P3: 68

Identification of ABC-JK2, a small molecule inhibitor of staphylococcal biofilm formation.

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Staphylococcus aureus is a major causative pathogen and forms biofilms. However, there are still no effective therapeutic agents for combatting biofilm-related infections. In this study, based on the concept that compounds specifically targeting biofilm formation would not exert high selective pressure for resistance, we aimed to identify compounds that inhibit biofilm formation by *S. aureus* without affecting bacterial growth. Biofilms were formed on 96-well flat-bottomed polystyrene plates in the absence or presence of compounds, and were quantified by the crystal violet-staining method. A high-throughput screening has been employed using 50,000 compounds with characteristic structures. Effects of the hit compounds on metabolic profile, gene expression profile, and cell morphology were evaluated by metabolomic analysis, microarray/quantitative real-time PCR, and thin-sectioning transmission electron-microscopy observation, respectively. One of the hit compounds, named anti-biofilm-compound JK2 (ABC-JK2), inhibited biofilm formation of several strains of *S. aureus* including MRSA and *Staphylococcus epidermidis*. ABC-JK2 decreased intracellular levels of glycolytic metabolites and increased the expression of genes related to peptidoglycan biosynthesis and hydrolysis in *S. aureus*. In addition, aberrant morphologies of *S. aureus* with thick cell walls and abnormal septa were observed in the presence of ABC-JK2. These results indicate that ABC-JK2 inhibits staphylococcal biofilm formation presumably by affecting glycolysis and quality control of cell wall. These findings might lead to innovative drug development for preventing and curing staphylococcal biofilm infections.

P3: 69

Identification of genetic transcriptional factors involved in non-*Candida albicans* *Candida* species biofilm development

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Candida albicans is the major cause of candidosis, however recently non-*C. albicans* *Candida* (NCAC) species have emerged as common pathogens. One of the most important virulence factors is the ability to form biofilms that have important clinical repercussions due to their increased resistance to antifungal therapy. In the case of *C. albicans*, the transcriptional network of biofilm formation is composed by six transcription factors (*BCR1*, *EFG1*, *TEC1*, *NDT80*, *ROB1* and *BRG1*). However, in the case of NCAC species little is known about the influence of these genes in biofilm formation. Thus, in order to identify targets to be used as biofilm controllers in NCAC species it was characterized the role of *BCR1*, *EFG1* and *TEC1* genes on *C. parapsilosis* and *C. glabrata* species biofilm formation. After planktonic cells and biofilms grown, the RNAs were extracted and the expression levels of *BCR1*, *EFG1* and *TEC1* compared by quantitative real time PCR. CFUs enumeration and crystal violet staining were used to quantify biofilm formation. The results demonstrated that in both *Candida* species all genes are expressed but in a species and lifestyle dependent manner. Specifically, in opposite to observed in *C. glabrata*, *BCR1* and *TEC1* expression levels, are higher in biofilm than in planktonic cells of *C. parapsilosis*. Interestingly the *EFG1* levels of expression was superior to 100% in both conditions for *C. parapsilosis*, however higher in planktonic cells. Thus, it is possible to assume that *BCR1*, *TEC1* and *EFG1* are biofilm regulators in *C. parapsilosis*, as in *C. albicans*, but not in *C. glabrata* and they could be suggested to be used as future targets to control *C. parapsilosis* biofilm formation.

P3: 70

Identification of putative nitric-oxide sensing protein domains that play a role in *P.aeruginosa* biofilm dispersal

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Previous studies have shown that low concentrations of nitric oxide (NO) can induce the dispersal of *Pseudomonas aeruginosa* biofilms and enhance the performance of antibiotics by modulating levels of intracellular c-di-GMP. However the NO-sensing pathway that leads to dispersal in *P. aeruginosa* remains to be fully elucidated. It is known that the GGDEF motif of DGC (diguanylate cyclase) and the EAL motif of PDE (phosphodiesterase) are responsible for the production and hydrolysis of c-di-GMP, respectively. The activity of these two motifs is hypothesised to be regulated by upstream NO sensor PAS or MHYT domains, resulting in c-di-GMP turnover. Our research uses PAO1 as model organism and looks into the functions of 13 genes encoding PAS/MHYT + GGDEF and/or EAL through knockout and phenotypic assays with NO treatment, as well as the structure and enzymatic activities of these 13 proteins. This work is being carried in collaboration with the Diamond light source Synchrotron for structural analysis of potential NO-binding domains. Phenotypic assays show that the *P. aeruginosa rbdA* mutant has lost its response to NO, while *dipA*, *pa0338* and *pa0285* have reduced motility compared to PAO1 wild type. This study will provide a deeper insight into NO signal transduction and its application as an adjunctive agent with various antibiotics for eliminating pre-established and persistent *P.aeruginosa* biofilms in a range of settings.

P3: 72

Influence of daptomycin resistance on MRSA biofilm formation

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Daptomycin (DAP) is an anti-MRSA (methicillin-resistant *Staphylococcus aureus*) drug. There is a risk of resistance in long-term treatment with it. The influence of DAP resistance on the phenotypes of MRSA is unclear. In this study, we examined biofilm formation of DAP-susceptible MRSA (DAP-S) and DAP-resistant MRSA (DAP-R) isolated before and after DAP treatment performed in clinical course of a patient. MICs of DAP for clinical isolates (DAP-S and DAP-R) used in this study were determined by Etest. Using next-generation sequencing data, sequence types (STs) and staphylococcal cassette chromosome *mec* (SCC*mec*) types of these isolates were assigned. Moreover, amino acid sequence of the multiple peptide resistance factor MprF was analyzed. Quantification and visualization of biofilms were performed with the crystal violet staining and confocal reflection microscopy method. Productivity of phenol-soluble modulins (PSMs) involved in MRSA biofilm formation was compared using quantitative real-time PCR and SDS-PAGE. MIC of DAP for DAP-S was 0.094 µg/ml, while DAP-R was 1.5 µg/ml. These strains isolated from a patient belonged to the same clone (ST1-SCC*mec*IV). DAP-R had a single point mutation in *mprF*, leading to an amino acid substitution (S295P). The biofilm biomass of DAP-R was much lower than DAP-S. Compared with DAP-S, higher expression of PSMs in DAP-R at the level of transcription was observed. It is suggested that DAP resistance leads to decreased biofilm formation via promotion of PSM production. By analyzing the influence of MprF mutation on biofilm formation, we can get a clue to prevent and control MRSA biofilm infections.

P3: 73

Influence of pipe materials on the effectiveness of biofilm removal with a cationic surfactant

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The efficacy of a treatment with benzyldimethyldodecyl ammonium chloride (BDMDAC) combined with mechanical removal was assessed against biofilms formed on stainless steel (SS) and high-density-polyethylene (HDPE). A rotating cylinder reactor (RCR) was used to form *Bacillus cereus* and *Pseudomonas fluorescens* single and dual-species biofilms. The minimum inhibitory concentration (MIC) of BDMDAC was 5 µg/mL for *B. cereus* and 15 µg/mL for *P. fluorescens*. Quorum sensing inhibition was also assessed with different concentrations of BDMDAC against a *Chromobacterium violaceum* strain. The MIC did not cause inhibition of quorum sensing, however, 150 µg/mL and 300 µg/mL of BDMDAC did inhibit quorum sensing. Moreover, selected phenotypical aspects of the biofilms formed on the RCR were evaluated. *P. fluorescens* biofilms had higher dry mass on both materials. *P. fluorescens* biofilms formed on SS presented the highest cell density, while no difference was found for biofilms formed on HDPE. The extracellular contents were distinct amongst all the biofilms. The treatment with BDMDAC removed more biofilm formed on HDPE. The mechanical treatment was more effective than the chemical one, but neither was effective for proper cleaning. *P. fluorescens* biofilms showed high resistance to the combined treatment, regardless of the surface where they were formed. Despite the material there was always a layer of at least 15% of persistent biofilm that resisted to treatments, which may lead to the growth of recidivist biofilms even after sanitation procedures.

P3: 74

Inhibition of adhesion of uropathogenic *Escherichia coli* to canine and feline uroepithelial cells by extracts from cranberry

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Uropathogenic *Escherichia coli* (UPEC) is the most important infectious cause of urinary tract infections (UTI) in dogs and cats. The UPEC isolates from pets share virulence similarities with strains responsible for UTI in humans. Dietary consumption of cranberries has long been associated with the maintenance of urinary tract health in humans. This protective activity is linked to decreased adhesion of UPEC to uroepithelial cells after consumption of cranberries. The present study was designed to evaluate if cranberry extract addition has any impact to prevent the attachment of UPEC to canine Madin-Darby Canine Kidney (MDCK) and Crandell-Rees Feline Kidney (CRFK) uroepithelial cells *in vitro*. When the extracts were present during bacterial growth or only during adhesion tests, a dose-dependent decrease of UPEC adhesion to all cell types was observed. Bacterial growth was weakly decreased in the presence of cranberry extracts showing that the anti-adherence effect did not require a bacterial growth inhibitory effect. In conclusion, the addition of cranberry extract has preventive effects on the *in vitro* bacterial attachment to canine and feline uroepithelial cells in a dose dependent way.

P3: 75

Inhibition of staphylococcal biofilm formation by marine sponge-derived bacterium *Streptomyces* sp.

D56

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Staphylococcus epidermidis, an opportunistic pathogen belonging to the coagulase-negative staphylococci (CoNS) ranks first among the causative agents of nosocomial and chronic biofilm-associated indwelling medical device-related infections. The restricted penetration of existing antimicrobials into these biofilms together with the alarming levels of resistance to antibiotics and host immune system rendered by the bacteria in the biofilms, has sharpened the need for development of new antibiofilm molecules. We aimed to evaluate the antagonistic potential of marine sponge-derived actinomycetes in inhibiting the biofilm formation of *S. epidermidis*. The biofilm forming reference strain *S. epidermidis* RP62A was employed as a model for searching antibiofilm compounds by the standard crystal violet assay. Results from *in vitro* assays, scanning electron and confocal microscopy, revealed that an organic extract derived from the marine sponge-associated bacterium *Streptomyces* sp. D56 significantly ($p < 0.0001$) inhibited the biofilm formation on polystyrene, glass and contact lens surfaces, without affecting the growth of *S. epidermidis*. Interestingly, the extract at the tested concentration (125 µg/mL) did not have any cytotoxic effects on human corneal epithelial cell line. These data suggest that compounds in *Streptomyces* sp. D56 extract selectively restrict the staphylococcal biofilm formation without interference with bacterial cell viability. These results highlight the potential of sponge-associated bacterium *Streptomyces* sp. D56 to prevent the intractable infections caused by staphylococcal biofilms.

P3: 76

Insights into *Pseudomonas aeruginosa* and *Candida albicans* consortia challenged by antimicrobials

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Ventilator associated pneumonia (VAP), an usual nosocomial infection in the intensive care units and the most common in mechanically ventilated patients, is a serious problem due to high mortality and morbidity rates associated. The presence of the endotracheal tube is the principal risk factor for developing VAP because its surface is prone to microbial adhesion and the formation of biofilms, deserving thus high attention in clinical settings. Cell-to-cell communication is an important mechanism of interaction between VAP microorganisms, being involved in the process known as quorum-sensing (QS) that regulate the expression of virulence. To evaluate bacteria fungi cross-talk in co-infection, the biofilm-forming ability of *Pseudomonas aeruginosa* and *Candida albicans*, individually or jointly, before and after antibiotic and antifungal co-treatment was tested. Biofilms were characterized in terms of total mass and cell viability. Results showed that no antimicrobial combination was successful in the binary biofilms eradication. In some cases, the tolerance of the polymicrobial consortia was higher than that of single biofilms, highlighting that *P. aeruginosa* and *C. albicans* established synergistic relationships. To gain knowledge helping to explain those interactions, a quantitative real-time PCR approach was followed to inspect the expression profiles of some cell-cell communication genes involved in biofilm resistance. To overcome the tolerance issues, new antimicrobial combinatorial approaches using QS-inhibitors are being tested. Some combinations involving chlorogenic acid and ciprofloxacin displayed promising anti-biofilm potential.

P3: 77

Mixed species biofilms of *Fusobacterium necrophorum* and *Porphyromonas levii* impair the oxidative response of bovine neutrophils in vitro

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Biofilms composed of anaerobic bacteria can result in persistent infections and chronic inflammation. Host immune cells have difficulties clearing biofilm-related infections, which can result in tissue damage. Neutrophils are a vital component of the innate immune system and help clear biofilms. The comparative neutrophilic response to biofilms versus planktonic bacteria remains incompletely understood, particularly in the context of mixed infections. The objective of this study was to generate mixed species anaerobic bacterial biofilms composed of two opportunistic pathogens, *Fusobacterium necrophorum* and *Porphyromonas levii*, and evaluate neutrophil responses to extracellular fractions of both biofilms and planktonic cells. Purified bovine neutrophils exposed to mixed-species planktonic bacteria showed elevated oxidative response compared to neutrophils exposed to biofilms composed of the same bacteria. Bacterial lipopolysaccharide (LPS) plays a significant role in the stimulation of neutrophils; biofilms produced substantially more LPS than planktonic bacteria under these experimental conditions. Removal of LPS significantly reduced neutrophil oxidative response to planktonic bacteria. Oxidative responses to LPS-removed biofilm fractions and LPS-removed planktonic cell fractions were similar. The limited neutrophil response to biofilms in this study supports the reduced ability of the innate immune system to eradicate biofilm-associated infections. LPS is likely important in neutrophil response; however, the presence of other extracellular, immune modifying molecules in the bacterial media also appears to be important in altering neutrophil function.

P3: 78

Natural products interfering with *Pseudomonas aeruginosa* quorum sensing system

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The multidrug-resistant bacterial biofilms represent a challenge in the treatment of persistent infections. One strategy focuses on quorum sensing inhibitors (QSIs). Quorum sensing (QS) is used by various bacteria to coordinate their group behavior in response to population density. Gram-negative bacteria use *N*-acyl-homoserine lactones (AHLs) for cell to cell communication. AHLs interact with cellular receptors and initiate the expression of target genes which regulate e.g. biofilm formation. Sessile cells of *Pseudomonas aeruginosa* are highly resistant to conventional antibiotics. Because quorum sensing plays an important role in infections caused by this human pathogen, compounds which are able to inhibit quorum sensing are required. QSIs can attenuate virulence without killing the bacteria and thus without creating evolutionary pressure and resistance development. The aim of our study was to investigate the production of QS molecules in *P. aeruginosa* and to evaluate the ability of selected natural products and a conventional antibiotic polymyxin to interfere with the QS system of *P. aeruginosa*. We optimized the method for determination of AHLs levels using *Agrobacterium tumefaciens* NTL4 (pZLR4) biosensor for this purpose. This biosensor contains an inserted plasmid that is responsible for the expression of β -galaktosidase in presence of AHLs. This enzyme cleave X-Gal to a blue product and spectrophotometric measurement can be applied. Using this approach, we found out that after application of natural product chitosan, production of AHL in *P. aeruginosa* is reduced (the β -galaktosidase expression is lower).

P3: 79

New phytochemicals as antibiotic coadjuvants against biofilms of *Staphylococcus aureus*

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The ability of five phytochemicals, reserpine, pyrrolidine, quinine, morin and quercetin, to prevent and control *Staphylococcus aureus* biofilms and to potentiate antibiotics was assessed. Prevention of bacterial adaptation to antibiotics and inhibition of efflux pumps by the phytochemicals was also evaluated on NorA overexpressing SA1199B. Biofilms grown for 24h in 96-well microtiter plates were exposed for 1h and 24h to phytochemicals either alone or combined with ciprofloxacin, tetracycline and erythromycin and analyzed regarding their culturability. Prevention of biofilm formation was assessed by growing biofilms for 24h in the presence of the drugs. Moreover, an adaptation experiment was conducted for 15 days by growing bacteria with increasing doses of ciprofloxacin in the presence of phytochemicals. Bacterial susceptibility was assessed by disc diffusion method. Ethidium bromide accumulation in SA1199B in the presence of phytochemicals was measured fluorometrically in a 96-well microtiter plate for 60min. The exposure of pyrrolidine and morin for 1h against biofilms allowed high decrease in culturable cells. Morin and quercetin significantly prevented biofilm formation. Synergism between antibiotics and phytochemicals was found especially against SA1199B biofilms. SA1199B cells growing in subinhibitory dosis of ciprofloxacin showed increased resistance, which was successfully reversed by quinine and morin. Reserpine and quercetin showed high efflux pump inhibition. This study emphasized the importance of co-therapies, such as phytochemicals and antibiotics association, in order to promote more efficient treatments and decrease antimicrobial resistance to antibiotics.

P3: 80

Novel perfume composition for the prevention and control of biofilms based on biotechnologically-produced molecules

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Biofilms are the source of persistent hygienic problems in industrial and domestic areas. Conventional disinfectants, which are often toxic and carcinogenic, may contribute to inefficient biofilm control and to the dissemination of resistance. Consequently, there is a necessity to develop new sustainable strategies for biofilm prevention. The main objective of PERFUMIC project is the development of a new perfume, which will be formulated in cleaning products, with antibiofilm and antimicrobial activity for the efficient disinfection and malodor removal. Instead of chemical biocides, such perfume is constituted by antibiofilm agents from biotechnological processes: microbial fermentation and enzymatic synthesis. Whereas *Lactobacillus plantarum* was selected as the appropriate microorganism because it can produce bacteriocins and organic acids, lipase was chosen as it is an enzyme able to synthesize monoglycerides and sugar esters. The production of these molecules included lab-scale and bioreactor biosynthesis. The most relevant microorganisms of target sectors were selected to validate antibiofilm and antimicrobial properties of the compounds. Antimicrobial activity was assessed by agar diffusion and minimum inhibitory concentration tests. Antibiofilm activity was analyzed with biofilm models grown over inert surfaces by two methods: cell count and electronic microscope (SEM). Promising results were obtained as biotechnologically-produced molecules were active at low concentrations. Moreover, these molecules were suitable to be placed into the organic matrix of typical perfumes of cleaning products.

P3: 81

Photodynamic inactivation of oral biofilms with tetrahydroporphyrin-tetratosylat (THPTS) as photosensitizer

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In the face of the increasing antibiotic resistance of bacterial species in the oral biofilm, 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 THPTS on the initial adhesion of oral bacteria and oral biofilm *in situ*. Individual upper jaw acrylic appliances were manufactured for six study participants and six BES were anchored on their proximal sites for 2 h or 3 days. For APDT with VIS + wIRA the irradiation time was 5 minutes. THPTS was used as photosensitizer in a concentration of 100 µg/ml. The untreated biofilms served as negative control and biofilms treated with 0.2% chlorhexidine (CHX) served as positive control. After the APDT-treatment, the colony forming units (CFU) were quantified, whereas the surviving bacteria were isolated in pure cultures and identified using MALDI-TOF, biochemical tests and 16S rDNA-sequencing. The bacterial vitality was determined using live/dead staining and confocal laser scanning microscopy (CLSM). THPTS-mediated APDT yielded a significant decrease of up to 3.7 and 5.4 log₁₀ CFU for initial and mature oral biofilms, respectively. The vital staining showed a high bactericidal effect of APDT against the initial (91%) and mature oral biofilm (75%). The diversity of the adherent oral microorganisms (*Streptococcus mitis*, *Streptococcus sanguis*, *Veillonella parvula*) has been significantly reduced. The APDT can be considered as a new promising supplementary therapy to treat periodontitis and periimplantitis.

P3: 82

Potent activity of citrus essential oils for the prevention and treatment of polymicrobial biofilms

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Mixed microbial infections with *Pseudomonas aeruginosa* and fungi capable of producing biofilms are commonly found in patients with chronic infections and constitute a significant health care burden. The aim of this study was to address the antimicrobial potential of selected essential oils (EOs) to prevent and treat biofilms formed by *P. aeruginosa* and pathogenic fungal isolates. The mechanism of antimicrobial activity of EOs is presented. The inhibition of biofilms formation was measured by crystal violet staining and visualized using fluorescent microscopy. The effect of EOs on *P. aeruginosa* quorum sensing and virulence was evaluated. Acyl-homoserine lactone (AHL) secretion was followed using *P. aeruginosa* PA14-R3 and *P. aeruginosa* PAOJP2 reporter strains. The effect of the EOs on fungal membrane integrity was addressed by propidium iodide uptake assay using flow cytometry. Pompha and greipfruit EOs affected *P. aeruginosa* virulence and biofilm formation through reduced AHL production. Importantly, the EOs treatments did not affect *P. aeruginosa* growth, making resistance unlikely to occur. EOs caused fast disintegration of fungal membrane inhibiting their growth and biofilm formation. Citrus EOs efficiently reduced formation of bacterial and fungal monomicrobial biofilms in concentrations up to 100 µg/ml. The same concentrations of EOs inhibited mixed biofilms formed by *P. aeruginosa* with *Aspergillus fumigatus* or *Scedosporium apiospermum*. Biofilms formed by *P. aeruginosa* with *A. terreus* stayed resistant to EOs. Pompha and greipfruit EOs showed a potent antibiofilm activity and could be used for the control of common polymicrobial infections.

P3: 83

Preliminary studies on the susceptibility of *Staphylococcus epidermidis* biofilm-released cells to antibiotics and ability to survive in the presence of human blood

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Worldwide, the formation of bacterial biofilms on the surface of medical devices is a major concern in health care systems due to their high tolerance to antibiotics and ability to evade the host immune system. Biofilm lifecycle involves three stages: 1) adhesion, 2) accumulation and maturation and 3) biofilm disassembly. Biofilm disassembly, in which cells from the biofilm are released to other sites, is thought to be one of the major causes of the emergence of serious complications such as sepsis and embolic events of endocarditis, as observed in *S. epidermidis* biofilm infections. Despite the clinical relevance of these cells, little is known about the phenotypic changes that occur after being released from the biofilm. To overcome this lack of knowledge, we performed a series of *in vitro* assays aiming to compare the susceptibility of planktonic, biofilms and biofilm-released cells to several antibiotics. In addition, the ability of these populations to evade circulating immune cells was also addressed. Interestingly, our results showed that biofilm-released cells presented a different phenotype when exposed to some antibiotics. However, regarding the ability to evade the circulating immune cells, no significant differences among the distinct populations were observed. Thus, these findings indicate that biofilm-released cells present a distinct antimicrobial tolerance that should be investigated in depth, in order to proficiently target, prevent and treat *S. epidermidis* biofilm-related infections.

P3: 84

Pseudoalteromonas sp. 3J6 prevent biofilm of *Vibrio tapetis*, a clam pathogen forming biofilms with spherical components

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Vibrio tapetis is a marine bacterium causing Brown Ring Disease (BRD) in the Manila clam *Ruditapes philippinarum*. *V. tapetis* biofilm formation remains unexplored despite the fact that it might be linked to pathogenicity. Our objectives were to characterize the *in vitro* biofilm formation of *V. tapetis* and to control it with the marine bacterium *Pseudoalteromonas* sp. 3J6 which produces anti-biofilm proteinaceous exoproducts. Biofilm structure was examined by confocal laser scanning microscopy and scanning electron microscopy. *V. tapetis* was able to form biofilms on a glass substratum within 24 h. Original spherical components of about 1-2 μm diameter were found at the biofilm surface. They contain DNA, proteins, and seemed to be physically linked to bacteria and of cellular nature. Transmission electron microscopy showed that the spherical components were devoid of internal compartments. *V. tapetis* biofilm was sensitive to *Pseudoalteromonas* sp. 3J6 and its exoproducts. In *V. tapetis* CECT4600-GFP - *Pseudoalteromonas* sp. 3J6 co-cultures, the latter outcompeted *V. tapetis* whatever the growth mode (planktonic or biofilm). When the glass was coated with a culture supernatant of *Pseudoalteromonas* sp. 3J6 (SN3J6) prior to inoculating *V. tapetis* CECT4600-GFP, the bacterial attachment was about 5-fold lower than in control experiment without SN3J6. *V. tapetis* failed to form a biofilm fully covering the entire glass surface, but generated only separated macrocolonies. This study was the first to investigate *V. tapetis* biofilm formation. We will further explore the potential application of *Pseudoalteromonas* sp. 3J6 as probiotic or as a source of new anti-biofilm molecule.

P3: 85

Sodium trimetaphosphate and hexametaphosphate impregnated with silver nanoparticles: characteristics and antimicrobial efficacy

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The aim of this study was to synthesize and characterize nanocompounds of silver nanoparticles (Ag) associated with sodium polyphosphates (trimetaphosphate-TMP or hexametaphosphate-HMP) and fluoride (NaF). Ethanolic solution containing 10g of TMP or HMP, 500 ppm of NaF and silver nitrate at 1 or 10% were prepared in the presence of a surfactant (ammonium salt of polymethacrylic acid). The nanocompounds were characterized by SEM and EDX mapped in 2D, and they were tested against *Candida albicans* (ATCC 10231) and *Streptococcus mutans* (ATCC 25175). The broth microdilution method (MIC) was firstly carried out followed by the biofilm quantification through the counting colony-forming units (CFUs), metabolic activity (XTT method) and the total biomass (Crystal Violet method). The results confirmed the formation of Ag nanoparticles associated with HMP or TMP. MIC values for Ag-TMP and Ag-HMP ranged from 100-800 µg/ml for *C. albicans* and 400-800 µg/ml for *S. mutans*, except for 10% Ag-TMP for both microorganisms. For all biofilm quantification methods conducted, *S. mutans* was more susceptible than *C. albicans*, getting a log of CFU reduction of approximately 4.5 at 400 µg/ml for both Ag-polyphosphates. Moreover, for *C. albicans* the drug concentration did not interfere on its effectiveness, especially for Ag-TMP. The Ag-polyphosphates demonstrated a significant antimicrobial activity against *S. mutans* and might be considered as anti-caries dental materials.

P3: 86

Soluble ficin disrupt bacterial biofilm

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Many opportunistic bacteria like *Staphylococcus*, *Micrococcus*, *Pseudomonas* form biofilms on chronic and acute dermal wounds retarding their healing, causing reinfection and sepsis. Several proteases like trypsin, chymotrypsin were reported to exhibit anti-biofilm properties degrading the backbone of the biofilm matrix and thereby speeding up the wound healing. Our results indicate that ficin, protease from the plant, efficiently degrades the structural components of biofilm matrix formed by *S. aureus*, *S. epidermidis* and *P. aeruginosa* although with less efficiency. The anti-biofilm effect of ficin was significantly more pronounced compared to trypsin, a protease that is widely used in wound treatment. Significance of the biofilm disruption activity has been also supported by fluorescent microphotographs. Moreover, presence of ficin also led to the increase of the antimicrobial efficiency of ciprofloxacin against biofilm-embedded cells of *S. aureus* and *P.aeruginosa*. While 24h antibiotic treatment did not lead to the increase of red-fluorescent dead cells of neither *S.aureus* nor *P. aeruginosa* embedded into the biofilm matrix, in the presence of ficin the fraction of viable cells decreased significantly. Ficin does not exhibit the cytotoxicity and does not affect the growth of adipose derived stem cells. Similarly, no genotoxic effects were observed in Ames test and SOS-chromotest. Accordingly, soluble ficin appears safe and beneficial for outer wound treatment to prevent the biofilm formation and reduce the reinfection risk.

P3: 87

Surface-adaptive, antimicrobially-loaded, micellar nanocarriers with enhanced penetration, bacterial targeting and killing efficiency in biofilms

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Biofilms cause persistent bacterial infections and are extremely recalcitrant to antimicrobials. Here we describe the preparation of surface-adaptive, Triclosan-loaded micellar nanocarriers showing enhanced biofilm penetration, combined with electrostatic targeting towards negatively-charged bacterial cell surfaces and Triclosan release due to degradation of the micelle core by bacterial lipases. First, it was established that mixed shell polymeric micelles (MSPM) consisting of a hydrophilic poly(ethylene glycol) (PEG) shell and pH responsive poly(β -amino ester) become positively-charged at pH 5.0, while being negatively charged at physiological pH. This is opposite to the pH dependent charge of single shell polymeric micelles (SSPM) possessing only a PEG shell that remains negatively charged at pH 5.0. The stealth properties of the PEG shell allows both SSPMs and MSPMs to penetrate biofilms, as demonstrated for fluorescent Nile red-loaded micelles using Confocal Laser Scanning Microscopy. Opposite to SSPMs however, only the positive charge adapted by MSPMs at pH 5.0 allows them to bind to staphylococcal cell surfaces that are negatively charged at both pH 5.0 and 7.4 and accumulate in a biofilm. Once bound, bacterial enzymes degrade the MSPM core to release its antimicrobial content and kill bacteria over the depth of a biofilm. This constitutes an very effective pathway to control infectious biofilms using antimicrobials.

P3: 88

The effect of silver and gold nanoparticles on *Pseudomonas aeruginosa* biofilm

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The nano-dimensions of the metals provide them entirely new properties, including high activity towards microorganisms. The antimicrobial properties of silver have led to broad interest in its nanostructures, but also gold, considered as more or less nonreactive metal, exhibits biological activity in nanoparticle form. In this study we focused on the direct effect of silver and gold nanoparticles (AgNP and AuNP) with average dimension 20 nm on planktonic and biofilm growth of opportunistic bacterium *Pseudomonas aeruginosa* NRRL B-59188. The minimum inhibitory concentration (MIC) of growth of *P. aeruginosa* planktonic populations determined in complex medium had a value of 16 mg / l for AgNP and 35 mg / l for AuNP. The negative effect of used nanoparticles on biofilm of *P. aeruginosa* was manifested already at above stated values of MIC and further toxic effects were proportional to the increasing concentration of nanoparticles. For example, application of 50 mg / l of AgNP caused, besides decrease of biomass in biofilm, 50% reduction of metabolic activity of biofilm cells (spectrophotometric method based on color reaction of cellular dehydrogenases with XTT). The presence of nanoparticles also caused oxidative stress, expressed as a significant increasing lipid peroxidation. Double of MIC was responsible for the almost complete inhibition of the production of N-acyl homoserine lactones, *P. aeruginosa* signaling molecules which are directly associated with biofilm formation. It was demonstrated that the resistance of *P. aeruginosa* to both studied types of nanoparticles does not substantially increased in biofilm phenotype, as it is common in antimicrobials.

P3: 89

The role of inter-kingdom interactions in chlorine resistance of biofilms formed by drinking water-isolated microorganisms

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Biofilms in drinking water distribution systems (DWDS) are responsible for several undesirable effects in water. One of the main drawbacks is their potential to protect pathogens from stress conditions. Microbial interactions in biofilms can benefit the survival of co-existing microorganisms, including the increased resistance to antimicrobials. Chlorination is the main widespread strategy used in DWDS for microbial control. Even if new and alternative strategies are being developed, it is conceivable that the future strategies still persist with chlorine due to economic and safety aspects. Therefore, the understanding on the efficacy of chlorine against biofilms is of utmost importance in order to improve the current strategies. The purpose of this work was to assess the effects of sodium hypochlorite (SHC) on the control of single and dual-species biofilm formation by selected filamentous fungi (*Penicillium expansum* and *Penicillium brevicompactum*) and bacterium (*Acinetobacter calcoaceticus*) isolated from DWDS. Biofilms were developed during 48 h in 96-wells microtiter plates under two hydrodynamic conditions (25 and 150 rpm). The effects of SHC at several concentrations (0.1, 0.5, 1, 10 and 100 mg/L) was tested. The results shown that, *P. brevicompactum* biofilms were extremely resistant to disinfection when compared with single-species biofilms of *P. expansum* and dual-species biofilms of *P. brevicompactum*-*A. calcoaceticus*. The association of *A. calcoaceticus* with both fungi seems beneficial, since the dual-species biofilms were more resistant to disinfection. The inactivation and removal occurred for high SHC concentrations. However, total biofilm control was not achieved.

P3: 90

The role of *sawR* in regulating virulence and biofilm formation in *Pseudomonas aeruginosa*

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Recent studies have shown that PA3133 (*sawR*), a probable transcription factor of *Pseudomonas aeruginosa*, is over-expressed in response to Surface Acoustic Waves (SAW). SAW is known to disrupt *P. aeruginosa* biofilm formation and antibiotic resistance. We attempt to uncover the regulatory role of *sawR* in the bioacoustic response of *P. aeruginosa*. A flow cell system was utilized to demonstrate that the biofilm of the *sawR* overexpressing strain is highly sensitive to antibiotic treatment and forms less biomass when compared to the wild type (WT) strain, mirroring the effects of SAW exposure on the WT. To examine the genetic effects of *sawR*, gene expression levels of the *sawR* overexpressing strain were compared to that of the WT using a microarray. *sawR* showed a significant impact on gene expression pattern, where several virulence-associated genes were down-regulated in the *sawR* overexpressing strain, while specific metabolic genes were up-regulated. *hmgA* is a metabolic gene that is down-regulated in the *sawR* overexpressing strain. It is known that in the absence of *hmgA*, strains hyper-produce a brown pigment called pyomelanin, which is also produced when *sawR* is overexpressed. The decreased expression of multiple virulence factors in the *sawR* overexpressing strain led us to examine whether *sawR*, when overexpressed, can reduce virulence. We used a HeLa cytotoxicity assay in which cytotoxic ability of the *sawR* overexpressing strain was compared to the WT and found that it decreased by approximately 50%. Our data suggests that *sawR* plays a central role in mediating the response to SAW and key phenotypes such as biofilm formation, antibiotic resistance and pigment production.

P3: 91

Use of bacteriophages to prevent and control *Salmonella* Enteritidis biofilm formation on poultry skins at refrigerated and room temperatures

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Salmonella is one of the leading worldwide foodborne pathogens responsible for illnesses and hospitalizations. *Salmonella*'s capacity to form biofilms contributes to its resistance and persistence in both host and non-host environments, and is especially important in food processing settings. Because cross-contamination still happens during food processing and preparation, other down-stream safety measures must be applied, like the use of control agents of foodborne pathogens in food products. Phages are the natural killers of bacteria, innocuous to human and animals, and good candidates to be used in the control of bacterial pathogens. In this work we aimed to characterize a *S. Enteritidis* phage, phi38, which was shown to have 4.3 kbp in size, dsDNA genome and to contain 60ORFs. We also evaluated whether the addition of phi38 on poultry skin samples could decrease the levels of *S. Enteritidis*. For this, two approaches were used: a preventive approach focusing on decreasing *Salmonella* colonization ability of phage-pretreated skins; and a control one, aiming to kill *Salmonella* biofilms already present in the poultry skins. The effect of these two approaches was investigated at refrigerated temperatures (-18 and 4°C) and also during 1 h at RT (22°C). While poor effectiveness was observed using phi38 to control and reduce *Salmonella* biofilms following *in vitro* contamination of skins (< 1 log reduction of CFU) at all tested conditions, the preventive approach showed promising results (> 2 log reduction of *Salmonella* colonization). In this way, this study endorses that phages can be used to prevent foodborne pathogen colonization and consequently to promote food safety.

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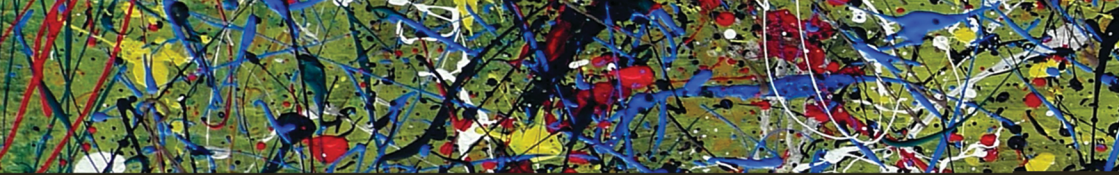
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Cristian Picioreanu (Netherlands)

Hans-Curt Flemming (Germany)

Joanna Yerran (UK)

Luís F. Melo (Portugal)

Marvin Whiteley (USA)

Thomas Neu (Germany)

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