Belgian Interdisciplinary Biofilm Research

First Meeting

December 20th 2013

Croix du Sud, auditorium 01, Carnoy building

UCL Louvain-La-Neuve

Funding

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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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Schedule of the first BIBR meeting

8h30-9h30: Welcome

9h30-10h30: Prof. Hans-Curt Flemming. The Biofilm Matrix: the Perfect Slime.

10h30-10h45: Coffee break

10h45-11h15: Dr Audrey Beaussart. Atomic Force Microscopy of microbial cell adhesion

11h15-11h45: Prof. P. Cornelis: *Pseudomonas aeruginosa* biofilms: from formation to dispersion

11h45-12h15: Prof. F. Van Bambeke. In vitro pharmacodynamics models for the study of antibiotic activity against bacterial biofilms

12h15-13h00: Lunch break

13h00-14h00: Poster session - Carnoy building (ground floor) rooms b006 and b091

14h00-14h15: Sandra Van Puyvelde. A time course transcriptome study of *Salmonella typhimurium* during the switch from planktonic to biofilm growth

14h15-14h30: Felice Mastroleo. Quorum sensing is needed for *Rhodospirillum rubrum* S1H cell dispersion

14h30-14h45: Pauline Modrie. On the fate of *Bacillus cereus* sensu lato in multi-species communities: biofilm formation and plasmid transfer

14h45-15h00: Rosemarie De Weirdt. The impact of low fluid shear near the gut mucosa on biofilm formation by health-promoting *Lactobacillus reuteri*

15h00-15h15: Katrien Forier. On the rational design of nanomedicines for the eradication of pulmonary biofilm infections

15h15-15h30: Coffee break

15h30-15h45: Nathalie Vandevelde . Activity of antibiotics in models of naïve and induced biofilms of *Streptococcus pneumoniae*

15h45-16h00: Quentin Zune. High-energy X-ray tomography analysis of a metal packing biofilm reactor for the production of lipopeptides by *Bacillus subtilis*

16h00-16h15: Catherine Philippart. Nano-Engineered Surfaces to Control the Development Of Bacterial Microcolonies

16h15-16h30: Soňa Kucharikova. Non-invasive bioluminescence imaging of mature *Candida albicans* biofilms developed in a subcutaneous mouse model

16h30-16h45: Nicolas Delattin. The plant-derived decapeptide OSIP108 inhibits *Candida albicans* biofilm formation without affecting cell viability

16h45-17h30: general discussion

Abstracts of oral presentations

Professor Hans-Curt Flemming. University of Duisburg-Essen, Germany.
Biofilm Centre

"The Perfect Slime - Contributions of the Matrix to the Emergent Properties of Biofilms"

In biofilms, microbial life is organized within a matrix of extracellular polymeric substances (EPS). This matrix keeps biofilms together and provides their mechanical stability. It allows for the formation of synergistic microconsortia, orchestrated degradation of recalcitrant organics, intense communication, expression of virulence factors, and extended tolerance to antibiotics and disinfectants. Extracellular enzymes are retained by the matrix and turn it into an external digestion system. Thus, the matrix enables the biofilm organisms to develop emerging properties, i.e., properties which are not predictable from those of planktonic microorganisms. Living in a biofilm is a quantum leap in terms of survival chances, spectrum of nutrients which can be utilized and complex organization of interactions. Thus, the matrix imparts biofilms with properties of a cooperative tissue, although it should not be neglected that, in spite of all cooperation, biofilms are also the stage of fierce competition. In summary, the matrix is a major reason for the evolutionary success of the biofilm mode of life.

Dr Audrey Beaussart. Professor Yves F. Dufrêne

Institute of Life Sciences, UCL, Louvain-la-Neuve

Atomic Force Microscopy of microbial cell adhesion.

Microscopy and microbiology have always been intimately connected. While classical light microscopy is useful for counting and identifying the cells as well as for determining their general morphological details, the resolution is limited to the wavelength of the light source and therefore information on the nanometer scale is not accessible. High-resolution images of microbial cells and biofilms are traditionally obtained using transmission and scanning electron microscopy. These approaches, however, are demanding and are limited by the requirement of vacuum conditions during the analysis, meaning that live cells cannot be investigated in aqueous solution. With its ability to observe and manipulate the microbial cell surface at nanometer resolution and in buffer solutions, atomic force microscopy (AFM) has recently opened up a wide range of novel opportunities in microbiology. Using topographic imaging, researchers can visualize the ultrastructure of live cells and their subtle modifications upon cell growth or treatment with drugs. Chemical force microscopy, in which AFM tips are modified with specific functional groups, enables investigators to image chemical groups on cell surfaces, and to directly measure their molecular-scale adhesion. Force spectroscopy with tips bearing bioligands offers a means to probe the localization and adhesion of single receptors on cells, such as cell adhesion proteins and antibiotic binding sites, while single-cell force spectroscopy quantifies the forces guiding whole cell adhesion. In this talk, I will discuss how we can use these AFM modalities in microbiology, emphasizing their potential for studying cell adhesion and biofilm formation in *Candida albicans*.

Professor Pierre Cornelis Microbiology, Vrije Universiteit Brussel

Biofilm forming in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an important opportunistic pathogen which causes a range of infections including chronic airways infections in cystic fibrosis patients. Biofilms play an important role in chronic infections and the forming and dispersion of biofilms has been extensively investigated. P. aeruginosa can switch from a planktonic form able to cause acute infections associated with the production of numerous virulence factors to a sessile form which causes chronic infections. This switch is caused by the balance between different sensors in the membrane and by the level of the internal signal molecule cyclic-di-GMP. Multiples cues and factors favor the formation of biofilms in this bacterium including the production of quorum sensing molecules, of siderophores, secondary metabolites (phenazines) and extracellular matrix (polysaccharides). Research is also going on to investigate which factors could be involved in the ageing and in the dispersion of biofilms, including the production of NO as a trigger of dispersion.

Professor Françoise Van Bambeke. Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université Catholique de Louvain, Bruxelles

In vitro pharmacodynamics models for the study of antibiotic activity against bacterial biofilms

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Biofilm-related infections are often persistent, mainy because they do not respond to antibiotics.

We have set up in vitro pharmacodynamic models allowing for the evaluation of the pharmacodynamic profile of activity of antibiotics against biofilms from commun human pathogens (*S. aureus, S. pneumoniae, P. aeruginosa*). Biofilms are exposed to a broad range of antibiotic concentrations, in order to obtain full concentration-response curves. Antibiotic activity is measured against viability within the biofilm or against biomass. Hill equations are fitted to the data and used to calculate pharmacodynamic parameters like relative potency or maximal efficacy of antibiotics.

These models allow thus to compare (a) antibiotics against a same bacterial strain or (b) a single antibiotic against different strains.

The data generated open many perspectives for the investigation of parameters that can affect antibiotic relative potency or maximal efficacy and for the study of new therapeutic options.

Related publications :

Bauer J, Siala W, Tulkens PM, Van Bambeke F. A combined pharmacodynamic quantitative and qualitative model reveals the potent activity of Daptomycin and Delafloxacin against *Staphylococcus aureus* biofilms. Antimicrobial Agents and Chemotherapy (2013) 57:2726-2737

Vandevelde NM, Tulkens PM, Van Bambeke F. Antibiotic activity against naïve and induced *Streptococcus pneumoniae* biofilms in an in vitro pharmacodynamic model. Antimicrobial Agents and Chemotherapy - in press

Sandra Van Puyvelde. Centre of Microbial and Plant Genetics. KU Leuven

A time course transcriptome study of *Salmonella typhimurium* during the switch from planktonic to biofilm growth

Salmonella has the ability to form structured, multicellular communities, called biofilms. The regulatory network controlling biofilm formation is complex, with many players that come in the act (1). We analyzed phenotypically initial biofilm formation during the first 24 hours. Hereto, we grew *Salmonella* biofilms in polystyrene petri dishes immersed with liquid medium, in which the bacteria were inoculated. Planktonic cells are defined as the free-living cells in the solution, while biofilm cells are the cells that are attached to the surface of the petri dish. Biofilms were phenotypically studied by counting the cell distribution between the planktonic and biofilm fraction, and by microscopy. The CFU counts of both the planktonic and biofilm fraction revealed a well-timed phenotypic switch from planktonic to biofilm growth. During this switch, planktonic growth reaches an optimum and starts decreasing significantly afterwards. Biofilm growth starts during this switch.
To unravel the dynamics in the regulatory cascades during initial biofilm formation, we subsequently conducted a time course transcriptome experiment using tiling arrays. Ten time points each were studied of both the planktonic and biofilm fraction (originating from the same setup), giving a global overview of the genetic profiles during the switch from planktonic to biofilm growth. Following, we performed a time-dependent and condition-dependent clustering, to distinguish changes in gene expression within each phase over the time course, and in between the 2 phases (planktonic versus biofilm). Our dataset identifies genes that play a role during the different phases of initial biofilm development, namely during: (i) preparation/determination of planktonic cells to become a biofilm cell; (ii) the switch from planktonic to biofilm growth and (iii) biofilm growth. For a set of confirmed genes with central roles in biofilm formation we could describe their dynamic behavior. This list of key players could be completed with additional genes, with unknown involvements in biofilm formation. Our study increases the understanding of the process of initial biofilm development, by studying a time lapse transcriptome profile of Salmonellae shifting from planktonic to biofilm growth.

References : Steenackers, Hermans *et al.* (2012), Food Res Int 45: 502-531

Felice Mastroleo. SCK-CEN, Mol

Quorum sensing is needed for *Rhodospirillum rubrum* S1H cell dispersion

In nature, bacteria can form complex bacterial communities that are closely associated with abiotic and biotic surfaces under certain environmental condition. A mechanism related to community behavior is quorum sensing (QS) where gram-negative bacteria can communicate with each other by producing and sensing certain concentration and types of molecules including N-acyl homoserine lactone (AHL). It allows bacteria to jointly control the expression of target genes and mount a co-operative response (e.g biofilm maturation, regulation of virulence, bioluminescence, etc.).
Currently we are investigating the use of the photosynthetic bacterium *R. rubrum* S1H in the context of the Micro-Ecological Life Support System Alternative (MELiSSA) project where organic waste could be converted by bacteria and higher plants into water, oxygen and food. However continuous culture of *R. rubrum* could lead to unwanted thick biofilm formation on the photobioreactor wall that could be linked to quorum sensing. Therefore the aim of this research is to characterize the QS system of *R. rubrum* S1H.
In that purpose, a knockout mutant of gene Rru_A3396 with a predicted autoinducer synthase function was constructed by allelic exchange. AHL molecules were extracted from whole-cell cultures of mutant and wild-type (WT) grown under MELiSSA-like conditions (light, anaerobic, and acetate as carbon source). Separation of AHLs was performed by thin layer chromatography (TLC) and detection was achieved using the reporter strain *Agrobacterium tumefaciens* NT1. In addition, WT and mutant were subjected to a low-shear stress experiment using rotating wall vessel (RWV) bioreactors to further characterize the mutant phenotype.
On the TLC plates, AHLs with acyl chains ranging from C4 to C12 carbon were detected in WT cultures whereas in the mutant, no signal was observed. Regarding mutant phenotype, a longer lag-phase, decreased pigment content and a decreased motility were observed. Interestingly, unlike WT, cell aggregation was observed in the mutant when cultured in the RWV. Moreover, under these low-shear stress conditions, only WT formed biofilm after 7 days of incubation while mutant did not.
Ongoing whole-genome transcriptomics and proteomics studies are helping us to further unravel the QS system of *R. rubrum* S1H and to assess possible consequences for its use in a closed-loop life support system.

Pauline Modrie. Laboratory of Food and Environmental Microbiology, UCL

On the fate of *Bacillus cereus* sensu lato in multi-species communities: biofilm formation and plasmid transfer.

Bacillus cereus group is composed of highly related species which display distinct virulence spectra adapted to specific habitats. The emergence of various ecotypes and pathotypes has been driven by the exchange of mobile genes [1] [2] [3]. The group includes *B. cereus* sensu stricto and its close relative *B. thuringiensis*. They persist in numerous microcosms including soil, human tissues and food products [4] [5] [6]. They are able to colonize biotic and abiotic surfaces and form biofilms [7] [8]. These structured communities are often composed of multiple species that are kept in close contact and interact. This study aimed to gain more insight into the ecology of the *B. cereus* group and the potential transmission of genetic material towards other food-borne pathogens. The main objectives were, (i) to analyze the formation of *B. thuringiensis* biofilms in multi-species communities using controlled flow cell experiments, and (ii) to evaluate to what extent biofilm lifestyle impacts on the fate of a plasmid from *B. cereus* group. Intra- and inter-species conjugation efficiencies have been monitored in flow cell and static biofilms. The 72-kb plasmid pAW63 originated from *B. thuringiensis* sv. kurstaki [9] was chosen as model to estimate the plasmid transfer amongst *B. thuringiensis* strains and towards other Gram-positive species. A large set of flow cell experiments were performed to characterize and quantify mono-, bi- and tri-species biofilm formation involving both *B. thuringiensis* and *Listeria ivanovii*, in combination, or not, with the strong biofilm producer *Staphylococcus epidermidis*. After 72 h, biofilms formed by the sole *B. thuringiensis* consisted in large floccular structures composed of long chains strongly anchored to microcolonies attached to the glass surface. When inoculated with S. epidermidis, a synergistic effect was observed, with chains of *Bacillus* anchored to *Staphylococcus* clusters, themselves strongly attached to the surface. Reciprocally *B. thuringiensis* formed plait structures on which cells of *S. epidermidis* could grow. This research revealed that the biofilm lifestyle positively impacts on intra- and inter-species conjugation of pAW63. The frequencies of intra-species pAW63 transfer in a flow cell biofilm (4,4 x 10-1 Transconjugant/Recipient) were about 10 times higher than in liquid LB medium (5 x 10-2 T/R). Inter-species pAW63 transfer from *B. thuringiensis* to *L. ivanovii* reached a frequency about 500 times higher in both flow cell and static biofilms (10-4 T/R) than in liquid medium (5 x 10-7 T/R).

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De Weirdt Rosemarie. Laboratory of Microbial Ecology and Technology, UGent.

The impact of low fluid shear near the gut mucosa on biofilm formation by health-promoting *Lactobacillus reuteri*

Mucosal gut microbes are considered to most profoundly impact human health because they make the closest contact with the host. The gut mucosal environment differs from that of the luminal environment in various ways that profoundly impact microbial life, e.g. by the presence of high concentrations of antimicrobial peptides and the transition from anaerobic conditions in the lumen towards micro-aerophilic conditions near the gut epithelium. In contrast to the lumen, the gut mucosa is furthermore characterized by a low fluid shear environment, which can be compared to a no-gravity environment. Such an environment was previously demonstrated to strongly impact the virulence and overall behaviour of pathogens such as Salmonella and Pseudomonas species. Here, we will provide new evidence that low fluid shear also impacts the commensal and health-promoting, mucosal microbe *Lactobacillus reuteri*. More specifically, a low fluid shear environment, enabled by growing *Lactobacillus reuteri* in a rotating wall vessel (RWV) bioreactor, was found to stimulate the formation of non-adhered *Lactobacillus reuteri* biofilm flocs. These biofilm flocs were achieved without the addition of an adherence site and were oval, freefloating units. We are currently investigating the impact of extracellular DNA on the formation of these biofilms. Our results implicate that mucosal colonization and biofilm formation by the health-promoting *Lactobacillus reuteri* in the gut mucosa may be the result of low fluid shear. Hence, disturbance of the low fluid shear in the gut mucosa, e.g. in case of inflammatory bowel disease, may inhibit its colonization and thereby impact overall health.

Katrien Forier. Centre for Nano- and Biophotonics, UGent

On the rational design of nanomedicines for the eradication of pulmonary biofilm infections

Treating chronic pulmonary infections in cystic fibrosis patients is still problematic. However, studies have shown increased antibiofilm activity when using nanoparticle encapsulated antimicrobials. In this work, the behavior of nanoparticles in CF sputum and bacterial biofilms was investigated. This information is key to the rational design of an optimized nanomedicine for treating pulmonary biofilm infections in CF patients.
The transport of pegylated, carboxylate or amine modified 100 & 200 nm polystyrene (PS) nanospheres was measured in CF sputum or *Burkholderia multivorans* biofilms using single particle tracking (SPT) microscopy. Next, the accessibility of biofilm clusters was investigated in function of particle size. Pegylated PS nanospheres (25-500 nm) were added to *B. multivorans* the biofilms and imaged using confocal microscopy. The penetration efficiency of the particles into dense bacterial clusters was quantified using ImageJ.
From the SPT experiments, it was clear that the pegylated nanospheres remained mobile in CF sputum and biofilms while the charged nanoparticles were immobilized by binding to sputum or biofilm components. When comparing SPT measurements of the pegylated particles in sputum obtained from different patients, qualitatively the same results were found although the absolute mobility was different between patients.. When comparing the accessibility of particles into dense bacterial clusters in function of particle size, it was observed that the 25 and 60 nm particles can penetrate efficiently in the clusters while this was decreasingly so for particles >100 nm, with 500 nm particles being almost entirely excluded from the clusters.

When developing a drug delivery nanocarier for the treatment of pulmonary biofilm infections, pegylation would be necessarily to enable the nanocarrier to reach the site of infection. To be able to efficiently penetrate into dense bacterial clusters in biofilms, the nanocarrier should preferably be less than 100 nm in diameter.

Nathalie Vandevelde. Cellular and Molecular Pharmacology, UCL, Brussels

Activity of antibiotics in models of naïve and induced biofilms of *Streptococcus pneumoniae*.

Chronic streptococcal infections are associated with in situ formation of biofilms. In these structures, bacteria can also exchange information, which can lead to behavior modifications. We have set up models of naïve and induced biofilms and used them to compare the matrix production and study antibiotic (AB) activity.

S. pneumoniae (capsulated [ATCC49619] and non capsulated [R6] strains) were grown in 96-well plates for 2, 4, 7 and 11 days. Naïve model was started from fresh bacteria grown on blood agar plate, and induced model, from planktonic cells collected from the medium of plates containing naïve biofilms having 6 days of maturity. AB activity was evaluated after 24 h of incubation (concentrations: 0.0001 to 1000-fold the MIC). Total biofilm mass (matrix + bacteria) was quantified by the OD at 620 nm of crystal violet (CV), and bacterial viability using the redox indicator resazurin (reduced in situ to fluorescent resorufin [RF] by living cells; [Lett. Appl. Microbiol.2008, 49: 249-54]). A Hill equation was fitted to the data to calculate maximal relative efficacy (Emax).

In the naïve model, CV OD increased from 0.2-1.55 to 40-180 between day 2 and day 11. This increase of biofilm thickness was accompanied by a reduction in antibiotic efficacy, especially for fluoroquinolones against bacteria. In the induced model, CV OD was higher than for the naïve model (between 2-11 at day 2 and 200-300 at day 11), leading to an even more important loss of activity against bacteria survival and on biofilm mass against 2-days biofilms. Over aging, fluoroquinolones (FQ) activity on survival remains the same and decreased against biofilm mass. At the opposite, ketolides showed an increased activity on survival for both strains and this phenomenon seems to be less dependent on biofilm thickness.

Conclusion: Biofilm production is independent of the non-capsulated or capsulated phenotype and is accelerated when bacteria had been previously in contact with a biofilm. AB activity globally decreases over aging in the naïve model but not in the induced model, demonstrating it is correlated to matrix thickness, which can oppose a permeability barrier to antibiotics.

Quentin Zune. Bio-Industries unit, Gembloux ABT ULg.

High-energy X-ray tomography analysis of a metal packing biofilm reactor for the production of lipopeptides by *Bacillus subtilis*

BACKGROUND: Whereas multi-species biofilm reactors are commonly used for the treatment of liquid and solid wastes, new strategies are progressively arising for the development of single species biofilm for the production of high-value metabolites. Technically, this new concept relies on the design of bioreactors able to promote biofilm formation and on the identification of the key physico-chemical parameters involved in biofilm formation.

RESULTS: An experimental setting comprising a liquid continuously recirculated on a metal structured packing has been used in order to promote *Bacillus subtilis* GA1 biofilm formation. The colonization of the packing has been visualized non-invasively by X-ray tomography. This analysis pointed out an uneven, conical, distribution of the biofilm inside the packing. Compared with a submerged culture carried out in a stirred tank reactor, significant modification of the lipopeptide profile has been observed in the biofilm reactor with a disappearance of fengycin and iturin fractions and an increase of the surfactin fraction. In addition, considering the biofilm reactor design, no foam formation has been observed during the culture.

CONCLUSIONS: The configuration of our biofilm reactor set-up allows for a higher surfactin production by comparison with a submerged culture while avoiding foam formation. Additionally, scale-up could be easily performed by increasing the number of packing elements.

Catherine Philippart. Institute of Condensed Matter & Nanosciences (BSMA), UCL, Louvain-la-Neuve, Belgium

Nano-Engineered Surfaces to Control the Development of Bacterial Microcolonies

The colonization of material surfaces by microorganisms remains a nagging problem, notably in the field of tissue engineering where bacterial contamination is the major cause of implant rejections. Therefore, the development of new strategies to limit bacterial activity on surfaces requires a better knowledge of the interactions involved between the bacteria and the surface. Although the physicochemical properties of the surfaces such as the charge, the roughness, the hydrophobicity and the chemistry are well-known to influence bacterial behavior, the understanding of the complex molecular mechanisms occurring at the nanometer scale between the surface and the cell remains a central question. Here, we investigated the response of bacteria to the variation of surface chemistry at the nanometer scale.

Combining nanoimprinting technique and surface modification, we produced large surfaces composed of nanometer-wide adhesive lines distributed in a non-adhesive background. We explored how these nanopatterned surfaces impact on the adhesion and the growth of gram negative *E. coli* and gram positive *L. Lactis*. Interestingly, it was shown that the nanopattern governed the collective behavior of bacteria which grew preferentially according to the pattern direction. Moreover, adhesion tests performed with *E. coli* mutants deleted for surface appendages (flagella, type I fimbriae, etc.) evidenced the role of these appendages on cell organization. Finally, viability tests performed in presence of an antibiotic substance revealed the higher sensibility of bacteria adhered onto patterned surfaces compared to non-patterned surfaces. These results show that bacteria can be manipulated through the surface, which could be of fundamental importance for rational design of material surfaces to control the adhesion and the proliferation of bacteria.

Soňa Kucharikova. LMCB, Department of Molecular Microbiology, KUL -VIB

Non-invasive bioluminescence imaging of mature *Candida albicans* biofilms developed in a subcutaneous mouse model.

Candida albicans biofilm development on biotic and/or abiotic surfaces represents a specific threat for immunocompromised patients. Although many in vitro models have been developed to follow up biofilm development, there is a crucial need for better understanding of this dynamic process under in vivo conditions. In our laboratory, we developed an in vivo subcutaneous rat model to study *C. albicans* biofilm formation (Řičicová *et al*., 2010). In this model, the implant of multiple Candida-infected devices to the back part of the animal provides a big advantage as it allows us to study several biofilms in one animal. Recently, this model was adapted to study *C. albicans* biofilm development in BALB/c mice, which significantly reduced costs and holds potential for biofilm research in different transgenic mice strains (a-Vande Velde *et al*., 2014). Despite advantages, the quantification of fungal biofilm biomass is traditionally analyzed post mortem, requiring host sacrifice. Hence, we aimed to make this model compatible for less invasive bioluminescence imaging (BLI), where detecting a quantifiable in vivo BLI signal from biofilms formed on the inside of catheters is challenging. We engineered a wild type strain, which expressed the *C. albicans* codon-optimized *Gaussia princeps* luciferase (gLuc) at the cell wall. gLuc activity from Candida cells and biofilms was measured and correlated with cell counts. The BLI signal was proportional to the amount of free-living yeast or hyphal cells. We could also detect a significant BLI signal above background (control biofilm) from in vitro and in vivo biofilms in live animals, and compared this signal with fungal biofilm biomass from explanted catheters. Better reproducibility of quantification (and therefore smaller test groups) can be achieved when mice are immunosuppressed when carrying biofilm-containing catheters. Additionally, BLI and Magnetic Resonance images provided information on the exact position in 3D of the catheters, which increases accuracy of signal quantification (b-Vande Velde *et al.,* 2013). Non-invasive imaging assay for quantifying in vivo biofilm formation adds immediate applications for the screening and validation of antimycotics under in vivo conditions, as well as for studies based on host-pathogen interactions, hereby contributing to a better understanding of the pathogenesis of catheter-associated infections.

Řičicová M., Kucharíková S., Tournu H. *et al*. 2010, Microbiology
a-Vande Velde G., Kucharíková S. *et al*., 2014, Methods in Molecular Biology
b-Vande Velde G., Kucharíková S. *et al*., 2013, Cellular Microbiology

Nicolas Delattin. Centre of Microbial and Plant Genetics, KU Leuven

The plant-derived decapeptide OSIP108 inhibits *Candida albicans* biofilm formation without affecting cell viability

We previously identified a plant decapeptide from *Arabidopsis thaliana*, OSIP108, which is induced in plants upon fungal pathogen infection. We assessed the effect of OSIP108 on planktonic *C. albicans* cells and on *C. albicans* biofilm formation, as *C. albicans* is one of the main fungal pathogens and biofilm formation plays a key role in infection processes.

Methods.
Biofilm formation in presence of OSIP108 and analogues was quantified using the cell titre blue method. In a first attempt to unravel its antibiofilm mode of action, we screened a library of 137 homozygous *C. albicans* mutants, affected in cell wall proteins and transcription factors involved in hyphal morphogenesis. Based on this screening, activation of the cell wall integrity pathway by OSIP108 was investigated by determining the degree of Mkc1p-phosphorylation in presence of OSIP108.

Results and conclusion.
We found that OSIP108 inhibits biofilm formation of *C. albicans* starting from 5 µM, without affecting viability of planktonic cells. A structure activity relationship study revealed that the cysteine on position 3 and the arginine on position 9 are the key amino acids for its antibiofilm activity. We identified 11 OSIP108-resistant *C. albicans* mutants, either defective in hyphal morphogenesis or in components important for cell wall integrity and structure. In line, we showed that OSIP108 activates the cell wall integrity pathway of *C. albicans*. Furthermore, inhibiting the yeast-to-hypha transition by specific components completely blocked the antibiofilm activity of OSIP108 pointing to the yeast-to-hypha transition as a crucial prerequisite in its antibiofilm activity.

Addendum

Sofiane El-Kirat-Chatel: oral communication and poster:

Single-Cell and Single-Molecule Analysis Deciphers the Localization, Adhesion and Mechanics of the Biofilm Adhesin LapA

Sofiane El-Kirat-Chatel (1), Audrey Beaussart (1), Chelsea D. Boyd (2), George A. O'Toole (2) and Yves F. Dufrêne (1)

(1) Université Catholique de Louvain, Institute of Life Sciences, Belgium.
(2) Geisel School of Medicine at Dartmouth, Microbiology & Immunology, Hanover, USA.

The large adhesin protein LapA mediates adhesion and biofilm formation by *Pseudomonas fluorescens*. Although adhesion is thought to involve the long multiple repeats of LapA, very little is known about the molecular mechanism by which this protein mediates attachment. Here we use atomic force microscopy to unravel the biophysical properties driving LapA-mediated adhesion. Single-cell force spectroscopy shows that expression of LapA on the cell surface via biofilm-inducing conditions (i.e., phosphate-rich medium) or deletion of the gene encoding the LapG protease (LapA+ mutant), increases the adhesion strength of *P. fluorescens* towards hydrophobic and hydrophilic substrates, consistent with the adherent phenotypes observed in these conditions. Substrate chemistry plays an unexpected role in modulating the mechanical response of LapA, with sequential unfolding of the multiple repeats occurring only on hydrophilic substrates. Biofilm induction also leads to shortening of the protein extensions, reflecting stiffening of their conformational properties. Using single-molecule force spectroscopy, we next demonstrate that the adhesin is randomly distributed on the surface of wild-type cells, and can be released into the solution. For LapA+ mutant cells, we found that the adhesin massively accumulates on the cell surface without being released, and that individual LapA repeats unfold when subjected to force. The remarkable adhesive and mechanical properties of LapA provide a molecular basis for the "multi-purpose" adhesion function of LapA, thereby making *P. fluorescens* capable of colonizing diverse environments. Our findings may represent a general mechanism among bacteria expressing analogous large adhesins promoting cell adhesion and biofilm formation.

List of posters

1	**Activity of Tobramycin in combination with clarithromycin against *Pseudomonas aeruginosa* (PA) biofilms in an artificial sputum medium (ASM) model**. Bassères Eugénie, Julien M. Buyck, Hamidou Traore, Paul M. Tulkens, Francis Vanderbist and Françoise Van Bambeke.
2	**Effects of Antibiotics on Biofilms of *Staphylococcus epidermidis* in vitro.** Claessens Jolien
3	**Repurposing as a means to increase the activity of Amphotericin B and Caspofungin against *Candida albicans* biofilms** Delattin Nicolas, K De Brucker, K Vandamme, E Meert, A Marchand, P Chaltin, B.P.A.Cammue and Karin Thevissen
4	**Does nicotine influence the kinetics of biofilm development in reference and clinical strains of *Streptococcus pneumoniae* ?** Diaz Iglesias Yvan, F. Van Bambeke and N.M. Vandevelde.
5	**The role of endolithic microbial biofilms in marine bioerosion** Golubić Stjepko Gudrun Radtke, Seong-Joo Lee and Jürgen Schneider
6	**PROFILING THE EXTRACELLULAR PROTEOME IN *BURKHOLDERIA CENOCEPACIA* GROWN UNDER BIOFILM AND PLANKTONIC CONDITIONS** Goza Agata, Gilles Brackman, Tom Coenye, Miguel Valvano and Bart Devreese
7	**Development of aerobic granular sludge** Henriet Olivier, Christophe Meunier, Paul Henry, Jacques Mahillon
8	**Forces driving the Attachment of Staphylococcus epidermidis to Fibrinogen-Coated Surfaces** Herman Philippe, Sofiane El-Kirat-Chatel, Audrey Beaussart, J. Geoghan, Thomas Van Zieleghem, Timothy J. Foster, Pascal Hols, J. Mahillon, and Yves F. Dufrêne.
9	**Cyanobacterial biofilms in the polar regions: Diversity and toxic metabolites** Kleinteich Julia.
10	**Revealing moxifloxacin activity against biofilms of *S. aureus* isolated from persistent infections by means of polycationic and amphiphilic substances.** Siala Wafi.
11	**Development of anti-biofilm agents and elucidation of their mode of action** Steenackers Hans
12	**The use of artificial cyanobacterial communities to assess bioinformatic pipelines for the analysis of 454 pyrosequencing data** Stelmach Pessi Igor, Pedro de Carvalho Maalouf, Haywood Dail Laughinghouse IV, Denis Baurain and Annick Wilmotte
13	**Single-Cell Force Spectroscopy of Pili-Mediated Adhesion** Sullan Ruby May A, Audrey Beaussart, Prachi Tripathi, Sarah Lebeer, Jos Vanderleyden and Yves F. Dufrêne.
14	***Candida albicans* alters the ofloxacin resistance of *Escherichia coli* in a dual-species biofilm** Tan Yulong, Karin Thevissen, Bruno Cammue and Katrijn De Brucker
15	**Study of the *Bacillis subtilis* ATCC21332 *pbpE-racX* operon in relation with the formation or disassembly of biofilms** Vanden Broeck Arnaud, Edwige Van der Heiden, Bernard Joris and Colette Duez
16	**The role of nucleotide biosynthesis in biofilm formation of *Escherichia coli* and *Salmonella*.** Yssel Anna
17	**COATIM – Development of antibiofilm coatings for implants** Katrijn De Brucker, B. Cammue, J. Michiels, J. Vanderleyden, P. Van Dijck, J. Vleugels, T. Bernardi, M. Erdtmann, F. Impellizeri, J. Kovac, Mirjam Fröhlich, A. Krona, G. Majdic, A. O'Neill and Karin Thevissen

18	**Single-Cell and Single-Molecule Analysis Deciphers the Localization, Adhesion and Mechanics of the Biofilm Adhesin LapA** Sofiane El-Kirat-Chatel, A. Beaussart, C. D. Boyd, G. A. O'Toole and Y. F. Dufrêne

Abstracts of Posters

The list is presented in alphabetical order of first authors

1. Bassères Eugénie[1] Julien M. Buyck,[1] Hamidou Traore,[2] Paul M. Tulkens,[1] Francis Vanderbist,[2] and Françoise Van Bambeke.[1]

1. Pharmacologie Cellulaire et Moléculaire, Louvain Drug Research Institute. UCL, Brussels. 2. SMB laboratories, Brussels, Belgium.

Activity of Tobramycin in combination with clarithromycin against *Pseudomonas aeruginosa* (PA) biofilms in an artificial sputum medium (ASM) model.

Macrolides improve respiratory function in cystic fibrosis patients, though inactive against PA. Yet, we previously demonstrated that macrolides regain activity on PA in media mimicking the infection site like bronchoalveolar lavage fluid (PMID 22573850). We have now investigated the activity of tobramycin-clarithromycin combinations on PA biofilms in a medium mimicking cystic fibrosis lung environment.

Methods: Strains: ATCC PAO1 and 39324 (stable mucoid). Biofilms were grown in 96 well-plates in ASM or Mueller Hinton Broth (MHB). Antibiotic effect on biofilm formation was examined after 24 h incubation (drugs added at the time of inoculation). Antibiotic effect on mature biofilms was tested on 12-days old biofilms exposed during 48h to antibiotics (0.125 to 250 µg/mL tobramycin or/and 0.5 to 100µg/mL clarithromycin). Biofilm biomass was evaluated by crystal violet staining and bacterial viability by fluorescein diacetate assay (PMID: 15567221).

Results: Biofilm formation was prevented in both media by tobramycin at concentrations ≥ MBC (Minimal Bactericidal Concentration = 0.5µg/mL) but at concentrations 6-7 dilutions lower than the MBC (= 1024µg/mL) for clarithromycin. On mature biofilms of PAO1 grown in ASM, tobramycin decreased viability from 60 to 70%, and clarithromycin from 10 to 40%; their combination was synergistic (70 to 80% decrease in viability). For ATCC39324, tobramycin reduced viability of 65 to 80% and clarithromycin, of 30 to 65%; their combination was more effective (80 to 90% reduction in viability).

Marginal effects were seen on biomass.
In biofilms grown in MHB, antibiotics alone showed similar effects than in ASM but combinations were not synergistic.

Conclusion: Clarithromycin increases tobramycin activity against PA (especially a mucoid strain) growing in biofilms in a medium representative of cystic fibrosis patients' sputum. These data further document that eukaryotic media can reveal specific antipseudomonal effects of macrolides; they also rationalize the interest of macrolide administration in cystic fibrosis patients.

2. Claessens Jolien. KU Leuven

Effects of Antibiotics on Biofilms of *Staphylococcus epidermidis* in vitro.

Biofilm-associated bacteria are characterized by a decreased susceptibility towards antibiotics. Routine assessment of antibiotic susceptibility, based on the planktonic growth of bacteria, therefore offers an insufficient prediction of the biofilm response. To assess the antibiotic susceptibility of biofilms, different methods were proposed. In the present study, the biofilms were evaluated after antibiotic treatment by staining with crystal violet to determine the total biofilm biomass and XTT to determine the cell viability. The effects of vancomycin, teicoplanin, oxacillin, rifampicin and gentamicin were assessed on preformed biofilms produced by eight clinical *Staphylococcus epidermidis* strains, isolated from proven biofilm-related infections. The antibiotics were applied separately and also combined with rifampicin.
We observed that the glycopeptides increased the total biofilm biomass, due to increased accumulation of living and dead bacteria. The major autolysin, AtlE was found to contribute in this increased accumulation. Increased production of PIA was not observed. In addition, the glycopeptides were not effective in killing biofilm-associated bacteria in biofilms produced by strong biofilm-producers, such as *S. epidermidis* strains 1457, 10b and 22c. For all strains analyzed, the effect of teicoplanin on the bacterial cell viability was more pronounced than the effect of vancomycin. Rifampicin, oxacillin and gentamicin were effective in killing biofilm-associated bacteria in all tested *S. epidermidis* strains.
Combining the glycopeptides with rifampicin, as it is often used in clinical practice, only resulted in an additional decrease of the bacterial viability in the case of vancomycin. However, the total biofilm biomass remained substantial. Combining rifampicin and gentamicin had an antagonistic effect on bacterial viability, as it is also mentioned in the literature. The total biofilm biomass was not influenced by combinations of rifampicin with oxacillin or gentamicin.
We concluded that combining crystal violet with XTT, gives information about the total biofilm biomass as well as the cellular viabilitiy. Use of the combined methodology showed that glycopeptides were not effective in eradicating *S. epidermidis* biofilms. Moreover, strains categorized as strong biofilm producers, are less susceptible to be killed by the glycopeptides when they are incorporated in a biofilm. Additionally we observed that teicoplanin is more efficient then vancomycin in killing biofilm-associated bacteria in vitro. In conclusion, we might question the efficacy of vancomycin for the treatment of biofilm-related infections.

3. Delattin Nicolas (1), K De Brucker (1), K Vandamme (2), E Meert (1), A Marchand (3), P Chaltin (3), B.P.A.Cammue (1), Karin Thevissen (1)

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(3) CISTIM, Heverlee, Belgium

Repurposing as a means to increase the activity of Amphotericin B and Caspofungin against *Candida albicans* biofilms

Biofilms of Candida species are often formed on medical devices and are resistant to currently available antifungal drugs. The aim of this study was to identify potentiators of amphotericin B (AmB) and caspofungin (CAS), the most commonly used antifungals, against Candida biofilms.

Methods: A library containing off-patent drugs was screened for potentiators that increase the in vitro activity of AmB against *C. albicans* biofilms. Biofilms were grown in 96-well plates and growth was determined by the cell titre blue (CTB) assay. Synergy between identified potentiators and the antifungals was further characterized in vitro using FICI values and in vivo using a worm biofilm infection model. In light of application of the compounds on implants, the effect of the compounds on the growth potential of MG63 osteoblasts was assessed.

Results: Pre-incubation of *C. albicans* biofilms with subinhibitory concentrations of the potentiators drospirenone, perhexiline maleate or toremiphene citrate significantly increased the activity of AmB against C. albicans and *C. glabrata* biofilms. Moreover, the potentiators act synergistically with CAS against *C. albicans* and C. glabrata biofilms (FICI < 0.5), while they do not affect growth potential of osteoblasts. Toremiphene citrate was selected to translate these in vitro findings to a worm and a rat *C. albicans* biofilm infection model. We demonstrate synergy between toremiphene citrate and CAS in curing the infection.

Conclusions: Our data demonstrate in vitro and in vivo potentiation of the antibiofilm activity of CAS by toremiphene citrate. Furthermore, our results pave the way for implant-related applications of the identified potentiators.

4. Diaz Iglesias Yvan, F. Van Bambeke, N.M. Vandevelde. Cellular and molecular Pharmacology. UCL

Does nicotine influence the kinetics of biofilm development in reference and clinical strains of *Streptococcus pneumoniae* ?

S. pneumoniae (SP) is a human pathogen frequently involved in acute exacerbations of chronic bronchitis (COPD). Tobacco smoking is one of the main risk factors for COPD. Our objective was to study the impact of nicotine on development of SP biofilm, using in vitro models of young to very mature biofilms.

Methods: 3 strains were used for the study, namely the reference strain ATCC49169 and 2 clinical isolates: N1 [smoking COPD patient] and SP1337 [CAP, child, non smoking]). Biofilms were obtained by culture in 96-well plates for up to 16 days, with medium replacement every 3 days (CA-MHB+2% glucose +5% lysed horse blood, supplemented or not with nicotine at 15.3 mg/L [urinary concentration; Br Med J. 1982, 284:1002-1004]. Total biofilm mass (matrix and bacteria) was quantified by staining with crystal violet (CV) followed by OD measurement at 570 nm.

Results: In control conditions, biofilm development proceeded following a two steps process over time for all strains. First, biofilm biomass increased following an ascending sigmoid function (knee point at 7 days) to reach a plateau that remained stable between days 10 and 12. Second, biomass decreased following a descending sigmoid function to reach a second plateau at much lower OD values at day 16 (OD values at day 7: 10-48; at day 12: 127-216; at day 16: 19-37. The addition of nicotine to the medium did not modify the biomass over time neither the kinetics of the whole process. Moreover, no systematic difference in biofilm biomass was observed between strains.

Conclusions: The biphasic evolution of biofilm mass overtime suggests that a disassembling process may take place in very mature biofilms. This phenomenon, already evidenced in other bacterial species, is thought help bacterial spread and recolonization of the host at other localizations, contributing to the persistence of infections. The fact that nicotine did not modify the kinetics of development of biofilms in vitro suggests that the adhesion of bacteria in the respiratory tract of COPD patients may rely rather on the pathophysiological condition than on a direct stress induced by nicotine on bacteria.

5. Golubić Stjepko[1,5] Gudrun Radtke[2], [3]Seong-Joo Lee and [4]Jürgen Schneider
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The role of endolithic microbial biofilms in marine bioerosion

Rocky limestone coasts are marked by a dark belt along the zone of the impact by tides and waves, which has long been considered to be the work of destructive physical and chemical forces of the ocean. A close microscopic view offers a completely different view, by unravelling dense populations of cyanobacteria and, to a lesser extent, microscopic algae and fungi in biologically complex interrelationships. These microbes are organized in several tears and follow tifferent of foodwebs. The destructive activity starts with microbial carbonate corrosion and is followed by invertebrate grazing pressure (gastropods, polyplacophores, echinoderms and, in the tropical areas, carid fishes. The communities of these internal biofilms are arranged along steep gradients of harsh environmental fluctuations in water supply, salinity and temperature and are also exposed to severe solar radiation.
The microbial community is supported by cyanobacterial populations that actively penetrate the rock leaving microboring patterns, closely conforming to the outlines of the organisms that make them. Boring patterns are often characteristic so as to enable the identification of microborers based on the boreholes alone. Cyanobacteria are the principal primary producers of these communities. They support trophic webs that range in scale from microscopic to macroscopic. In the lower intertidal and subtidal ranges, where ecological conditions are less harsh, the cyanobacteria are joined by microscopic green and red algae. High population density and corresponding productivity of these microbial communities have a significant geological impact that conists of microbial bio-corrosion, followed by bio-abrasion by grazers, and finally by the impact of waves on the biologically weakened coastal rocks. The lithological and sedimentological consequences of this biointegrated activity have excellent preservation potential, so that they contain important biologial and environmental information for the understanding of the fossil record, especially regarding their ecological indication value for paleoecological and paleobathymetric reconstructions and modelling.

6. Goza Agata[1], Gilles Brackman [2], Tom Coenye[2] , Miguel Valvano [3] , Bart Devreese [1]

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PROFILING THE EXTRACELLULAR PROTEOME IN *BURKHOLDERIA CENOCEPACIA* GROWN UNDER BIOFILM AND PLANKTONIC CONDITIONS

Burkholderia cenocepacia has emerged as problematic cystic fibrosis pathogen due to antibiotic resistance and high transmissibility. It develops in biofilms on lung epithelial cells and can survive intracellularly in macrophages, influencing antibiotic susceptibility.

Recent evidence suggests cooperation between the type II and type VI bacterial secretory pathways in intracellular bacterial survival (Rosales-Reyes, 2012). However, little is known about the actual extracellular proteins secreted by these two systems. The main objective of this study was to characterize the secreted protein repertoire of T2SS and T6SS of *B. cenocepacia* K56-2 under the biofilm and planktonic mode of growth.

The extracellular proteins present in the culture supernatant of *B. cenocepacia* WT, ΔT2SS, ΔT6SS strain and grown under biofilm conditions employing a CDC bioreactor model with a synthetic CF sputum medium, were analyzed by Gel-LC-MS/MS using an LTQ-FTICR mass spectrometry system.

Comparing secreted proteins from various strains revealed their dependency from T2SS or T6SS. Apart of known T2SS-dependent virulence factors like ZmpA, ZmpB and phospholipase C, we identified a range of hydrolytic enzymes and solute transporters. For instance, we discovered a putative chitinase that might interact with host cell glycans contributing to a pathogenic potential of bacteria.

Rosales-Reyes R, Skeldon AM, Aubert DF, Valvano MA. Cell Microbiol.14(2):255-73

7. Henriet Olivier, Christophe Meunier, Paul Henry, Jacques Mahillon. MIAE-UCL

Development of aerobic granular sludge

The discharge of untreated wastewaters in the environment is responsible of the rapid degradation of aquatic ecosystems. The current leading technology to treat important volumes of wastewater is the activated sludge process, where the dissolved pollution is degraded by free floating microbial consortia called “flocs”. The major drawback of these loose aggregates is their low settling velocity, which imposes large clarifiers to separate them from treated water. An emerging technology aims to improve this process by selecting consortia that form aerobic granules. As dense aggregates, they settle significantly faster than flocs, which may reduce both capital and operational cost of treatment plants. However, conditions to obtain, and maintain, granules are still challenging, and the role of constituting microorganisms on both granule formation and pollution removal is largely unknown.

This research aims to develop efficient batch reactors that can promote granulation from different wastewater sources. The performances of the reactors are recorded and the dynamics of microbial communities are assessed. The expected outcomes of the project are both fundamental and applied, as it will give a better understanding of aerobic granulation and it will be the first step towards the implementation of this technology in the Walloon Region.

Stable granulation has already been achieved with artificial wastewater. Feeding is to be done from the bottom of the reactor, within the granular sludge bed. Cycles include a short settling time to select dense granules and a long feeding phase followed by an aeration phase to favor accumulating organisms. The major problem is the development of filamentous bacteria on the periphery of the granules. At least two different species are involved, including *Microthrix* sp., a bacterium that causes foaming in activated sludge plant.

The bacterial composition of granules is very different between reactors, which seems to confirm that there is not a single microbial community that leads to granules. Several genera have been identified, including the Rhodocyclaceae genera Zoogloea, *Thauera* and *Dechloromonas*.

The effect of granule density on bacterial composition has been assessed with Denaturing Gradient Gel Electrophoresis (DGGE) technique. Three reactors with different operating conditions were tested. Granules were separated on a sucrose gradient and collected in three fractions.
So far, DGGE profiles of granules have not clustered in sucrose fractions.

A preliminary experiment to assess the effect of granular size has been conducted. Granules were separated in 3 classes. The largest granules are clustered, while medium and small-sized granules are not. Complementary work is ongoing to confirm this trend.

8. Herman Philippe, Sofiane El-Kirat-Chatel, Audrey Beaussart, Joan A. Geoghan, Thomas Van Zieleghem, Timothy J. Foster, Pascal Hols, Jacques Mahillon, and Yves F. Dufrêne. UCL Louvain-La-Neuve

Forces driving the Attachment of Staphylococcus epidermidis to Fibrinogen-Coated Surfaces

Cell surface proteins of bacteria play essential roles in mediating the attachment of pathogens to host tissues and, therefore, represent key targets for anti-adhesion therapy. In the opportunistic pathogen *Staphylococcus epidermidis*, the adhesion protein SdrG mediates attachment of bacteria to the blood plasma protein fibrinogen (Fg) through a binding mechanism that is not yet fully understood. We report the direct measurement of the forces driving the adhesion of *S. epidermidis* to Fg-coated substrates using single-cell force spectroscopy. We found that the *S. epidermidis* -Fg adhesion force is of ∼150 pN magnitude and that the adhesion strength and adhesion probability strongly increase with the interaction time, suggesting that the adhesion process involves time-dependent conformational changes. Control experiments with mutant bacteria lacking SdrG and substrates coated with the Fg β6-20 peptide, instead of the full Fg protein, demonstrate that these force signatures originate from the rupture of specific bonds between SdrG and its peptide ligand. Collectively, our results are consistent with a dynamic, multi-step ligand-binding mechanism called "dock, lock, and latch".

9. Kleinteich Julia. Centre d'Ingénierie des Protéines. Lab. de Physiologie, génétique et diversité bactériennes, ULg

Cyanobacterial biofilms in the polar regions: Diversity and toxic metabolites

The freshwater systems of the Arctic and Antarctic are typically dominated by extensive biofilms mainly composed by cyanobacteria. Despite of their pivotal role as the predominant primary producers in these ecosystems, there is little information as to their diversity and physiology. In the temperate and tropical regions cyanobacteria are known to produce a range of (toxic) secondary metabolites, but was only recorded a few times in the polar regions.

In this study cyanobacterial mats from various locations in the Arctic (Svalbard, Baffin Island) and the Antarctic (Byers Peninsula, Adelaide Island) were investigated using 16S rRNA gene pyrosequencing and Automated Ribosomal Intergenic Spacer Analysis. Mat cyanobacteria genera and species diversity was characteristic for cold ecosystems, comparable amongst mats, and correlated best with the geographical location of the mat. Leptolyngbya and Phormidium usually predominated the mat species composition on Adelaide Island.

The cyanobacterial toxin microcystin was detected in one mat sample from Baffin Island as well as in 26 out of 27 mats (10 - 300 ng / g organic mass) on Adelaide Island. The neurotoxin saxitoxin was recorded in a cyanobacterial mat from Baffin Island and parts of responsible genes (*sxtA*) detected. For the first time in the Antarctic, cylindrospermopsin was detected in 21 of 30 mats (2 - 156 ng CYN / g organic mass). The latter finding was confirmed via Liquid Chromatography-Mass Spectrometry and by the presence of the *cyrAB* and *cyrJ* genes.

These results indicate that the potential for toxin production in the cyanobacterial communities of the Arctic and Antarctic is higher than previously thought. Moreover, the highly divers mats are likely to harbour a variety of metabolites which could be of use to the scientific community.

10. Siala Wafi. UCL

Revealing moxifloxacin activity against biofilms of *S. aureus* isolated from persistent infections by means of polycationic and amphiphilic substances.

Moxifloxacin has low activity against *S. aureus* biofilms (Bauer, Siala *et al.*2013). Positively-charged hydrophilic molecules and cationic detergents prevent *S. aureus* biofilm formation (Kolodkin-Gal *et al*. 2012). We have examined whether these 2 types of molecules could also modulate moxifloxacin activity on preformed biofilms, in relation with moxifloxacin diffusibility through the biofilm. Norspermidine was selected as hydrophilic polycation, and caspofungin (antifungal echinocandin) as amphiphilic polycation because, contrary to detergents, it has no intrinsic activity on *S. aureus*.

Methods: Biofilms were grown for 24 h in 96-wells plates, using 7 clinical isolates from recurrent infections, and exposed for 48 h to increasing concentrations of moxifloxacin, combined with 200µM norspermidine or 73µM caspofungin. Biofilm mass was quantified by crystal violet absorbance; viability in the biofilm, using the redox indicator resazurin. Antibiotic diffusion through the biofilm was determined as described by anderl *et al*.

Results: Caspofungin and norspermidine alone were ineffective. Moxifloxacin caused 50 % reduction in viability at concentrations < 20mg/L for 2/7 strains. This effect was reached for 6/7 strains when combined with caspofungin, with no change in biomass. Norspermidine did not show any synergistic effect. Moxifloxacin diffusibility was > 70% through 6/7 biofilms, but only 20% through the biofilm remaining resistant to moxifloxacin-caspofungin combinations.

Conclusion: A cationic amphiphile like caspofungin improves moxifloxacin potency against *S. aureus* biofilms, provided the antibiotic can diffuse through the structure. Molecular mechanisms responsible for synergy are being studied.

11. Steenackers Hans. S&P-CMPG, KU Leuven

Development of anti-biofilm agents and elucidation of their mode of action

The objective of our interdisciplinary research consortium is the development of anti-biofilm agents that specifically target biofilms without affecting the bacterial growth. These specific anti-biofilm agents have the advantage of being less prone to resistance development than classical biocides or antibiotics. Moreover, elucidation of their biofilm-specific mode of action (MOA) reveals insight into the molecular events of biofilm formation and is likely to result in the identification of new cellular targets for biofilm inhibitors. The anti-biofilm agents have potential to be used in a preventive fashion (e.g. as coatings on medical implants and devices) or to eradicate biofilms (possibly in combination with antibiotics or disinfectants). *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Escherichia coli* are the model organisms, against which the anti-biofilm agents are being developed. Our development strategy consists of (i)in vitro and in silico screenings of compound libraries for anti-biofilm agents, (ii) chemical synthesis of a series of analogues of the identified hits, (iii) structure-activity relationship studies (SAR) and structure-toxicity relationship studies, (iv) mode of action studies of the lead compounds combining wet lab (transcriptomics, gene reporter fusion libraries and/or artificial evolution) and bioinformatics approaches and (v) further evaluation and development of the lead compounds (e.g. synergy studies, use of more applied test systems, multispecies biofilms…).

Results. *An in silico screening revealed a 2N-subsituted 2-aminoimidazoline as biofilm inhibitor. Analogues (~250) with either a 2-aminoimidazoline or a 2-aminoimidazole scaffold were synthesized by using newly developed chemistry. (4)5-phenyl-2-amino-1H-imidazole was found to have a moderate inhibitory activity. However, substitution of the N1-position, the 2N-position and the 4(5)-phenyl ring enhanced the activity up to 100 times (IC50 ~1µM). MOA studies revealed three possible cellular targets of the imidazoles, which are currently under further investigation. An experimental evolution experiment was performed to identify additional targets and obtained resistant pools of clones are being investigated in great detail via full-genome sequencing, RNA-seq and bio-informatics analyses. *A high throughput screening of >20,000 small-molecules resulted in the identification of 140 anti-biofilm agents. Three compound families were selected for which an early SAR study has been performed. MOA studies were performed using a hereto developed reporter fusion library. This library is a fast and easy accessible assay for MOA studies against *Salmonella* biofilms. Studies in four different conditions for three of the inhibitors revealed several pathways involved the (in)direct repression of the CsgD biofilm master regulator and attachment processes, coupled with changes in motility, virulence related process and metabolism.

12. Stelmach Pessi Igor[1], Pedro de Carvalho Maalouf[1], Haywood Dail Laughinghouse IV[1], Denis Baurain[2] and Annick Wilmotte[1]

1 - CIP, Physiologie, génétique et diversité bactériennes and 2 - Phylogenomics of Eukaryotes, University of Liège, Belgium

The use of artificial cyanobacterial communities to assess bioinformatic pipelines for the analysis of 454 pyrosequencing data

In frame of the BELSPO project CCAMBIO, we study the biodiversity and biogeography of cyanobacterial mats that cover the bottom in Antarctic lakes and meltwaters. We start to use next-generation sequencing (NGS) technologies. However, this generation of a very high number of sequences comes with the cost of relatively high error rates for individual reads, which can lead to overestimation of diversity due to the generation of spurious Operational Taxonomic Units (OTUs) consisting of erroneous sequences. Therefore, a correct assessment of microbial diversity using NGS relies on robust bioinformatic tools in to correct for biases. Here we report on community structures recovered for two artificial cyanobacterial communities applying three commonly used and two recently published bioinformatic pipelines. Artificial communities were constructed using DNA isolated from 22 strains from the BCCM/ULC Collection (http://bccm.belspo.be/about/ulc.php). DNA was extracted from individual cultures and pooled at equal (community Art1) or tiered (community Art2) concentrations. The V3-V4 hypervariable region of the 16S rRNA gene was used [Nübel et al (1997) Appl Environ Microbiol 63: 3327-3332], and amplicons were sequenced on a 454 GS FLX Titanium platform. Data was demultiplexed and submitted to 5 bioinformatic pipelines for quality control of reads, removal of chimera and OTU clustering: (I) “shhh.flows (450 flows)”, (II) “shhh.flows (360-720 flows)” and (III) “Sliding Window (Q35, 50 bp)”, using MOTHUR [Schloss et al (2011) PLOS One 6: e27310]; (IV) “fastq_maxee” and (V) “fastq_truncqual”, using UPARSE [Edgar (2013) Nat Methods 10: 996-998]. Average sequence length varied considerably. The number of OTUs obtained in each pipeline also varied significantly. While 22 and 21 OTUs were obtained for pipelines IV and V, respectively, pipelines I-III generated a surprisingly high number of OTUs (199, 317 and 289, respectively). Despite differences in the number of OTUs, the relative abundance of each strain did not differ significantly between pipelines. Overall, community structures observed using the UPARSE protocol (pipelines IV and V) were the most consistent with the expected results. These findings show the importance of assessing the performance of different bioinformatic pipelines using artificial communities, in order to reduce the effects of PCR and sequencing errors, which can lead to distorted community structures estimates.

13. Sullan Ruby May A, Audrey Beaussart, Prachi Tripathi, Sarah Lebeer, Jos Vanderleyden and Yves F. Dufrêne. Institute of Life sciences. UCL

Single-Cell Force Spectroscopy of Pili-Mediated Adhesion

Although bacterial pili are known to mediate cell adhesion to a variety of substrates, the molecular interactions behind this process are poorly understood. We report the direct measurement of the forces guiding pili-mediated adhesion, focusing on the medically-important probiotic bacterium *Lactobacillus rhamnosus* GG (LGG). Using non-invasive single-cell force spectroscopy (SCFS), we quantify the adhesion forces between individual bacteria and biotic (mucin, intestinal cells) or abiotic (hydrophobic monolayers) substrates. On hydrophobic surfaces, bacterial pili strengthen adhesion through remarkable nanospring properties that, presumably, enable the bacteria to resist high shear forces in physiological conditions. On mucin, nanosprings are more frequent and adhesion forces larger, reflecting the influence of specific pili-mucin bonds. Interestingly, these mechanical responses are no longer observed on human intestinal Caco-2 cells; rather, force curves exhibit constant force plateaus with extended ruptures reflecting the extraction of membrane nanotethers. These single-cell analyses provide novel insights into the molecular mechanisms by which piliated bacteria colonize surfaces (nanosprings, nanotethers), and offer exciting avenues in nanomedicine for understanding and controlling the adhesion of microbial cells (probiotics, pathogens).

14. Tan Yulong, Karin Thevissen, Bruno Cammue and Katrijn De Brucker,

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Candida albicans alters the ofloxacin resistance of _Escherichia coli_ in a dual-species biofilm

In nature, biofilms are often composed of multiple species. These species influence the cellular and biochemical composition of the biofilm, and can influence industrial and clinically relevant outcomes such as drug resistance. In this study we compared the resistance of *Escherichia coli* to ofloxacin in biofilms consisting of *E. coli* alone or of *E. coli* in combination with *Candida albicans*. Survival of *E. coli* upon treatment with ofloxacin was quantified using a c.f.u. assay in single and mixed species biofilms. *C. albicans* significantly increased resistance of *E. coli* to ofloxacin in mixed biofilms. However, the supernatant of *C. albicans* biofilms alone did not increase resistance of *E. coli* biofilm cells to ofloxacin, indicating that *C. albicans* does not secrete molecules that induce ofloxacin-resistance of *E. coli* biofilm cells. Currently, we are investigating whether the increased ofloxacin-resistance of *E. coli* biofilm cells in a mixed biofilm is caused by the extracellular matrix of *C. albicans*.

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Study of the *Bacillis subtilis* ATCC21332 *pbpE-racX* operon in relation with the formation or disassembly of biofilms

Bacillus subtilis is a PGPR (Plant Growth Promoting Rhizobacterium) Gram positive organism and a model for studying the in vitro formation or disruption of biofilms. At the liquid/air interface of standing cultures, *B. subtilis* forms thick pellicles of limited lifetimes. Some D-amino acids have been reported among the factors playing a role in the disassembly of *B. subtilis* biofilms: double *ylmE* and *racX* mutants (in which both racemases YlmE and RacX are absent) show a delay in pellicle disruption [I. Kolodkin *et al*. Science (2010) 328:627-629]. The RacX encoding gene is part of a bicistronic operon in which the first gene (*pbpE*) codes for a Penicillin-Binding Protein, the PBP4* whose function is not characterized in *Bacillus*.

Our studies aim to delete the complete *pbpE-racX* operon and compare the phenotypes of mutants and parental strains *B. subtilis* ATCC21332 or ATCC6051 in standing cultures. The substrate specificity of the purified RacX racemase is currently under investigation as well as the functional characterization of PBP4*, a protein possessing a lipocalin-like domain.

16. Yssel Anna. KU Leuven

The role of nucleotide biosynthesis in biofilm formation of *Escherichia coli* and *Salmonella*.

Bacterial biofilm formation is tightly controlled by the response of genetically encoded elements to environmental cues. Changes in the both the external surroundings and the intracellular space must be sensed and processed, to enable the cell to adjust and survive. The down regulation of motility and the concurrent upregulation of biofilm components are controlled by signaling molecules such as c-di-GMP, (p)ppGpp and cAMP.
Given that the aforementioned molecules are derived from nucleotides, we hypothesize that the alteration of purine and pyrimidine biosynthesis pathways may be a significant point of control of biofilm formation.
A collection of single gene knockout mutants, affected in the purine and pyrimidine pathways, were complemented with a concentration range of adenine and uracil and assayed for biofilm formation. Mutants in the de novo pathways, leading to UMP and IMP, were severely affected in both planktonic and biofilm growth. A concentration range was identified where planktonic growth could be restored and biofilm growth enhanced by the addition of adenine or uracil. Furthermore our results suggest that the balance between GMP and AMP production may be important for biofilm formation.
When the wild-type strains were assayed for biofilm formation in the presence of excess nucleotides, no change in phenotype was observed. This indicates that it is a shortage of purines or pyrimidines that influence biofilm formation.
To better understand the effect that perturbations in the de novo nucleotide biosynthesis pathways have on the genetic regulation of biofilm formation, time-lapse gene expression studies were conducted using fluorescent reporter fusions. The results showed that when the de novo pathways are out of commission, then the expression of genes involved in curli biosynthesis, motility and long polar fimbria are significantly affected.
To better quantify the effect that disruptions of the purine and pyrimidine pathway have on the pools of nucleotide derived signaling molecules we have performed mass-spectrometry analysis. This would allow us to identify which nucleotides (or derived compounds) are important for biofilm formation.
Our results, and that of others, imply that a strong, yet complicated link between biofilm formation and nucleotide biosynthesis exists. Inhibition of nucleotide biosynthesis could affect biofilm formation in various ways, including blocking the production of signaling molecules or by interfering with DNA replication. With this work we hope to shed some light on the role of nucleotides in biofilm formation.

Poster addenda

17. De Brucker Katrijn [1], Bruno Cammue[1], Jan Michiels[1], Jos Vanderleyden[1], Patrick Van Dijck[2], Jef Vleugels[3], Thierry Bernardi[4], Martin Erdtmann[5], Frédéric Impellizeri[6], Jasminka Kovac[7], Mirjam Fröhlich[8], Annika Krona[9], Gregor Majdic[10], Alex O'Neill[11], and Karin Thevissen[1]

COATIM – Development of antibiofilm coatings for implants

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Biofilms, groups of microorganisms that stick together on different surfaces within the human body and escape conventional antibiotic treatment, are an increasing challenge in the medical field. These biofilms are typically found on medical devices like implants. The most recent generation of implants with open porosity enables fast osseointegration, but also presents an increased risk of microbial biofilm-associated infection. Biofilm-associated infections are responsible for 15-25% of implant failure, and necessitate burdensome and costly revision surgery.

Until now, biocidal implant coatings have been developed that are based on either the release of silver ions, which are toxic upon accumulation, or on conventional antibiotics that have poor activity against microorganisms in biofilms. Therefore, the COATIM consortium, consisting of 5 SMEs and 5 universities/research institutes, was established with support from the European Commission (Seventh Framework Program – FP7; www.coatim.eu). COATIM aims to develop the next generation of implant coatings containing novel potent proprietary antibiofilm molecules (ABMs) with inhibitory activity against microbial biofilms.

In COATIM, these ABMs are grafted or deposited on small titanium implant substrates, as a model for dental and orthopaedic implants. Next, the ABM-coated implants are evaluated for in vitro and in vivo activity in resisting microbial infection without compromising osseointegration. Finally, the ABM-coating is applied on complex orthopaedic and dental implants, allowing the exploitation of the results by industry. In parallel, the antibiofilm mode of action of the ABMs is unraveled.

18. El-Kirat-Chatel Sofiane (1), Audrey Beaussart (1), Chelsea D. Boyd (2), George A. O'Toole (2) and Yves F. Dufrêne (1)

Single-Cell and Single-Molecule Analysis Deciphers the Localization, Adhesion and Mechanics of the Biofilm Adhesin LapA

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The large adhesin protein LapA mediates adhesion and biofilm formation by *Pseudomonas fluorescens*. Although adhesion is thought to involve the long multiple repeats of LapA, very little is known about the molecular mechanism by which this protein mediates attachment. Here we use atomic force microscopy to unravel the biophysical properties driving LapA-mediated adhesion. Single-cell force spectroscopy shows that expression of LapA on the cell surface via biofilm-inducing conditions (i.e., phosphate-rich medium) or deletion of the gene encoding the LapG protease (LapA+ mutant), increases the adhesion strength of *P. fluorescens* towards hydrophobic and hydrophilic substrates, consistent with the adherent phenotypes observed in these conditions. Substrate chemistry plays an unexpected role in modulating the mechanical response of LapA, with sequential unfolding of the multiple repeats occurring only on hydrophilic substrates. Biofilm induction also leads to shortening of the protein extensions, reflecting stiffening of their conformational properties. Using single-molecule force spectroscopy, we next demonstrate that the adhesin is randomly distributed on the surface of wild-type cells, and can be released into the solution. For LapA+ mutant cells, we found that the adhesin massively accumulates on the cell surface without being released, and that individual LapA repeats unfold when subjected to force. The remarkable adhesive and mechanical properties of LapA provide a molecular basis for the "multi-purpose" adhesion function of LapA, thereby making *P. fluorescens* capable of colonizing diverse environments. Our findings may represent a general mechanism among bacteria expressing analogous large adhesins promoting cell adhesion and biofilm formation.

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