Belgian Interdisciplinary Biofilm Research

Second Meeting

December 12th 2014

Croix du Sud, Carnoy building,
Salle des Séminaires ISV
UCL Louvain-La-Neuve

Funding

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Sponsoring

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BIBR (Belgian Interdisciplinary Biofilm Research) is a contact group supported by the F.R.S.-FNRS that aims to create a meeting point for Belgian researchers active or willing to start projects on biofilms. We intend to include both clinical and environmental topics, our particular interest lies in the interdisciplinary approaches to study the biofilms.

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Florence Vande Capelle (UCL)

Schedule of the second BIBR meeting

9h00 - 10h00 : Welcome

10h00 -11h00 : Prof. J.-M. Ghigo **(**Unité de Génétique des Biofilms, Paris, France**)**. How original is the bacterial biofilm lifestyle? The quest for biofilm-specific functions

11h00 - 11h20 : Coffee break

11h20 - 11h5**0** **:** Prof. Tom Coenye (Laboratory of Pharmaceutical Microbiology (LPM), UGent) *Staphylococcus aureus* biofilms: interactions with *Staphylococcus epidermidis* and novel approaches to treatment

11h50 - 12h2**0** : Dr B. Horemans (Division of Soil and Water Management, KU Leuven). Cross-species metabolism of natural dissolved organic carbon enhances the synergistic removal of xenobiotics

12h20-13h00 : Lunch break

13h00-14h00 : Poster session

14h15-14h45 : Dr P. Lassaux and Dr C. van de Weerdt (GIGA, ULg and Symbiose Biomaterials) Active antibiofilm surfaces: Alliance between applied research and industrial development

14h45-15h30 : Oral presentations – Session I (3 x 15 min.)

Ruby May Sullan, UCL. Binding Forces of *Streptococcus mutans* P1 Adhesin

Agata Goza, Lab. Protein Biochemistry & Biomolecular Engineering L-ProBE, UGent Biofilm-associated T2SS-dependent secretome of antibiotic resistant *Burkholderia cenocepacia*

Florence Vande Capelle ELI-UCL
Enzymatic control of mature *Staphylococcus epidermidis* biofilms

15h30-15h50 : Coffee break

15**h**50-16h20 **:** Oral presentations – Session II (2 x 15 min.)

Sandra Condori, University of Mons.
Acyl homoserine lactone-based quorum sensing and biofilm formation in the photosynthetic bacterium *Rhodospirillum rubrum* S1H

Soňa Kucharikova
Clinical Implications of oral candidiasis: Host tissue damage and disseminated bacterial disease

16h20-16h40 : General discussion

Abstracts of oral presentations

Professor Jean-Marc GHIGO, Genetics of Biofilms Unit, Department of Microbiology

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How original is the bacterial biofilm lifestyle? The quest for biofilm-specific functions

Abstract: In all environments, bacteria commonly attach to surfaces and form biofilms, dense communities often containing multiple species. Bacterial biofilms constitute a major source of infection in medical and industrial settings, as biofilm bacteria survive hostile conditions that normally eradicate planktonic cells. In light of the profound phenotypic modifications during the switch from planktonic to a biofilm lifestyle, it was early hypothesized that bacterial biofilms could constitute reservoirs of yet unknown biofilm-associated biological functions of both fundamental and clinical relevance. I will review the relentless quest for such key biofilm-specific determinants and its contribution to the understanding of the biological resources used by bacteria to operate within biofilm communities. I will also discuss how functions or molecules specifically produced within biofilms may lead to new strategies to diagnose, limit or eradicate biofilm-associated infections.

Professor Tom Coenye (Laboratory of Pharmaceutical Microbiology (LPM), UGent)

Staphylococcus aureus biofilms: interactions with _Staphylococcus epidermidis_ and novel approaches to treatment

Staphylococcus aureus is an important (nosocomial) pathogen and many *S. aureus* infections are biofilm-related. In addition, many *S. aureus* isolates show a reduced susceptibility to frequently-used antibiotics. *Staphylococcus epidermidis* is a commensal skin bacterium that is also an opportunistic pathogen that can colonize indwelling medical devices, including endotracheal tubes.

As *S. aureus* and *S. epidermidis* are often co-isolated from biofilms, we wanted to investigate the mutual influence of these organisms on each other. To this end we used RNASeq (Illumina sequencing) to measure differences in gene expression between single and dual-species biofilms. *S. aureus* genes encoding virulence-associated proteins and *S. epidermidis* genes involved in antibiotic resistance were upregulated in dual species biofilms. In contrast, genes involved in ureum metabolism were downregulated (in both species) in dual species biofilms. In addition, we noticed that overall, genes encoding enzymes involved in central metabolism were downregulated. For *S. aureus* we were able to confirm that there is a link between between decreased metabolic activity, decreased urease activity, and an increased pH in dual species biofilms compared to single species biofilms.

As biofilm-related *S. aureus* infections are difficult to treat with antibiotics alone, alternative treatments are urgently needed. We have been exploring the use of the *S. aureus* quorum sensing inhibitor hamamelitannin (HAM). HAM is a non-peptide analog of RIP and increases biofilm susceptibility to glycopeptide antibiotics (as well as to other antibiotics) and leads to increased *in vivo* survival during bacterial infection in invertebrate animal model systems. I will present details on the effect of the use of HAM and will also present recent data obtained about the mode of action of this QS inhibitor and how it increases susceptibility.

Dr B. Horemans (Division of Soil and Water Management, KU Leuven)

Cross-species metabolism of natural dissolved organic carbon enhances the synergistic removal of xenobiotics

Benjamin Horemans, J Vandermaesen, P Breugelmans, E Smolder, D Springael
KU Leuven, Belgium

The main source of carbon, energy and nutrients for heterotrophic microbial biofilm communities is the naturally available dissolved organic matter (DOM). Its quality and quantity influences enzymatic activity, growth and species composition. Research on DOM affecting specific functionality such as pesticide degradation is scarce. As a model community, a triple-species microbial biofilm which metabolically cooperates in degrading the herbicide linuron was studied in continuously fed flow chamber systems. The linuron degrading consortium consists of *Variovorax* sp. WDL1 transforming linuron to 3,4-dichloroaniline (3,4-DCA) and N,O-dimethylhydroxylamine (N,O-DMHA). WDL1 uses 3,4-DCA as C-source but inefficiently and its excretion supports growth of *Comamonas testosteroni* WDL7. *Hyphomicrobium sulfonivorans* WDL6 grows on N,O-DMHA. The consortium biofilm was first grown on DOM formulations of natural origin with varying biodegradability. Synergism was observed in the multi-species biofilm for a wide variety of natural organic compounds and DOM formulations of varying biodegradability, hereby extending its metabolic reach beyond each strain's individual capabilities. Apart from niche complementarity, the consortium oxidized more carbon sources due to a synergistic cooperation between WDL1 and WDL7 making more carbon sources accessible. In addition, biofilms grown on DOM formulations, visualized with CLSM by strain specific fluorescent labelling, revealed close spatial proximity of WDL1 and WDL7. This juxtaposition of the strains was essential for the immediate removal of linuron when applied to the different DOM fed biofilms in a later stage. Natural C-sources assure a continuation of metabolic cooperation in pollutant degrading microbial communities, a necessary feature for the complete and efficient removal of pollutants.

Dr P. Lassaux and **Dr C. van de Weerdt** (GIGA, ULg and Symbiose Biomaterials, s.a)

Active antibiofilm surfaces: Alliance between applied research and industrial development

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Biofilms are highly structured communities of bacteria that can form on living surfaces such as human tissue and non-living surfaces, such as medical devices or industrial settings. Those biofilms are responsible for at least 65% of all human infections and place a huge burden on our society. Once biofilms are formed, it is almost impossible to eradicate them, thus it is important to develop new strategies to prevent bacterial biofilm formation. The strategy developed by our laboratory to respond to this threat is the molecular biomimetic approach, which consists in drawing its inspiration from Nature's biological structures to design practical materials and systems with remarkable properties. Over the past 8 years our laboratory has been applying this strategy in several applied collaborative projects to confer antibiotic and antibiofilm properties to various materials.

Since microorganisms can undergo cycles from planktonic to biofilm form and vice-versa, they possess the "tools" needed to degrade a biofilm matrix. And indeed in 2004, Kaplan described the gene and the protein involved in the degradation of matrix polysaccharide biofilm in *Actinobacillus actinomycetemcomitans*. This soluble glycoside hydrolase, DispersinB, has then been cloned and expressed in our lab to be coated on different surfaces using different approaches. The antibiofilm properties of those surfaces were then evaluated in static (test derived from ISO 22196 norm) and in dynamic (Biostream collaboration with Thomas Vanzieleghem). Our surfaces proved to be active. Symbiose Biomaterials, a young start-up, is currently developing industrial applications from the results obtained by the laboratory of molecular biomimetics. In particular, it exploits the potentiality of DispersinB for imparting antibiofilm activity to industrial surfaces, using technologies like DOPA-based nanogels for enzyme immobilization.

Ruby May Sullan, UCL

Binding Forces of *Streptococcus mutans* P1 Adhesin.

Central to the initiation and maturation of biofilms are the bacterial cell surface adhesion molecules (adhesins) that mediate initial attachment between the cell and a wide range of surfaces. *Streptococcus mutans*, a primary etiological agent associated with human dental caries, adheres to immobilized salivary agglutinin (SAG) contained within the salivary pellicle on the tooth surface through P1, a cell surface multifunctional adhesin that is also capable of interacting with extracellular matrix proteins. This may be of particular importance outside of the oral cavity as *S. mutans* has been associated with infective endocarditis and detected in atherosclerotic plaque. Despite the biomedical importance of P1, its binding mechanisms are not completely understood. In this work, we use atomic force microscopy-based single-molecule and single-cell force spectroscopy to quantify the nanoscale forces driving P1-mediated adhesion. Single-molecule experiments show that full-length P1, as well as fragments containing only the P1 globular head or C-terminal region, bind to SAG with relatively weak forces (~50 pN). In contrast, single-cell analyses reveal that adhesion of a single *S. mutans* cell to SAG is mediated by strong (~500 pN) and long-range (up to 6000 nm) forces. This is likely due to the binding of multiple (~10) P1 adhesins to self-associated gp340 glycoproteins. Such cooperative, long-range character of the *S. mutans*-SAG interaction would therefore dramatically increase the strength and duration of cell adhesion. We also demonstrate, at single-molecule and single-cell levels, the interaction of P1 with fibronectin and collagen, as well as with hydrophobic, but not hydrophilic, substrates. The binding mechanism (strong forces, cooperativity and broad specificity) of P1 provides a molecular basis for its well-recognized adhesive properties. Our methodology represents a valuable approach to probe the multifunctional behaviour of bacterial adhesins and offers a tractable methodology to assess anti-adhesion therapy.

BIOFILM-ASSOCIATED T2SS-DEPENDENT SECRETOME OF ANTIBIOTIC RESISTANT *BURKHOLDERIA CENOCEPACIA*

Agata Goza [1], Maarten Dhaenens [2], Gilles Brackman [3], Tom Coenye [3], Miguel Valvano [4] , Dieter Deforce[2] and Bart Devreese [1]

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The Gram-negative bacterium *Burkholderia cenocepacia* is an opportunistic pathogen of immunocompromised individuals and patients suffering from Cystic Fibrosis (CF). *B. cenocepacia* is among the most frequently isolated species in sputum of patients with CF and exhibits a high intrinsic multidrug resistance. Several virulence strategies are recognized that mediate the adaptation of *B. cenocepacia* to the host environment. One of them is the secretion of protein virulence factors by specialized protein secretion systems, including the type 2 secretion system (T2SS). Interestingly, the impact of T2SS in the context of biofilm formation in *B. cenocepacia* K56-2 has not yet been investigated.

The main objective of this study was to characterize the secreted protein repertoire of T2SS of *B. cenocepacia* K56-2 under the biofilm-associated mode of growth. The extracellular proteins were collected from the culture supernatant of WT and ΔT2SS strain, grown under biofilm conditions employing a CDC bioreactor model with a synthetic CF sputum medium. Proteins were analyzed by data-independent acquisition (DIA) (MSE) with travelling wave ion mobility spectrometry (TWIMS). Further, we used the confocal laser scanning microscopy to characterize the basic structure of *B. cenocepacia* biofilms of the WT and various secretion systems knockout strains.

Quantitative and qualitative differences of secreted proteins from various strains revealed their dependency from T2SS in a biofilm conditions. Apart of known T2SS-dependent virulence factors like ZmpA we identified a range of hydrolytic enzymes and lipase. For instance, we discovered an exported chitinase that might interact with host cell glycans contributing to a pathogenic potential of bacteria. Moreover, some proteins might exhibit moonlighting properties.

Our results indicate that the label-free proteomic approach serves as a powerful tool to analyze the biofilms-secreted proteins. To our knowledge this is the most comprehensive proteomic study of *Burkholderia cenocepacia* grown under biofilm-associated conditions.

Enzymatic control of mature *Staphylococcus epidermidis* biofilms

Thomas Vanzieleghem[a], **Florence Vande Capelle**[a], Mathias Schmelcher[b], Martin Loessner[b] and Jacques Mahillon[a]

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[b]: Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland

Commensal bacteria of human skin and mucosa, *Staphylococcus epidermidis* is now regarded as an opportunistic pathogen. The virulence of this species, encountered in about 30% of cases of chronic nosocomial infections associated to indwelling medical devices, is primarily due to its biofilm-forming ability. Biofilms display very little sensitivity to antibiotics and continuously release planktonic cells, therefore causing acute infections. In case of antibiotherapy failure, the medical staff has no choice but to surgically remove infected implants although it means higher risks for patients. The development of novel strategies to combat biofilm infections is thus needed. In that sense, enzymatic control of biofilms offers both qualitative and quantitative advantages.

This study aims to apprehend the synergistic effects of different classes of enzymes on mature *S. epidermidis* biofilms. Enzymes used in this work are a dispersin B analogue β-hexaminidase, lysostaphin, and two phage lysins (peptidoglycan hydrolases) from phages Twort and K.

S. epidermidis biofilms are grown in 96-well plates, incubated in presence of different enzymatic cocktails for 2 hours and stained according to a protocol described by Wang *et al*. in 2014. Absorbance measurement at 540nm indicates the quantity of biofilm left in the wells. Statistical experiment design has allowed us to quantify interactions between the four enzymes, but also the impact of each one on the biofilm degradation while reducing the number of assays.

After the results analysis with JMP©, an ideal enzymatic cocktail could be defined. The dispersin B analogue and lysostaphin appeared indispensable to clear the wells from any biofilm residues whereas the two phage lysins did not significantly participate to biofilm eradication. Statistical analysis also highlighted the rinsing steps in the experimental procedure as critical factors affecting reproducibility. Perspectives of enzymatic control of biofilms are numerous, ranging from prevention to curative treatment, notably as potentiating agents for antibiotics.

Sandra Condori, University of Mons

Acyl homoserine lactone-based quorum sensing and biofilm formation in the photosynthetic bacterium *Rhodospirillum rubrum* S1H.

MELiSSA (Micro-Ecological Life Support System Alternative) has been conceived as a 5 compartments microorganisms and higher plants recycling system for long haul space flights. *Rhodospirillum rubrum* S1H colonizes compartment II and grows under light anaerobic conditions (LAN) using acetate as carbon source (MELiSSA conditions). Previous work reported that continuous culture of the bacterium in a photobioreactor lead to thick biofilm formation, leading to bioreactor arrest. The aim of this research is to investigate the relation of the unknown quorum sensing (QS) system of *R. rubrum* S1H (wild type, WT) and biofilm formation under MELiSSA relevant culture conditions. In this context we have constructed a mutant strain named M68 that does not produce acyl homoserine lactones (AHLs) signaling molecules. We have compared the transcriptomic and proteomic profile of WT and M68 under MELiSSA conditions. In addition, we have studied biofilm formation development of WT and M68 in a flow cell system under light microaerobic conditions. Transcriptomic results showed that 8% (326 genes) of the genome of M68 was statistically significant differentially expressed. In addition, transcriptomic and proteomic results have shown that *R. rubrum* QS system regulates diverse biological processes i.e. photosynthesis, energy generation and carbon metabolism. Regarding biofilm formation preliminary results under light microaerobic conditions have shown that WT formed an aggregation-like biofilm structure. Mutant M68 did not form biofilm however some cells remained attached to the flow cell surface. In summary, *R. rubrum* has a cell-to-cell communication system based on AHLs and it regulates diverse biological processes under MELiSSA conditions, including biofilm formation. To our knowledge this is the first report where QS is linked to biofilm formation in *R. rubrum* S1H under light microaerobic conditions. Further flow cell experiments under MELiSSA conditions will help us to study biofilm formation and to test substances with known anti-biofouling properties.

Clinical Implications of oral candidiasis: Host tissue damage and disseminated bacterial disease

Soňa Kucharikova Laboratory of MoL Cell Biology, KU Leuven, Eric Kong, Patrick Van Dijck and Mary Ann Jabra-Rizk

The clinical significance of polymicrobial interactions, particularly those between commensal species with high pathogenic potential, remains largely understudied. Although the dimorphic fungal species *Candida albicans* and the bacterium *Staphylococcus aureus* are common co-colonizers of humans, they are considered leading opportunistic pathogens. Oral candidiasis specifically, characterized by hyphal invasion of oral mucosal tissue, is the most common opportunistic infection in HIV+ and immunocompromised individuals. In this study, building on our previous findings, a mouse model was developed to investigate whether the onset of oral candidiasis predisposes the host to secondary staphylococcal infection. Findings demonstrated that in mice with oral candidiasis, subsequent exposure to *S. aureus* resulted in systemic bacterial infection with high morbidity and mortality. Histopathology and scanning electron microscopy of tongue tissue from moribund animals revealed massive *C. albicans* hyphal invasion coupled with *S. aureus* deep tissue invasion. The crucial role of hyphae in the process was demonstrated using a non-hyphae producing and a non-invasive hyphae producing mutant strains of *C. albicans*. Further, in contrast to previous findings, *S. aureus* dissemination was aided but not contingent upon the presence of the Als3p hyphal-specific adhesion. Importantly, impeding development of mucosal *C. albicans* infection by administering antifungal fluconazole therapy protected the animals from systemic bacterial disease. The combined findings from this study demonstrate that oral candidiasis may constitute a risk factor for disseminated bacterial disease warranting awareness in terms of therapeutic management of immunocompromised individuals.

List of posters

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2	**Quantifying the forces guiding microbial cell adhesion using single-cell force spectroscopy.** Audrey BEAUSSART, UCL
3	**Caspofungin (CAS), Colistin (CST) and Polymyxin B (PMB) Enhance Moxifloxacin (MXF) Activity against *Staphylococcus aureus* Biofilms by Disturbing Matrix exopolysaccharides.** Wafi SIALA, UCL
4	**Preservation of quality and stability of a fusion protein produced in fungal biofilm reactor with metal structured packing.** Quentin ZUNE - ULg-Gembloux ABT
5	**Microbial diversity in aerobic granular sludge.** Olivier Henriet, Christophe Meunier, Paul Henry, Jacques Mahillon
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8	**Antibiotic activity against different *Pseudomonas aeruginosa* (PA) phenotypes in an Artificial Sputum Medium biofilm model.** E. Bassères, P. M. Tulkens, F. Van Bambeke

Abstracts of posters

Sofiane El-Kirat-Chatel, UCL-ISV

The binding force of the staphylococcal adhesin SdrG is remarkably strong.

SdrG is a cell surface adhesin from *Staphylococcus epidermidis* which binds to the blood plasma protein fibrinogen (Fg). Ligand binding follows a 'dock, lock and latch' model involving dynamic conformational changes of the adhesin that result in a greatly stabilized adhesin-ligand complex. To date, the force and dynamics of this multistep interaction are poorly understood. Here we use atomic force microscopy (AFM) to unravel the binding strength and cell surface localization of SdrG at molecular resolution. Single-cell force spectroscopy shows that SdrG mediates time-dependent attachment to Fg-coated surfaces. Single-molecule force spectroscopy with Fg-coated AFM tips demonstrates that the adhesin forms nanoscale domains on the cell surface, which we believe contribute to strengthen cell adhesion. Notably, we find that the rupture force of single SdrG-Fg bonds is very large, ~ 2 nN, equivalent to the strength of a covalent bond, and shows a low dissociation rate, suggesting that the bond is very stable. The strong binding force, slow dissociation and clustering of SdrG provide a molecular foundation for the ability of *S. epidermidis* to colonize implanted biomaterials and to withstand physiological shear forces.

Audrey BEAUSSART, UCL

Quantifying the forces guiding microbial cell adhesion using single-cell force spectroscopy

Single-cell force spectroscopy (SCFS) is a powerful atomic force microscopy (AFM) modality in which a single living cell is attached to the AFM cantilever to quantify the forces that drive cell-cell and cell-substrate interactions. Although various SCFS protocols are well established for animal cells, application of the method to individual bacterial cells remains challenging. Here, we present a nondestructive protocol for single-bacterial cell force spectroscopy that we have developed, which combines the use of colloidal probe cantilevers and of a bioinspired polydopamine wet adhesive. A single living cell is picked up with a polydopamine-coated colloidal probe, enabling us to quantify the adhesion forces between the cellular probe and solid substrates or another live cell. This novel technique represents a generic platform for studying the molecular mechanisms of cell adhesion, which has already allowed us to decipher the molecular details governing the adhesion of several probiotics and pathogens. SCFS was used to elucidate the specific and nonspecific forces driving the adhesion of the probiotic *Lactobacillus plantarum* to biotic and abiotic surfaces. By applying SCFS measurements to genetically modified microorganisms, the molecular bases of LapA adhesins of the soil colonizer *Pseudomonas* fluorescence have been explored. Additionally, the peculiar mechanoproperties of bacterial appendages in the pathogen *Pseudomonas aeruginosa* and the probiotic *Lactobacillus rhamnosus* GG have been revealed. Bacterial pili showed nanospring behaviors that are believed to enable the bacteria to withstand high mechanical forces in their natural environment. Finally, the molecular interactions governing the association of the two nosocomial pathogens *Staphylococcus epidermidis* and *Candida albicans* in mixed biofilms were quantified.

We anticipate that the approach will be useful to researchers interested in understanding the molecular bases of cell adhesion in a broad range of microorganisms. Advanced SCFS technologies could also contribute to the development and screening of anti-adhesion molecules that are capable of inhibiting the adhesion of pathogens or controlling the adhesion of beneficial bacteria.

Wafi SIALA, UCL

Caspofungin (CAS), Colistin (CST) and Polymyxin B (PMB) Enhance Moxifloxacin (MXF) Activity against *Staphylococcus aureus* Biofilms by Disturbing Matrix exopolysaccharides.

Objective: Biofilms are poorly responsive to anti-staphylococcal antibiotics. Our aim was to examine whether combining MXF with amphiphilic drugs without antistaphylococcal activity such as CST, PMB or CAS, could improve its activity on biofilms from reference strains and clinical strains from epidemic clones (acute infections).

Methods: Biofilms were grown for 24 h in 96-well plates in Trypticase Soy Broth (TSB) supplemented with 2% NaCl and 1% glucose. 24h-old (mature) biofilms were exposed for 48 h to a broad range of MXF conc. (to obtain full conc.-response curves), combined with CAS, CST, or PMB at 2 X MIC. Viability in biofilms was quantified using the redox indicator resazurin (1). MXF penetration in biofilms was assessed by confocal microscopy (λexc 400 nm / λem 520 nm). Exopolysaccharides (EPS) were extracted and assayed as described by Kolodkin-Gal *et al* (2) and Albalasmeh *et al.* (3) EPS macromolecular size was measured by dynamic light scattering.

Results: CAS, CST, and PMB had MICs of 40, 50, and 50 mg/L, respectively and did not show any activity by themselves against biofilms at 2x MIC. MXF alone was poorly active (50 % reduction in viability reached only for ATCC33591 and 1 clinical isolate at conc. ≤ 20mg/L). Combination with CAS, CST or PMB allowed reaching 50 % reduction for 6/7, 3/7 and 4/7 clinical strains respectively. CAS, CST or PMB increased up to 30-fold MXF penetration, reduced approx. 3-fold EPS content, and decreased at least 300-fold EPS size for biofilms formed by strain 2005/179 (high responder) but not by 2003/651 (poor responder).

Conclusion: Amphiphilic molecules like CAS, PMB or CST reduce EPS content and size in biofilms, which may contribute to enhance MXF penetration and activity. Combination with amphiphiles appears thus as a promising strategy to act upon *S. aureus* biofilms, warranting the search of more potent molecules.

Quentin ZUNE - ULg-Gembloux ABT

Preservation of quality and stability of a fusion protein produced in fungal biofilm reactor with metal structured packing

Potentialities of filamentous fungal biofilm have been highlighted in bioprocesses involving production of recombinant proteins or secondary metabolites. Fungal biofilm fermentation requires a support allowing surface adhesion for biofilm growth and synthesis of the biomolecule of interest. However, supports tested in previous studies present low specific area and have some difficulties to be relevant at larger-scale. The lab-scale design of a fungal biofilm reactor should consider the ability to be scaling-up. In this study, a metal structured packing providing high specific area and stacking easiness, replaces the agitation axis of a stirred tank bioreactor in order to promote biofilm growth of a recombinant strain of *Aspergillus oryzae*. This latter contains a fusion gene *gla*::*gfp* under the control of a promoter specifically induced in solid-state fermentation *glaB*. In this work, we describe and compare the production of the fusion protein in a biofilm reactor and a stirred tank reactor. On the contrary of our expectations, the highest fusion protein productivity occurs in a high stirred rate culture whereas it reaches a satisfying level in biofilm conditions. In this sense, *glaB* promoter activation seems to be more dependent of shear stress than biofilm conditions because a low stirred rate culture does not induce fusion protein synthesis. However, high shear stress involves a biomass leakage as well as a loss of quality of the fusion protein. Moreover, 2D-gel electrophoresis highlights preservation of the fusion protein integrity produced in biofilm conditions whereas a great proteolysis affects fusion protein recovery in the high stirred rate culture. The "soft" operating conditions set for the fungal biofilm growth in our experimental device allow continuous implementation of the process and ensure a good stability and quality of the recombinant protein. Further experiments will investigate the control of biofilm thickness and intensification of the bioprocess with stacking of several packings.

Microbial diversity in aerobic granular sludge

Olivier Henriet, Christophe Meunier, Paul Henry, Jacques Mahillon

The discharge of untreated wastewaters in the environment is responsible of the rapid degradation of aquatic ecosystems. The current leading technology is the activated sludge process, where the dissolved pollution is degraded by free-floating consortia (flocs). The major drawback of these loose aggregates is their low settling velocity. An emerging technology aims to improve this process by selecting consortia that form aerobic granules. As dense aggregates, they settle significantly faster than flocs. However, the role of constituting microorganisms on both granule formation and pollution removal is still largely unknown.

This research aimed to assess the bacterial diversity of granular sludge produced from both urban and industrial wastewaters. Bacterial diversity was assessed by denaturing gradient gel electrophoresis and tag-encoded FLX-amplicon pyrosequencing.

Successful granulation was achieved with brewery, dairy and urban wastewaters. The bacterial diversity of the initial flocular sludge responded to change in the reactor operating conditions applied to generate granules. However, the bacterial composition of granules was different between reactors, which seem to confirm that there is not a single microbial community that leads to granules. It was also shown that bacterial diversity differed between granules from the same reactor. Fast settling granules contained a higher population of "*Candidatus accumulibacter*" than slow settling granules. Members of this genus accumulate intracellular polyphosphate and polyhydroxyalcanoate and it is hypothesized that these inclusions increase the density of the cell, resulting in an improved sedimentation.

Elien PEETERS, KU leuven elien.peeters@biw.kuleuven.be

Magnetic nanobiosystems for biofilm control in greenhouse irrigation and reverse osmosis systems

Biofilms are complex, highly variable, surface-associated communities of microorganisms, embedded in a self-produced matrix. A typical characteristic of biofilms is that they cause very persistent contaminations and infections, which causes major problems in many different application fields, including medicine, household and industry. This project focuses on the development of a novel delivery strategy for anti-biofilm molecules, based on covalent coupling with magnetic nanoparticles. Proof-of-concept will be provided for this novel technology in two specific applications: biofilm eradication in irrigation pipes from hydroponics and biofouling reduction on reverse osmosis membranes. In order to build realistic in vitro biofilm models for anti-biofilm testing, in-situ biofilms from irrigation pipes and RO membranes are currently being characterized in depth, both with regard to species and matrix composition. Remarkably, our analysis indicated the presence in the irrigation pipes of many species that can potentially harm the harvest. In vitro screening of a first set of nanoparticles against a simple monospecies biofilm resulted in the identification of a magnetic nanoparticle with specific, non-biocidal anti-biofilm activity. Interestingly, preliminary experiments indicate that the nanoparticle shows a synergistic activity with ampicillin and a novel small molecule anti-biofilm molecule. These compounds are currently being coupled to the nanoparticle and the resulting 'nanobiosystems' will eventually be tested in the more complex, realistic biofilm models.

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Unraveling interspecies interactions in a linuron degrading bacterial consortium.

Different bacterial species are often organized in multispecies bacterial consortia (MBC) which enables them to cooperatively perform complex tasks. Interspecies interactions have been suggested to be important for the well-functioning of MBCs but supporting information is poor. This poster shows how a differential transcriptomic approach can be used to identify genetic features determining interspecies interactions in an MBC degrading synergistically the phenylurea herbicide linuron. The consortium consists of three bacterial strains belonging to three different genera, i.e., *Variovorax*, *Comamonas testosteroni* and *Hyphomicrobium sulfonivorans*. Both metabolic and physical interactions have been described before. The transcriptomes of the member bacteria when grown together as a consortium are compared to the transcriptomes representing monospecies growth conditions. For both conditions, member bacteria were grown as a biofilm in continuous flow chambers. An RNAseq approach was successfully used to show differential expression of several genes that could be associated with interspecies interactions in the linuron degrading MBC.

Sputum Medium biofilm model.

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Background: PA is the main cause of respiratory failure in cystic fibrosis patients. Its persistence in the lung is associated with its capacity to grow in biofilms, against which antibiotics are considered to be poorly active. We set up an in vitro model allowing for pharmacodynamic comparison of antibiotic activity against PA biofilms growing in Mueller Hinton Broth (MHB) or in Artificial Sputum Medium (ASM; PMID:15947432) in order to better mimic the physicochemical conditions prevailing in the lung of CF patients.

Methods: Reference strains (PAO1 and stable mucoid ATCC39324) were grown in MHB or ASM in physical surface treated 96-well plates for up to 12 days with medium replacement every 48h and exposed thereafter to antibiotics at concentration ranging from 0.003 to 100 xMBC during 24h. Biofilm biomass and bacterial viability within biofilms were quantified using crystal violet and fluorescein diacetate (PMID: 15567221) assays. In parallel, MBC were determined on planktonic cultures and in both media by CFU enumeration.

Results: All MBCs were 4-8 dilutions higher in ASM than in MHB except for MEM against the mucoïd strain (see Table). Adding either mucin (CAZ, CST) or DNA (CIP, AMK) to MHB brought MBC to levels close to those observed in ASM. ASM favors the development of mature biofilms for both strains within 15 days (biomass plateau values from 3 to 8 for PAO1; 2 to 12 for 39324). In most cases, antibiotics were less active on biomass than on bacterial viability, less effective in ASM than in MHB (lower Emax) except AMK. CIP, AMK were the most effective and potent (C50) in both media (MER in MHB) but had a lower effect on 39324's biomass. CAZ and CST were less effective and potent, especially in ASM for CST. Antibiotics remained unable to eradicate all bacteria within the biofilms.

Conclusions: Our model confirm the observations of higher antibiotic resistance in cystic fibrosis biofilms and point out the need to use relevant medium to predict antibiotic activity regarding interactions with the medium and modulation of bacteria metabolism/phenotype.

Antibiotic activity against different *Pseudomonas aeruginosa* (PA) phenotypes in an Artificial

Antibiotic	Strain	MHB					ASM				
		MBC	viability		biomass		MBC	viability		biomass	
			E_{max}	C_{50}	E_{max}	C_{50}		E_{max}	C_{50}	E_{max}	C_{50}
Ciprofloxacin (CIP)	**PAO1**	0.06	-85.11	1.13	-71.95	0.15	4	-72.5	0.21	-61.15	0.18
	39324	0.06	-76.34	0.38	-55.73	0.79	16	-66.29	0.04	-29	>1600
Colistin (CST)	**PAO1**	1	-51.69	29.4	-60.75	60.06	16	-36.23	>1600	-54.85	22.88
	39324	1	-65.46	24.96	-44.04	>100	32	-35.15	>3200	-40	>3200
Ceftazidime (CAZ)	**PAO1**	1	-41.53	>100	-25.49	>100	256	-40.96	>25600	-31.23	>25600
	39324	1	-73.84	0.28	-54.73	0.56	128	-10.04	>12800	-13.4	>12800
Amikacin (AMK)	**PAO1**	1	-83.19	3.3	-65.5	9.24	64	-75.04	0.4	-70.15	0.29
	39324	1	-78.66	1.32	-61.57	7.42	16	-77.64	0.66	-34.44	>1600
Meropenem (MER)	**PAO1**	0.5	-86.36	0.51	-65.65	1.98	64	-60.74	0.19	-43.13	>6400
	39324	0.125	-59.35	0.1	-34.47	>12.5	0.5	-37.78	>50	-19.1	>50

MBC and C_{50} in mg/L
Emax in percentage of inhibition compared to control (untreated biofilms)
C_{50} and Emax were calculated with the Hill equation of the sigmoidal concentration-response curve from 2 or 3 independent experiments

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