Biofilms IV

Communities Bridging Disciplines
4th International Conference
1 – 3 September 2010
Winchester, UK

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Conference Handbook

Biofilms IV
4th International Conference

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About the Conference

Microbial biofilm research is now a feature of many scientific disciplines (e.g. microbiology, biotechnology, engineering, ocean science) impacting across diverse fields of application (medicine, environment, industry). The objective of the meeting is to capitalize on the multidisciplinary nature of this exciting research topic by fostering discussion and exchange of ideas and methods between the various research areas.

The conference will be a continuation of three previous international European biofilm meetings held in Osnabrück (2004), Leipzig (2006) and Munich (2008). Each of these conferences provided an excellent and stimulating environment for interdisciplinary discussion. The aim of the conference series is to provide a European platform for biofilm research activities and to create an interface to biofilm research in other countries.

Conference Themes

- Microbial communities in disease
- Community ecology and evolution
- Global scale biofilm systems
- Surface engineering and biofilm tribology
- Novel biotechnology and bioengineering
- Structural dynamics and emergent properties of biofilms
- Modulation of biofilm communities
- Signalling and communication in biofilms
- Biofilm development: a multidisciplinary approach

International Scientific Committee

- Thomas Bjarnsholt, University of Copenhagen, Denmark
- Hilary Lappin-Scott, Swansea University, UK
- Carsten Matz, Helmhotz Centre for Infection Research, Germany
- Andrew McBain, University of Manchester, UK
- Henny van der Mei, University of Groningen, The Netherlands
- Thomas Neu, Helmholtz Centre for Environmental Research, Germany
- Matthew Parsek, University of Washington, USA
- Cristian Picioreanu, Delft University of Technology, The Netherlands
- Alex Rickard, University of Michigan, USA

Local Organising Committee, University of Southampton

- Luanne Hall-Stoodley
- C. William Keevil
- Paul Stoodley
- Jeremy Webb, Chair

Local co-ordinators:

- Sam Collins
- Rob Howlin
- Susie Sherwin
- Sandra Wilks

Conference Venue

Winchester is a beautiful historic city in southern England known for its 11th-century cathedral and 12th-century castle. The conference lectures, exhibition and posters will take place in the Stripe Building of the University of Winchester's King Alfred campus, on Sparkford Road, Winchester.

For those who have pre-booked to attend the conference dinner, this will be held on 2nd September at Winchester Cathedral, situated on The Close, Winchester. Please see below for further details.

Registration Times

Registration will be from 09:00 – 13:00 on 1st September, from 08:00 on 2nd and from 08:30 on 3rd September. This will take place in the main foyer of the Stripe Building at the University of Winchester.

Accommodation

For those delegates who have registered for the full conference, including accommodation, this is located at the University of Winchester. Breakfast will be served between 07:30 and 08:30 in the Food Hall at the main campus.

Check in is from 15:00 on 1st September 2010. A room has been allocated in the Stripe Building for your baggage. During registration, please tell us if you need to store your luggage.

Check out is by 10:00 on 3rd September. Please return your key to the conference reception desk in the Stripe Building. You can bring your bags to the lecture on that day and they will be stored until the end of the conference.

For delegates who have booked an additional night's accommodation on 3rd September, check out is by 10:00 on 4th September. Please return your key to reception before departing.

Refreshments and Meals

Tea, coffee and lunches on 1st, 2nd and 3rd September is included in your delegate fee. Tea and coffee will be served in the Stripe Building. Lunch will be served in two sessions in the Food Hall of the University of Winchester. You will be handed a voucher for lunch when you register and these will be issued to delegates on a first come, first served basis.

Conference Dinner, Thursday 2nd September

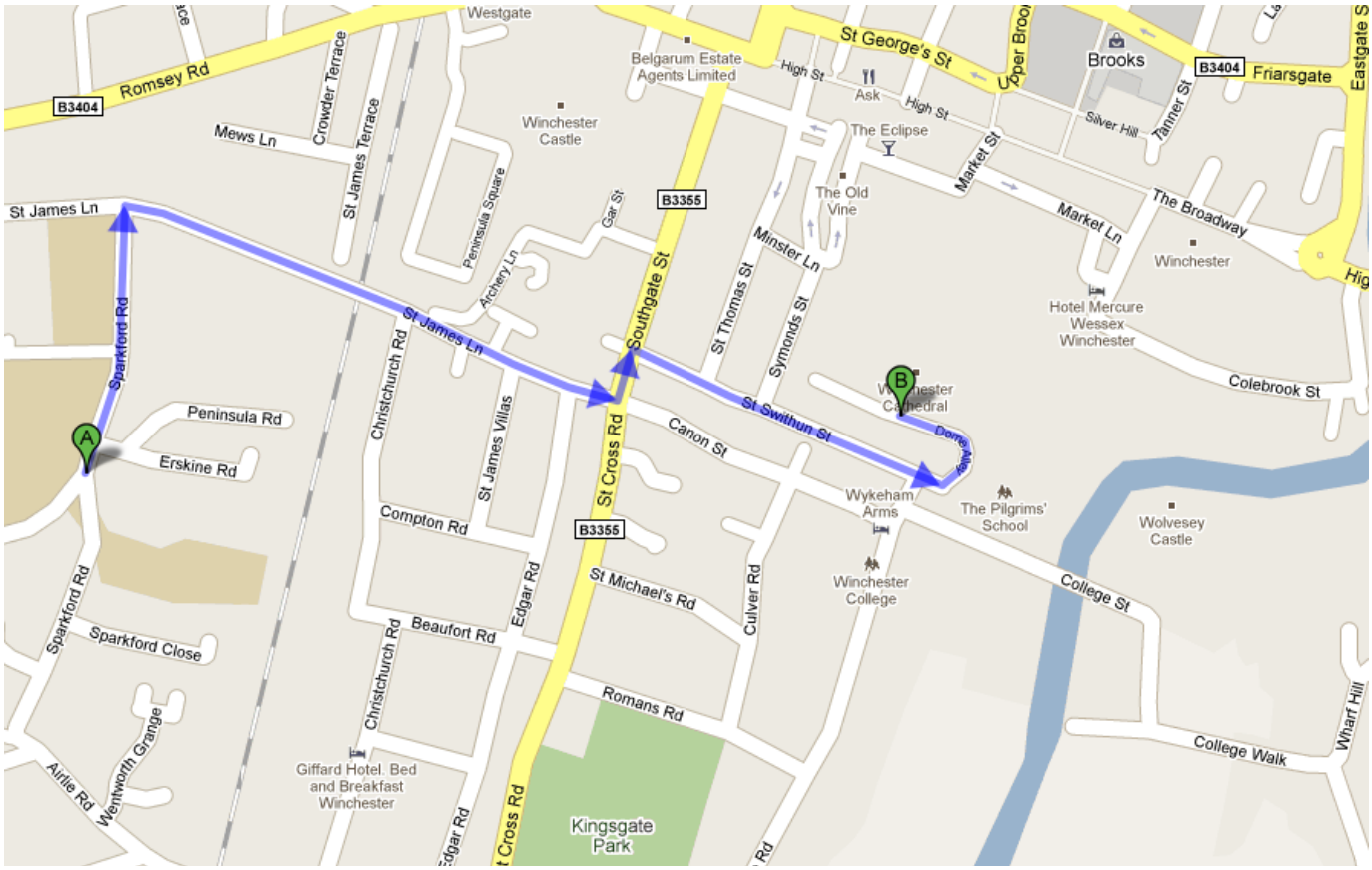
The conference dinner and drinks reception will be held at Winchester Cathedral. The evening will begin at 19:30 and end by midnight. You will need to arrange your own transport to and from the venue or you can walk; this will take approximately 20 minutes, please see the map below.

Local Taxi Numbers

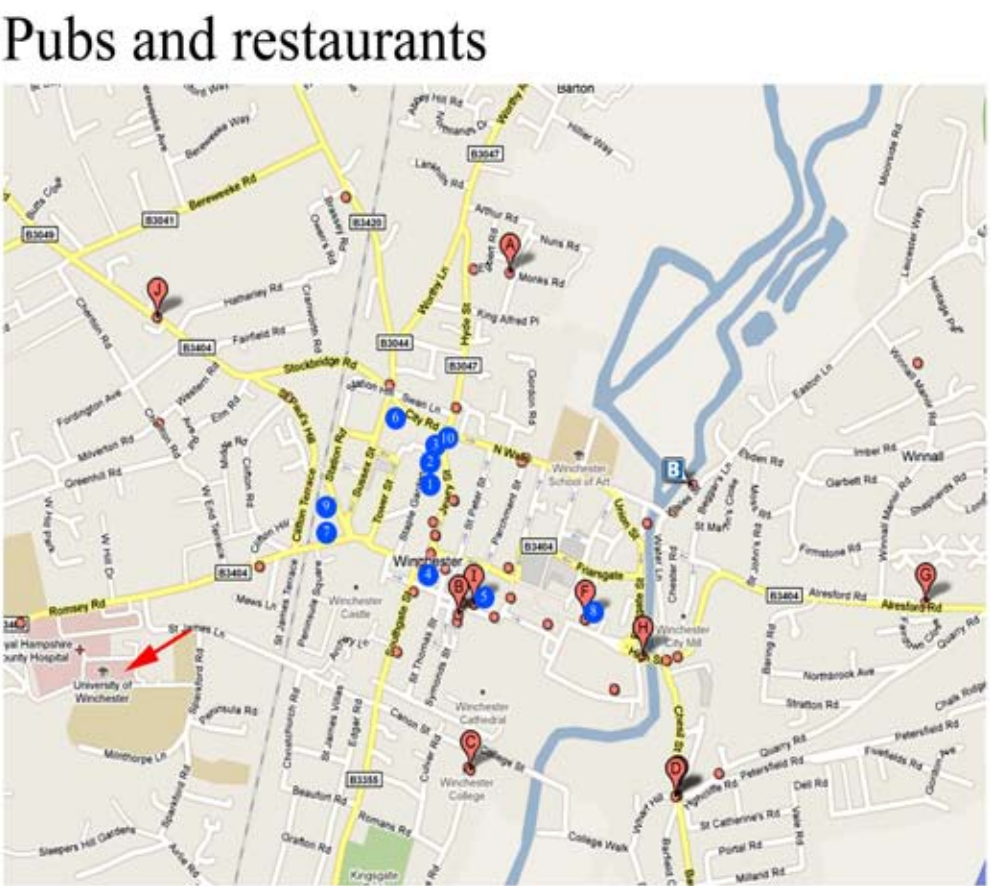
City Taxis - 01962 841212
Wintax - 01962 878737
Twyford Cars - 01962 712238

Route to Winchester Cathedral

The route from the University of Winchester (A) to Winchester Cathedral (B) is marked in blue on the map below.



Map of Pubs and Restaurants in Winchester



Popular Pubs

(A) King Alfred
Saxon Road

(B) The Old Vine- Pub and restaurant
8 Great Minster Street

(C) Wykenham Arms
75 Kinsgate Street

(D) The Black Boy
1 Wharf Hill

(E) The Black Rat Restaurant
88 Chesil Street

(F) Crown & Anchor
168 High Street

(G) The Golden Lion
99 Alresford Road

(H) The Bishop on The Bridge
1 Hight Street, Winchester

(I) The Eclipse
25 The Square

(J) The Roebuck Inn
57 Sstckbridge Road

Top 10 Restaurants

(1) Loch Fyne, 18-19 Jewry Street

(2) Prezzo, 16 Jewry Street

(3) Brasserie Blanc, 19-20 Jewry Street

(4) Zizzi's. 57 High Street

(5) Ask, 101 High Street

(6) Gurkha Chef, 17 City Road

(7) Gourmet Rajah Restaurant, Romsey Road

(8) Gandhi Indian Cuisine, 163 High Street

(9) Tanoshii Fusion, 12 Upper High Street

(10) The Porterhouse, 24 Jewry Street

Conference venue

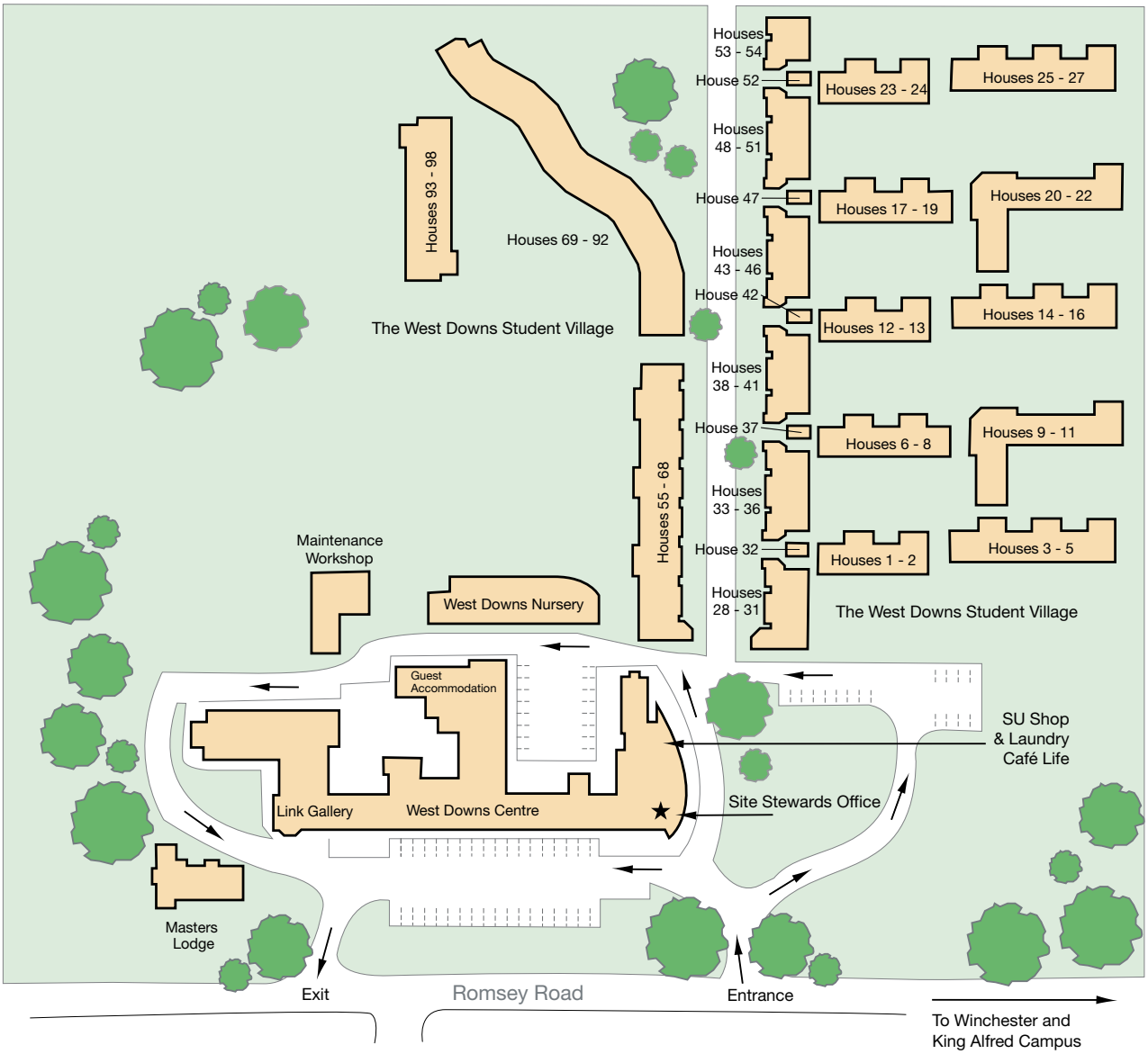
Restaurants in Winchester

Name / Address	Description
Bistro La Place 9 Great Minster St Winchester Tel: 01962 864004	French/International cuisine located close to the cathedral
Brasserie Blanc 19-20 Jewry Street Tel: 01962 810870	One of eight Raymond Blanc restaurants, serving good quality, freshly prepared food at affordable prices
Dim T 8-9 Jewry Street Tel: 01962 843000	Serves high standard oriental food in a well presented atmosphere
Gandi's Restaurant 163 High Street Tel: 01962 863940	Good Indian food in pleasant surroundings
Golden House Chinese Restaurant 6 St Cross Road Tel: 01962 840626	Excellent food in charming surroundings, with emphasis on friendly and personal service
Hotel Du Vin Restaurant 14 Southgate Street Tel: 01962 841414	Award winning and immensely popular bistro offering superb fresh food cooked in simple style
Loch Fyne Restaurant 18 Jewry Street Tel: 01962 872930	A good fish restaurant, prices suit all pockets. All dishes are individually prepared. Set in a beautifully converted Elizabethan building
Charles House Chinese Restaurant 3 Eastgate St Winchester Tel: 01962 854919	Lovely Chinese restaurant 5 minutes walk from the city centre
Pizza Express 1 Bridge Street Tel: 01962 841845	Pizza and pasta restaurant
Tanoshii Fusion 12 Upper High Street Tel: 01962 820288	A modern restaurant serving a variety of Thai and Japanese cuisine
Buddys Diner 5 Jewry Street Tel: 01962 860006	Very funky American diner – very good burgers & milkshakes
The Gurkha Chef 17 City Road Tel: 01962 842843	Traditional Nepalese food
The Old Vine 8 Great Minster St Winchester Tel: 01962 854616	Nice pub/restaurant with good atmosphere
The Slug and Lettuce 12/13 The Square Tel: 01962 850666.	Bar and Restaurant. British Food International Menu
The Willow Tree Pub 14 Durngate Terrace Tel: 01962 877255	
The Wykeham Arms 75 Kingsgate Street Tel: 01962 853834	

Finding your way around the King Alfred Campus

THE UNIVERSITY OF WINCHESTER

Finding your way around West Downs



THE UNIVERSITY OF WINCHESTER

Final Programme

	Wednesday, 1st September	
	09:00 – 13:00	**Registration**
	12:00 – 13:30	**Buffet lunch**
	13:30	**Welcome, followed by** **The Integration of the Biofilm Concept into the Diagnosis and Treatment of Musculoskeletal Infections** ***Keynote Speaker:*** *Bill Costerton – Allegheny-Singer Research Institute, USA*
Microbial communities in disease	14:10	**Session 1: Microbial communities in disease** Biofilms and chronic infections ***Chair and Invited Speaker:*** *Thomas Bjarnsholt, University of Copenhagen Denmark*
	14:30	Differential response of human keratinocyes to diffusible products from planktonic and biofilm cultures of *Staphylococcus aureus* *Garth James, Montana State University, USA*
	14:50	Polymicrobial, pathogenic biofilms in pediatric adenoids: a culture-independent approach to diagnosing biofilm infections *Luanne Hall-Stoodley, University of Southampton Wellcome Trust CRF, UK*
	15:10	The influence of selected autochthonous bacteria on the establishment of methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a novel wound simulator *Angela Oates, University of Manchester, UK*
	15:30 – 16:00	**Coffee break**
Surface engineering and biofilm tribology	16:00	**Session 2: Surface engineering and biofilm tribology** Antimicrobial effect of polymer brush coatings on staphylococcal biofilms ***Chair and Invited Speaker:*** *Henny van der Mei, University of Groningen, The Netherlands*
	16:20	From nanotechnology to microbiology - antibiofilm activity of nanosized magnesium fluoride *Ehud Banin, Bar-Ilan University, Israel*
	16:40	Antibiofilm coatings developed from a bio-inspired approach *Thomas Blin, University of Rouen, France*
	17:00	Tribological behaviour of oral mixed biofilms *Helena Cruz, DEB - University of Minho, Portugal*
	17:20 – 19:00	**Welcome Drinks and Poster Session**

	Thursday, 2nd September	
	08:30	**Biofilms on macrobenthic ecosystem engineers** ***Keynote Speaker:*** *Staffan Kjelleberg – University of New South Wales, Australia*
Global scale biofilm systems	09:00	**Session 3: Global scale biofilm systems** Mitigation and mediators of global change ***Chair and Invited Speaker:*** *Hilary Lappin-Scott, Swansea University, UK*
	09:20	Utility of biofilms in geologic carbon sequestration *Robin Gerlach, Montana State University, USA*
	09:40	Pathogen interactions within phylloplane biofilms: importance of the VBNC state *Bill Keevil, University of Southampton, UK*
	10:00	Biophysical basis for the geometry of coniform biofilms *Alexander Petroff, Massachusetts Institute of Technology, USA*
	10:20 – 10:50	**Coffee break**
Community ecology and evolution	10:50	**Session 4: Community ecology and evolution** Influences on the composition and stability of microbiome communities during development ***Chair and Invited Speaker****: Andrew McBain, University of Manchester, UK*
	11:10	Mutability of polyspecies bacterial biofilm community members *Larisa Magdanova, Institute of Ecology and Genetics of Microorganisms, Russian Federation*
	11:30	The significance of oxygen gradients on the evolution of biofilm formation by *Pseudomonas fluorescens* SBW25 in static liquid microcosms. *Anna Koza, The SIMBIOS Centre, University of Abertay Dundee, UK*
	11:50	Quantification of food web interactions on semi-natural biofilms. *Martina Erken, Helmholtz Centre for Environmental Research, Germany*
	12:10 – 13:40	**Lunch break**
Novel biotechnology and bioengineering	13:40	**Session 5: Novel biotechnology and bioengineering** Mass-spring models for individual-based microbial interactions and biofilm formation ***Chair and Invited Speaker****: Cristian Picioreanu, Technical University of Delft, The Netherlands*
	14:00	Study of antibiotics diffusion-reaction in biofilms by dynamical fluorescence (FLIM,FCS) *Karine Steenkeste, ISMO, France*
	14:20	Transcriptomic studies of putative gene clusters involved in biofilm formation & EPS biosynthesis of *Acidithiobacillus ferrooxidans* *Söeren Bellenberg, University of Duisburg-Essen, Germany*
	14:40	Effects of different acidophilic, moderately thermophilic microorganisms on attachment and biofilm formation on pyrite *Nanni Noel, University of Duisburg-Essen, Germany*
	15:00 – 15:30	**Coffee break**

Structural dynamics and emergent properties of biofilms	15:30	**Session 6: Structural dynamics and emergent properties of biofilms** Biofilm matrix functionality ***Chair and Invited Speaker**: Thomas Neu, Helmholtz Centre for Environmental Research*
	15:50	Biofilm physical properties from the inside *Nelly Henry, Institute Curie, France*
	16:10	Bacillus subtilis biofilms on immersed surfaces: there is a life below the pellicle *Romain Briandet, INRA, France*
	16:30	Role of eDNA in biofilms of *Shewanella oneidensis* MR-1 *Kai Thormann*
	16:50 – 18:00	**Poster session**
	19:30	**Conference dinner – Winchester Cathedral** **Poster prize announcement**
	Friday, 3rd September	
Modulation of biofilm communities	09:10	**Session 7: Modulation of biofilm communities** The driving and shaping of biofilm functions by protozoan predation ***Chair and Invited Speaker**: Carsten Matz, Helmholtz Centre for Infection Research, Germany*
	09:30	Identification of small molecule inhibitors of biofilm formation by *Salmonella Typhimurium* and *Pseudomonsa aeruginosa* and investigation of their mode of action *Hans Steenackers, Catholic University of Leuven, Belgium*
	09:50	Environmental triggers and intracellular signaling of biofilm dispersal *Nicolas Barraud, University of New South Wales, Australia*
	10:10	Analysis of multiple hypotheses for antibiotic tolerance of *Staphylococcus epidermidis* in biofilms *Phil Stewart, Montana State University, USA*
	10:30 – 11:00	**Coffee break**
Signalling and communication in biofilms	11:00	**Session 8: Signalling and communication in biofilms** Autoinducer-2: role in inter-species interactions between bacteria indigenous to the human microbiome ***Chair and Invited Speaker**: Alex Rickard, University of Michigan, USA*
	11:20	A novel cell-to-cell communication molecule, cis-2-decenoic acid, acts to induce bacteria to transition from a biofilm lifestyle to an active disseminating lifestyle *David Davies, Binghamton University, USA*
	11:50	Remote control of antibiotic resistance within bacterial communities *Jean-Marc Ghigo, Institut Pasteur, France*
	12:10	Denitrification and nitric oxide production in laboratory, in vivo and ex vivo biofilms of the oral cavity and nasopharynx. *Paul Stoodley, University of Southampton, UK*
	12:30 – 14:00	**Lunch break**

Biofiilm development: a multidisciplinary approach	14.00	**Session 9. Biofilm development: A multidisciplinary approach** The role of the Pel polysaccharide in Pseudomonas aeruginosa biofilm development ***Chair and Invited Speaker****: Matt Parsek, University of Washington, USA*
	14:20	dATP/ATP in biofilm formation and pathogenesis *Chuanwu Xi, University of Michigan, USA*
	14:40	Regulatory systems required for biofilm development and the maintenance of the planktonic mode of growth *Karin Sauer, Binghamton University, USA*
	15:00	Growth and development of 3-dimensional structures in biofilms and cancer - an integrated approach *Samuel Collins, University of Southampton, UK*
	15:20	**Long-term evolutionary dynamics of *Pseudomonas aeruginosa* in a human host environment** ***Keynote Speaker:*** *Soeren Molin – Technical University of Denmark*
	15:50	**Closing comments, departure**

Keynote Abstracts

The Integration of the Biofilm Concept into the Diagnosis and Treatment of Musculoskeletal Infections
Bill Costerton, Allegheny-Singer Research Institute, USA

Bacteria live predominantly in complex matrix-enclosed communities, that differ profoundly from the swarms of planktonic cells that were previously visualized as inhabiting natural and pathogenic ecosystems. Within these biofilms the bacteria cells undergo profound genomic changes, to equip themselves to prosper in the micro-niches they inhabit, and they carry out horizontal gene transfer on a scale never seen in planktonic populations. Metabolic coordination is achieved by the active juxtaposition of the cells of physiologically cooperative species, and a system of water channels is maintained for nutrient acquisition and waste removal. The consistency of the biofilm community is that of a viscous solid, and the whole community can move, in a coordinated fashion, in response to shear forces.

Within the biofilm community cells can communicate by means of diffusible chemical signals, that can control growth rates, and even the most complex of cell "behaviours". In addition to communication by diffusible signals, cells can communicate directly, by means of signals enclosed in membrane vesicles, and by a newly discovered process of cell envelope fusion to form "zippers" between adjacent cells. Cells within biofilms are also connected by means of very long nanowires, that can conduct currents between electrically different areas of the community, and may play a role in cell-cell communication. Bacteria in biofilms can also produce extracellular structures of very considerable complexity, that may serve as guides for social activities, or as protective "cages" for community security.

Cells from these highly sophisticated communities express a profoundly different phenotype from that of their planktonic counterparts, and one of the consequences of this difference in gene expression is that they grow only very poorly (if at all) when removed from the ecosystem and placed on the surfaces of agar plates. In practical terms, the biofilm bacteria that cause virtually all device related and other chronic bacterial infections, are not detected by routine cultures. This failure of cultures to detect bacteria in chronic biofilm infections poses an enormous difficulty in the management of infections in Orthopaedics, and we will present evidence that these organisms can be much more effectively detected and identified by modern DNA-based methods.

Biofilms on macrobenthic ecosystem engineers
Staffan Kjelleberg, School of Biotechnology and Biomolecular Sciences and Centre for Marine Bio-Innovation, The University of New South Wales, Sydney, Australia and School of Biological Sciences, College of Science, Nanyang Technological University, Singapore

No Abstract Received

Long-term evolutionary dynamics of *Pseudomonas aeruginosa* in a human host environment
Soeren Molin, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark

Understanding the molecular mechanisms of adaptation to new environments is of fundamental importance in biology. Laboratory evolution experiments have led to important findings relating organism adaptations to genomic evolution. However, continuous monitoring of long-term evolution has been lacking for natural systems limiting our understanding of these phenomena *in situ*. We characterize the evolutionary trajectory of a lineage of a clinically important opportunistic pathogen *Pseudomonas aeruginosa* as it adapts to the airways of cystic fibrosis patients over 35 years. Sampling isolates from multiple hosts over several decades, we find limited diversification in spite of the highly structured and complex environment. Interestingly, genomic changes accumulate at a constant rate while rapid global phenotypic changes occur in the early phases of adaptation. Early adaptation was followed by limited changes in the later stages accompanied by genomic signatures of negative selection. Three global regulatory mutations are responsible for most of the phenotypic adaptations and appear to position the strain for its subsequent colonization of over 40 patients within the clinic. Over the course of this study, the lineage underwent over 200,000 generations and, as such, this study represents the longest bacterial evolution experiment reported to date capturing early steps of bacterial speciation.

Invited Speaker Abstracts

Biofilms and chronic infections
Thomas Bjarnsholt, University of Copenhagen, Denmark

For bacteria, aggregation or biofilm formation is an important survival mechanism in almost any environment since; bacteria living in biofilms are very well protected against antibiotics and the host defense. Recent visualizations of bacterial aggregates in several chronic infections (chronic otitis media, cystic fibrosis, infection due to permanent tissue fillers and chronic wounds) highlights a trend in both the distribution (such as where in the wound bed) and organization (monospecies or multispecies microcolonies). It seems as these clinical biofilms differ in distribution and organization compared to commensal biofilms (dental and intestine) and biofilms in natural ecosystems (soil). The observations of the chronic biofilm infections point towards a tendency of low bacterial diversity and sovereign monospecies biofilm aggregates even though the infection in which they reside most often is multispecies. In contrast to this, commensal and natural biofilm aggregates contain multiple species that are believed to co-exist, interact and form biofilms with high bacterial and niche diversity. The pros and cons of this hypothesis are discussed.

Antimicrobial effect of polymer brush coatings on staphylococcal biofilms
Henny van der Mei, University of Groningen, The Netherlands

Biomaterial-associated-infection (BAI) remains the number one cause of prosthetic implant failure, despite the development of various state-of-the-art strategies to control BAI after implantation. Microbial adhesion is considered to be the onset of BAI and leads to formation of a biofilm in which microorganisms are embedded in a complex extracellular polymeric matrix where they are resistant against antibiotic treatment and the host immune system. Polymer brush-coatings are currently the most promising non-adhesive coatings as they reduce the adhesion of various bacterial strains and yeasts by orders of magnitude. Full prevention of adhesion has never been achieved by these coatings. One might argue that the few adhering bacteria to the brush can initiate a biofilm formation process which eventually implies that brush-coatings, at least when used as the only tool to control infection, are non-effective. We have studied adhesion strength of bacterial strains to pristine and brush-coated silicone rubber and monitored *ica*-expression and growth of adhering staphylococci into a biofilm. Biofilms grew on both brush-coated and pristine silicone rubber, while the *ica*-expression was much lower and the viability of biofilms on brush-coatings was higher than on pristine silicone rubber. We have the impression that staphylococci do not recognize the highly hydrated polymer brush-coating as a surface and are thus not stimulated to express the *ica*-operon. By consequence, biofilms on polymer brush-coatings remain susceptible to antibiotics, contrary to biofilms formed on other biomaterial surfaces.

Mitigation and mediators of global change
Hilary Lappin-Scott, Swansea University, UK

No abstract received.

Influences on the composition and stability of microbiome communities during development
Andrew McBain, University of Manchester, UK

Biofilm communities associated with the human body are notable for their taxonomic diversity and their compositional stability, which is i) maintained despite continued microbial influx, ii) manifested as individual-specific microbiotas and ii) mechanistically poorly understood. This presentation will use the oral cavity and the gastrointestinal tract microbiotas as paradigms to consider variables that are responsible for the development, maintenance and stability of individual-specific microbiotas. Key influences on this phenomenon include microbial factors such as colonisation-resistance within complex microbial climax communities and competitive processes, particularly during colonisation. Host-specific factors also play a role and these include the innate and adaptive immune systems, as well as the comparatively well understood extrinsic influences including diet, the use of antibiotics and other drugs and lifestyle.

Mass-spring models for individual-based microbial interactions and biofilm formation
Cristian Picioreanu, Katherine Celler, Department of Biotechnology, Delft University of Technology, The Netherlands, Iris Hoedl, Tom Battin, Department of Freshwater Ecology, University of Vienna, Austria

Numerous modeling studies addressed various questions of biofilm development, but only few studied the importance of mechanical factors. Mechanics plays a role in different biofilm formation stages and at different spatial scales. First, *cell flexibility and motility* are important not only in the initial stages of substratum colonization and colony spreading, but also in the later starvation stages, biofilm dispersal and formation of fruiting bodies. Second, *biofilm deformation*, mainly due to the action of liquid flow forces, has several consequences for biofilm detachment, transport and consumption of substrates, streamers formation and their oscillations, and on the overall biofilm architecture.

This presentation will illustrate the use of mass-spring models, as one numerical approach to study individual-based mechanical interactions among microorganisms. One example demonstrates that cell flexibility can be an important factor affecting both the movement of single cells and the alignment of cell populations. In particular, we studied how collisions between gliding *Myxobacterium* cells facilitate reorientation of cells upon contact and, thus, alignment of the whole population - crucial stage for the development of multicellular fruiting bodies. Here we created a computational mass-spring model of a flexible rod-shaped cell that glides on a substratum periodically reversing direction of movement. The model was formulated in terms of experimentally measurable mechanical parameters, such as engine force, bending stiffness and drag coefficient. We investigated how cell flexibility and motility engine type and force affected the pattern of cell gliding and the alignment of populations of 500 to 10000 mechanically interacting cells. It was found that a flexible cell powered by engine force at the rear of the cell, as suggested by the slime extrusion hypothesis for myxobacteria motility engine, would not be able to glide in the direction of its long axis. A population of rigid reversing cells could indeed align due to mechanical interactions between cells, but cell flexibility impaired the alignment. It has also been shown that a population of self-propelled stiff rods can form clusters due to mechanical interactions (Figure 1).

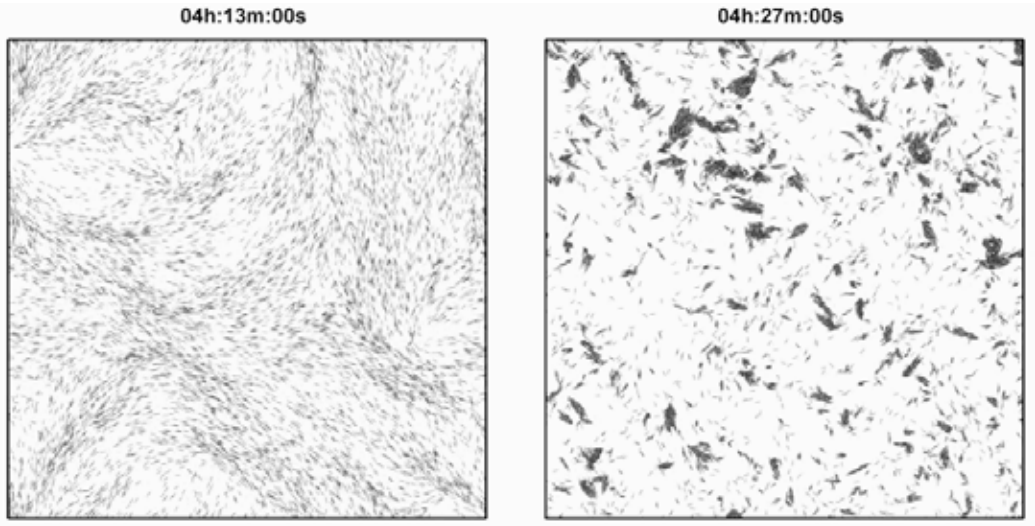


Figure 1. Population of 10000 motile myxobacteria (stiff rods) aligning in streams when periodically reversing movement direction (left), or forming clusters without movement reversal (right).

Another example presents a computational model developed to explain how filamentous *Diatoma* colonies form in rivers in different flow patterns. Significant aspects of biofilm formation, such as microbial growth, sticking and attachment, were considered and a mass-spring mechanical model was developed to simulate movement of filaments induced by the water flow. Several model parameters, such as the diatom density, size, filament geometry and growth rate, attachment rate, as well as the water velocity and its variation in time were experimentally determined. Other parameters, such as the elastic coefficients of the model springs, were assumed and their influence was assessed. Model results describe qualitatively formation of the different observed colony architectures built by zig-zag *Diatoma* filaments. When run at river bottom valley conditions, under slower and multidirectional flow, intricate dome-shaped structures and colonies formed. Alternatively, when run at river bottom ridge conditions, where flow is fast and mostly unidirectional, the model showed the formation of long, dreadlock-type filament structures (Figure 2).

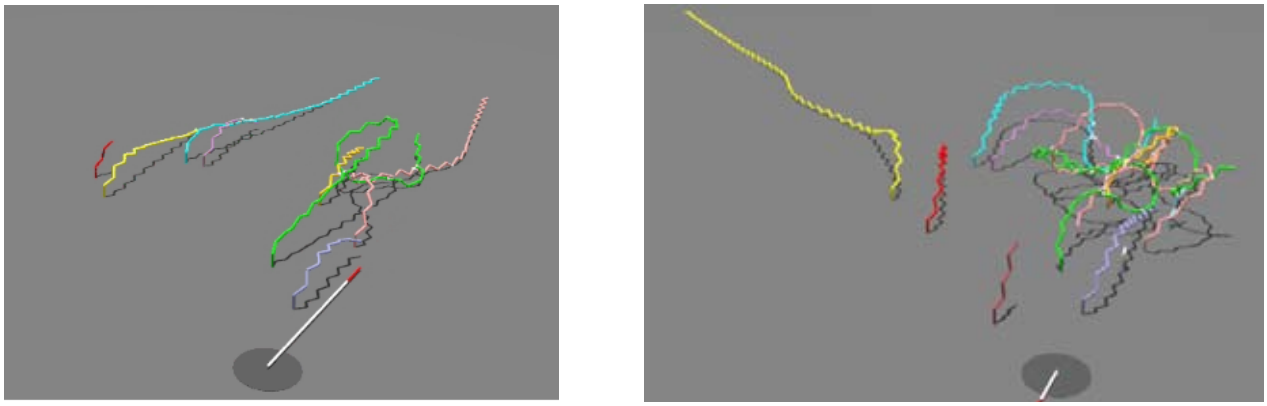


Figure 2. Colonies of zig-zag Diatoma filaments developed in fast and mostly unidirectional flow - river bottom ridge conditions (left) and slow and all-directions flow - river bottom valley conditions (right).

In conclusion, we show how other mass-spring models are particularly useful computational tools to study cell motility, spreading of microbial colonies made of mixed cell morphotypes or interactions between fluid flow and biofilm leading to detachment and streamer oscillations.

Biofilm matrix functionality

Thomas R. Neu[1], John R. Lawrence[2]
[1]Helmholtz Centre for Environmental Research – UFZ, Magdeburg, Germany
[2]Environment Canada, Saskatoon, Saskatchewan

Environmental microbial communities are composed of Archaea, Bacteria and micro-Eucarya embedded into a polymer matrix. This matrix, also known as extracellular polymeric substances (EPS) is produced by many different microbial species. The polymer matrix represents a complex mixture of chemically different polymers with largely unknown properties and functions. The EPS maybe identified as polysaccharides, proteins, nucleic acids, amphiphilic compounds and refractory polymers. The polymers are interacting with the substratum, with other cells as well as with dissolved, colloidal and particulate components. In this presentation we present a once again extended concept of ideas with respect to the function of EPS in biofilm systems. The roles of EPS may be considered at various levels of functionality and chemical activity: 1) constructive, 2) adsorptive, 3) adhesive, 4) active, 5) surface active, 6) informative, 7) nutritive, 8) locomotive, 9) redox-active, 10) conductive. In short, the EPS matrix may serve as versatile component involved in many functions and processes of bio-films and bio-aggregates. Furthermore it is very likely that some polymers have multiple functions and that several EPS functions are still unknown.

Neu TR, Lawrence JR (2009) Extracellular polymeric substances in microbial biofilms. In: Moran A, Brennan P, Holst O, von Itzstein M (eds) Microbial glycobiology: Structures, relevance and applications. Elsevier, San Diego, pp 735-758

The driving and shaping of biofilm functions by protozoan predation

Carsten Matz, Helmholtz Centre for Infection Research, Braunschweig, Germany

Biofilm growth and survival in the environment and many technical systems are constrained by the action of associated phagocytic mikroeukaryotes, the protozoa. Grazing by protozoa is considered to be a major source of bacterial mortality in most aquatic and terrestrial ecosystems. We hypothesize that biofilms serve as bacterial refuge against protozoan predation thus allowing long-term stability and persistence, and that the coexistence with biofilm-associated protozoa promotes pathogenic life-styles in bacteria. To test this, our studies assess (a) the capacity of protozoa to efficiently control biofilms, (b) the role of biofilm-specific defence strategies against grazing, (c) the molecules and cellular targets used to suppress trophic regulation and to exploit the 'eukaryotic niche' presented by protozoa, and (d) the overall effects of selective grazing on community structure and function. Our experiments reveal that bacteria growing in biofilms are less vulnerable to predation than planktonic cells. Widespread resistance against predators is mediated by the interplay of biofilm-specific traits such as exopolymer production, cellular cooperation, inhibitor secretion, and phenotypic variation. Selective predation is suggested to promote bacterial life in the biofilm niche and to govern structure-function relationships and associated biogeochemical transformations. There is increasing evidence that some of the bacterial pathogenicity traits may have their origin specifically in successful antipredator adaptations. Parallel selective pressures in and outside the human host may result in cross-adaptations of bacterial pathogens.

Autoinducer-2: Role in Inter-species Interactions between Bacteria Indigenous to the Human Microbiome

Alex Rickard, Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, USA

The healthy human body is composed of 10-100 times more bacteria than host eukaryotic cells. These bacterial cells are either resident commensal species or transient pathogenic species. It is likely that inter-bacterial communication occurs within and between these resident commensal and transient pathogenic communities. Autoinducer-2 (AI-2) is proposed to be a universal signal molecule that mediates communication between different species of bacteria, many of which are indigenous to the human microbiome. We propose that these bacteria use AI-2 to modulate inter-species interactions when co-localized with one-another within human biofilm communities. Changes in these interactions alter the ecology of the biofilm communities. It is the aim of this presentation to describe the impact of AI-2 on inter-species bacterial interactions and community development of these biofilms. Our findings from studies on dental plaque and chronic wound biofilms will be described. For example, when grown together in biofilms under conditions relevant to the human oral cavity, AI-2 promotes mutualism between the commensal dental plaque bacteria *Streptococcus oralis* and *Actinomyces oris* and also promotes competition between *S. oralis* and *Streptococcus gordonii*. Changes in the concentration of AI-2, in the nanomolar range, alter the degree of mutualism or competition. While studies of AI-2 mediated interactions between bacteria common to chronic wounds are still in their infancy, we have discovered that many commensal species produce AI-2 while transient pathogenic species do not. It is possible that these pathogenic species can detect AI-2 from commensal species and coordinate their behavior accordingly. Such a

possibility is supported by our ability to detect AI-2 in saliva and chronic wounds. A model that describes the role of AI-2 in the development of human communities and the expansion of transient pathogenic communities within the human microbiome will be presented.

The role of the Pel polysaccharide in Pseudomonas aeruginosa biofilm development
Matt Parsek, University of Washington, USA

Bacterial extracellular polysaccharides can contribute to the formation of surface-attached communities called biofilms. *Pseudomonas aeruginos*a is a model organism for the study of biofilms and produces three extracellular polysaccharides that have all been implicated in biofilm development, Alginate, Psl and Pel. Significant work has been conducted on the roles of alginate and Psl in biofilm development. However, we know little regarding Pel. In this study, we demonstrate that Pel can serve multiple functions in biofilms. Using a novel assay involving optical tweezers, we demonstrate that Pel is crucial for maintaining cell-to-cell interactions in a PA14 biofilm, serving as a primary structural scaffold for the community. Deletion of *pelB* resulted in a severe biofilm deficiency in the lab strain PA14 but not in PAO1. In PAO1 Psl appears to be the primary biofilm structural exopolysaccharide. Furthermore, we demonstrate Pel plays a second role in biofilms by enhancing resistance to aminoglycoside antibiotics in PA14. This protection occurs only in biofilm populations. We show that expression of the *pel* gene cluster is enhanced on a surface compared to liquid cultures. Thus, we propose that Pel is a capable of playing both a structural and protective role in *P. aeruginosa* biofilms.

Offered Oral Abstracts

Session 1: Microbial communities in disease

Differential response of human keratinocyes to diffusible products from planktonic and biofilm cultures of *Staphylococcus aureus*
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Recent reports have demonstrated that chronic cutaneous wounds are often inhabited by biofilms and this is considered detrimental to wound healing. However, the mechanisms by which biofilms inhibit healing are poorly understood. We performed transcriptional analyses of human keratinocytes after four hours of in vitro exposure to diffusible products formed by biofilm and planktonic cultures of *Staphylococcus aureus*. Genes up-regulated by biofilm-conditioned medium (BCM) relative to planktonic conditioned medium (PCM) included those coding for Interleukin 6 (IL-6) and Interleukin 8 (IL-8). Production of these cytokines was further evaluated using enzyme-linked immunosorbent assay. With a short exposure time to BCM or PCM, HK produced more of these cytokines, in agreement with the transcriptional analysis. However, with longer exposure times (e.g. 24 hours), the HK produced more IL-6 and IL-8 when exposed to PCM relative to BCM. One of the major pathways regulating the expression of these chemokines is the mitogen activated protein kinase (MAPK) cascade. Transcriptional analysis also indicated that several transcription factors involved in the MAPK cascade were upregulated in BCM-treated cells. Using specific inhibitors of MAPK pathways, we found that the MAPK cascade, particularly p38 and ERK pathways, to be more important for IL-6 and IL-8 production for PCM-treated HK relative to BCM-treated HK. Overall, these results suggest that HK may respond to diffusible products from biofilms using different inflammatory pathways.

Polymicrobial, pathogenic biofilms in pediatric adenoids: a culture-independent approach to diagnosing biofilm infections
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Biofilms of pathogenic bacteria are present on the middle ear mucosa of children with chronic otitis media (COM) and these mucosal biofilms may contribute to pathogen persistence and recalcitrance to antibiotic treatment. One hypothesis suggests that the adenoid is a reservoir for pathogenic bacteria in children with COM. To investigate this hypothesis, samples were obtained from 35 children undergoing adenoidectomy for COM or obstructive sleep apnea (OSA). We used a novel, culture-independent molecular diagnostic methodology, the Ibis T5000 Universal Biosensor System to interrogate the microbial diversity within adenoid biopsies, followed by fluorescence in situ hybridization (FISH) and confocal microscopy (CM) to investigate the in situ distribution and organization of pathogens in the adenoids to determine if pathogenic bacteria exhibited criteria characteristic of biofilms. Using this combination we demonstrated that adenoids from both diagnostic groups were colonized with polymicrobial biofilms. *Haemophilus influenzae* was present in more

adenoids from the COM group (P = 0.005), but there was no significant difference between the two patient groups for *Streptococcus pneumonia* or *Staphylococcus aureus*. FISH, lectin binding and antibodies specific for host epithelial cells demonstrated that pathogens were aggregated, surrounded by a carbohydrate matrix, and associated with the epithelium, consistent with criteria for bacterial biofilms. The combination of a broad-based culture independent methodology with FISH to visualize pathogenic biofilms represents a powerful approach towards identifying, and potentially diagnosing, biofilm-associated infections.

The Influence of Selected Autochthonous Bacteria on the Establishment of Methicillin Resistant Staphylococcus aureus and Pseudomonas aeruginosa in a Novel Wound Simulator
Angela Oates, Andrew McBain, University of Manchester, UK

Several variables influence the ecological fate of autochthonous bacteria on human skin, including nutrient availability and colonisation resistance. In this study, a novel wound fine-celled foam (FCF) biofilm reactor was used to determine the ecological fate of MRSA and *Pseudomonas aeruginosa* when introduced to established populations of *Staphylococcus saprophyticus* or *Corynebacterium xerosis* under nutritional conditions broadly reflective of healthy skin and chronic wounds. *S. saprophyticus* and *C. xerosis* were grown on axenically on fine-celled foam substrata and maintained with artificial sweat. Following the establishment of culture equilibrium these were exposed to c. 7.0 Log_{10} CFU/ml of *S. aureus* (MRSA) or *P. aeruginosa* for 20min, rinsed with saline and incubated for a further 24hrs with either artificial sweat or serum. When compared to non-colonised controls, prior colonisation with *S. saprophyticus* resulted in a 99% reduction in MRSA and a 75% reduction in *Pseudomonas aeruginosa* whilst prior colonisation by C. *xerosis* resulted in a significant reduction of 91% in *P. aeruginosa* when grown in artificial sweat. However, when the media was switched to artificial serum MRSA or *P. aeruginosa* populations were not significantly reduced by the established populations of S. *saprophyticus* and *C. xerosis*. **Conclusion:** Colonisation resistance could be simulated in the wound FCF biofilm reactor and the outcome of immigration was markedly influenced by i) the species of established bacterium and ii) nutrient availability.

Session 2: Surface engineering and biofilm tribology

From nanotechnology to microbiology - antibiofilm activity of nanosized magnesium fluoride
Ehud Banin, Aharon Gedanken, Edith Kahana, Sivan Elias, Bar-Ilan University, Israel

The ability of bacteria to develop antibiotic resistance and colonize abiotic surfaces by forming biofilms is a major cause of medical implant-associated infections and results in prolonged hospitalization periods and patient mortality. This raises the urgent need to find novel approaches to inhibit bacterial colonization of surfaces. One approach comes from recent progress in nanotechnology, which offers an opportunity for the discovery of novel compounds with antimicrobial activity as well as the use of "nano-functionalization" surface techniques.

Here we present the antibiofilm activity of metal fluoride nanomaterials. Using microwave synthesis we synthesized MgF2 nanoparticles and demonstrate their ability to inhibit biofilm development of common pathogens. The nanoparticles attach and penetrate into the cells, causing disruption in membrane potential and induce membrane lipid peroxidation. Based on these findings we further explored the possibility of using the MgF2 Nps to coat surfaces and inhibit biofilm formation. A microwave synthesis and coating procedure was utilized to coat glass coupons. The MgF2 coated surfaces effectively restricted biofilm formation of the tested bacteria**.** This study emphasizes the potential of using metal fluoride nanoparticles as a new approach for the design of sterile surface coatings that may be useful for various medical applications.

Antibiofilm coatings developed from a bio-inspired approach
Thomas Blin, Viswas Purohit, Jérôme Leprince, Karine Glinel, Ludovic Galas, Thierry Jouenne, Xavier Laloyaux, Alain Jonas, University of Rouen, France

Biofilms are responsible for many industrial problems such as the clogging or the corrosion of industrial equipments. More dramatically, they also serve as a reservoir for the development of pathogen infections. Therefore, there is a great interest to fabricate materials preventing the formation of biofilms. Various approaches based on the immobilisation of bactericidal substances such as silver or ammonium derivatives, antibiotics or chloride derivatives have been extensively tested. However, they are not completely satisfying regarding their efficiency, their potential toxicity or their role in the emergence of multi-resisting bacteria. Besides these synthetic approaches to protect synthetic surfaces, some living organisms have developed highly efficient strategies to counter bacterial adhesion. For instance, amphibians secrete a thin skin mucus containing antibacterial peptides (AMP's) to protect themselves against bacterial attachment. Here, we present the preparation of antibacterial coatings directly bio-inspired from the amphibian strategy. Macromolecular thin films based on biocompatible poly(ethylene glycol) derived polymers or polysaccharides were grafted onto substrates of various natures and geometries. Then magainin I, an AMP produced by claw frog was grafted onto these macromolecular layers via a hetero-linker allowing the accessibility of the peptide. The antibacterial

properties of the resulting layers were evidenced against various micro-organism such as *L. ivanovii*, *P. aeruginosa* or *E. coli*. Additionally, smart coatings switching their surface properties from bactericidal to cell-repellent with temperature were prepared by grafting the peptide onto a thermoresponsive macromolecular layer. Our strategies should be advantageously adapted to coat various materials or items used in medicine or food industries.

Tribological behaviour of oral mixed biofilms

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The use of dental implants has been increasing even though failures do occur. The presence of wear debris and oral microorganisms can contribute to infections and jeopardize implant integration. The aim of this work was to study the influence of mixed biofilms in the tribological behaviour of commercially pure titanium for dental implants under different concentrations of fluoride. Samples of titanium with two different surface topographies were used. Mixed biofilms of Candida *albicans* and *Streptococcus mutans* were formed on both surfaces at 37 °C in a tryptic soy broth containing mucin, peptone, yeast extract and sucrose. After 8 days, biofilm biomass was analysed by crystal violet staining method. Biofilm biomass was significantly higher for the samples with higher roughness. Some samples with biofilms were analysed under friction (using a force of 100 mN) in an artificial saliva solution (Fusayama) without or with different concentrations of fluoride (30 and 227 ppm). It was verified that the coefficient of friction (COF) decreased in the presence of biofilms. Moreover, samples with more biomass (0.4 µm of roughness) presented the lowest values of COF. Concerning the effect of the presence of fluoride, although there were no significant differences on the COF for 30 ppm, for 227 ppm a transition regimen was observed. These results were confirmed by sample observation under scanning electron microscopy.

In conclusion, it can be highlighted that biofilm formation on dental implants can significantly affect the tribological behaviour of titanium, namely, the presence of biofilms reduces the release of wear debris.

Session 3: Global scale biofilm systems

Utility of Biofilms in Geologic Carbon Sequestration

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Geologic carbon sequestration involves the injection of CO_2 into underground formations including oil beds, deep un-minable coal seams, and deep saline aquifers with temperature and pressure conditions such that CO_2 will likely be in the supercritical state.

Four trapping mechanisms are proposed to play significant roles in the deep geologic sequestration of CO_2: formation trapping, capillary trapping, solubility trapping, and mineral trapping.

Our research has shown that microbial biofilms are capable of enhancing formation trapping, solubility trapping, and mineral trapping under conditions found in deep brine aquifers.

i) We have demonstrated that engineered microbial biofilms are capable of reducing the permeability of rock cores at pressures and temperatures, which would be found in the presence of supercritical CO_2.
ii) The biofilms have been demonstrated to be resistant to supercritical CO_2.
iii) Biofilms precipitate CO_2 in the form of calcium carbonate ($CaCO_3$), which resists dissolution by brine and $scCO_2$.
iv) Microbial activity can increase CO_2 solubilization in brine thus improving solubility trapping.

Pathogen interactions within phylloplane biofilms: importance of the VBNC state

Bill Keevil, Arinder Gill, Nicola Gibbins, Jeremy Webb, Jennifer Warner, University of Southampton, UK

The phylloplane is an essential global ecosystem, experiencing hostile fluctuating environmental stresses, yet host to diverse microbial colonists and plant pathogens forming biofilm. Recently, disease outbreaks caused by fresh produce consumption have turned interest to survival of zoonotic pathogens on the phylloplane; despite strong epidemiological evidence suspected pathogens are frequently undetectable. This study aims to understand the size and spatial distribution of bacterial biofilm communities on the salad phylloplane and assess their role in zoonotic pathogen attachment and survival as viable but nonculturable (VBNC) forms. Spinach or watercress leaves were examined for biofilm and exopolymeric substances (EPS) using EDIC/EF microscopy. Microorganisms were confirmed and quantified using culture, DAPI and BacLight staining. Leaves were also spiked with *Salmonella* Typhimurium, *S.* Thompson or *Escherichia coli* O157:H7, some carrying gfp fluorescent markers, and cell-cell or cell-phylloplane interaction monitored continuously using real time fluorescence imaging. Microcolonies and EPS slime were observed in leaf margins, between margins and around stomata. Spiked cultures of motile *S.* Thompson showed subpopulations with different attachment strategies including directly binding to the leaf surface or to biofilm, and also chemotactic swimming into stomata. *ΔrpoS*

and *ΔcrlΔcsgB* or *flhC* mutant strains lacking curli fimbriae or flagella, respectively, were unable to attach to abiotic polystyrene but only the *flhC* mutant showed reduced attachment on the phylloplane. *E. coli* O157 attached specifically to stomata guard cells in a characteristic pattern. Biofilm communities and pathogens proved highly resistant to oxidative stress, even 100 ppm chlorine, and became VBNC, confirmed using the cell elongation assay.

Biophysical basis for the geometry of coniform biofilms

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Stromatolites may be Earth's oldest macroscopic fossils, however, it remains controversial what, if any, biological processes are recorded in their morphology. Although the biological interpretation for the morphology of these ancient biofilms is often confounded by the influence of sedimentation, conical stromatolites form in the absence of sedimentation and are, therefore, considered to more robust records of biophysical processes. A qualitative similarity between conical stromatolites and modern microbial mats suggests a photosynthetic origin for ancient stromatolites. To better understand ancient fossils, we seek a quantitative relationship between the geometry of conical stromatolites and the biophysical processes controlling their growth. Through a combination of field work, laboratory experiments, and mathematical models, we show that these biofilms are shaped by the diffusion of nutrients. By comparing high spatial resolution (nanoSIMS) measurements of the chemical composition of laboratory and field samples to theoretical predictions, we quantify the competition for nutrients between bacteria. We find that the metabolism of each bacterium is constrained by the macroscopic geometry of the biofilm, thus linking form to physiology. These biofilms are also shaped by the competition for nutrients surrounding the mat. Notably, the organization of a field of stromatolites is set by a diffusive time scale over which structures compete for nutrients. The centimeter-scale spacing between modern and many ancient stromatolites corresponds to a rhythmically fluctuating metabolism with a period of approximately 20 hours. The correspondence between the observed spacing and the day length provides quantitative support for the photosynthetic origin of conical stromatolites throughout geologic time.

Session 4: Community ecology and evolution

Mutability of polyspecies bacterial biofilm community members

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One of the supposed extraordinary biofilm tolerance causes is hypermutagenesis. Different researches confirm association of bacterial hypermutability with their adaptation to antimicrobial agents. Furthermore, there are reports about resistance development stimulation by usual environmental factors. Biofilm growth is accompanied with high concentration of quorum sensing signals and metabolic byproducts and low concentration of nutrients. Such stress factors activate different mechanisms of temporary mutageneses which can produce stable hereditary mutators. In the course of our work we carried out: 1) isolation and identification of the swimming pool bacterial biofilm community members; 2) isolates' mutation rates determination by the fluctuation method ; 3) estimation of species' mutability variances and correlations with biocide resistance and biofilm forming ability. Basic microbial community included such species as Pseudomonas aeruginosa, Pseudomonas alcaligenes, Acinetobacter lwoffii, Ralstonia pickettii, Flavobacterium indologenes, Cytophaga aquatilis, Bacillus cereus. The results demonstrate 1) positive correlation between resistance to usually used biocides and average mutation rate of different bacterial species; 2) low-scale (B. cereus, P. aeruginosa, P. alcaligenes, A. lwoffii) and high-scale (P. aeruginosa, B. cereus) intraspecies mutation rates heterogeneity; 3) bacterial populations clustering on the base of mutation rates and biofilm forming abilities; 4) stability of isolates' mutability differences. Results confirm our suggestions about existing of week and more strong mutator alleles mix in bacterial populations and association of such diversification with the biofilm mode of growth.

The significance of oxygen gradients on the evolution of biofilm formation by *Pseudomonas fluorescens* SBW25 in static liquid microcosms

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Adaptive radiation of *Pseudomonas fluorescens* SBW25 results in the emergence of the Wrinkly Spreader (WS) which produces a biofilm colonising the air-liquid (A-L) interface of static microcosms. These have opposing O_2 and nutrient gradients which may drive the radiation of bacterial populations and the emergence of novel, adaptive genotypes, but until now the significance of O_2 availability has not been explicitly examined. We have investigated the establishment of O_2 gradients by SBW25 colonists as well as growth and biofilm–formation under high and low–O_2 conditions. A significant change in the O_2 profile was observed within 20 min of inoculation with SBW25 colonists and before significant population growth had occurred. O_2 was rapidly depleted from the liquid column, and the transition zone between high and low–O_2 regions moved to within

200 µm of the surface after 5 days. SBW25 grew nine times faster in high–O_2 conditions which also favoured the emergence of WS-like genotypes, indicating that O_2 availability was growth-limiting in this experimental system. There was a fitness advantage to biofilm-formation under high but not low-O_2 conditions, suggesting that the cost of biofilm production could only be compensated when O_2 levels above the A-L interface were high. These findings demonstrate that O_2 availability and value can explain WS success in evolving populations in static microcosms. WS biofilms were also bisected by the transition zone, producing high and low–O_2 environments which may enable further diversification and succession to occur in these populations.

Quantification of food web interactions on semi-natural biofilms.

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Protozoa-bacteria interactions on biofilms were mostly studied under well defined laboratory conditions. Insights on food web interactions in natural biofilm communities are hardly available. We investigated ciliate-flagellate-bacteria interactions within semi-natural biofilms in flow cells connected as a river bypass system with the help of video microscopy. Individuals of different species (the flagellates *Neobodo designis*, *Rhynchomonas nasuta*, *Planomonas* sp. and the ciliate *Acineria* sp.) were continuously filmed and the videos analysed regarding the food preferences (microcolonies vs. single bacterial cells), individual grazing rates, handling time, search time and loss rates due to predation.

The three flagellates species preferred single bacteria cells within the biofilm. Bacterial microcolonies were attacked by the flagellates but bacteria from within the microcolonies were not ingested. Furthermore, we could show that different food uptake strategies apply for the different flagellate species. While *N. designis* and *R.nasuta* show preferences for loosely attached bacteria, *Planomonas* sp. preferred firmly attached bacteria. The ciliate *Acineria* sp. grazed on benthivorous gliding flagellates rather than on planktivorous sessile ones and thus had a strong impact on the taxonomic and functional composition of the protozoan community within the biofilm. Our method of applying video microscopy in combination with experimental flow cell set-ups provide direct insights into biofilm food webs.

Session 5: Novel biotechnology and bioengineering

Study of antibiotics diffusion-reaction in biofilms by dynamical fluorescence (FLIM, FCS)

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In natural environments, bacteria grow on biotics surfaces (mucosa, tissues...) or abiotic surfaces (medical equipments, food surfaces workshops...). They are usually embedded in a matrix of exopolymers which leads to communities organized in heterogeneous structures, the biofilms. When these biostructures are composed of pathogenic bacteria, they are frequently tolerant to the action of antibiotics and, therefore, extremely difficult to eradicate. Currently, the mechanisms involved in the development of biofilm resistance to antimicrobial agents are not fully elucidated. To better understand these processes and improve the inactivation of pathogenic biofilms, it is now necessary to understand the dynamics of diffusion-reaction of antimicrobial molecules within them, at the molecular level.

For this purpose, we present here the first local measurements of the diffusion-reaction of fluorescent-labelled vancomycin antibiotic in *Staphylococcus aureus* biofilms, structures of medical interest. Molecular diffusion-reaction measurements were performed by a non-invasive and sensitive photophysical technique, the fluorescence correlation spectroscopy (FCS). In addition, *in situ* assessments of fluorescence lifetime (FLIM) were achieved on target antibiotics in suspension or biofilms to highlight the interactions between these molecules and the organic constituents of biofilms.

We illustrate that both the FCS and FLIM techniques offer new methodological perspectives for the mechanistic understanding, at the molecular level, of cellular reactivity and of the biofilms tolerance towards antimicrobial agents.

Transcriptomic studies of putative gene clusters involved in biofilm formation & EPS biosynthesis of *Acidithiobacillus ferrooxidans*

Söeren Bellenberg, Wolfgang Sand, Mario Vera, University of Duisburg-Essen, Germany

Bioleaching is the dissolution of metal sulfides, such as pyrite (FeS_2) by bacterially-driven oxidation processes. Selective attachment and biofilm formation on sulfidic mineral surfaces by *Acidithiobacillus ferrooxidans* is important for this process. Uronic acid residues present in the Extracellular Polymeric Substances (EPS) bind ferric ions, which are the main oxidative agents for pyrite dissolution. Consequently, the EPS-matrix is a reaction space, coupling the bacterial respiratory chain with the chemical oxidation of pyrite. EPS composition of iron(II)- and pyrite grown cells is similar, but more EPS are produced by cells attached to solid substrates. Cells grown on elemental sulfur exhibit a different EPS composition, with stronger hydrophobic properties,

leading to a diminished bacterial attachment on pyrite. Bioinformatic analysis of *At. ferrooxidans* complete genome sequence revealed at least two gene clusters probably involved in capsular EPS production and biofilm formation. In order to understand the molecular and physiological adaptations of this bacterium to a biofilm-lifestyle we have compared their expression patterns using RNA extracted from cells grown on different energy sources by quantitative RT-PCR. Preliminary results show substrate specific differences in gene expression patterns. These studies have also been extended to attached and planktonic cell subpopulations in pyrite cultures at different times of the biofilm formation process.

Effects of different acidophilic, moderately thermophilic microorganisms on attachment and biofilm formation on pyrite

Nanni Noel, Bianca M. Florian, Mario Vera, Wolfgang Sand, University of Duisburg-Essen, Germany

Bioleaching is the dissolution of metal sulfides by bacterial oxidation processes. Attachment of leaching organisms to mineral surfaces enhances the dissolution. To prevent acid mine drainage (AMD) or to optimize industrial bioleaching, attachment and the accompanying microbial leaching efficiency has to be optimized. Here the interactions between moderately thermophilic bacteria in bioleaching and biofilm formation on pyrite were studied. We are dealing with three open questions: "Which member of a mixed culture is the first colonizer of a pyrite surface?" "Which member needs a precolonization by others?" "Have homoserine lactones (AHLs) effects on moderately thermophiles?"
Strains of *Acidithiobacillus* and *Leptospirillum* were tested. To investigate attachment and leaching behavior on pyrite standardized tests were used with and without addition of different AHLs. For visualization a combination of Atomic Force- (AFM) with Epifluorescence Microscopy (EFM) was used plus DAPI staining and FISH. Attachment experiments with mixed cultures of *Leptospirillum ferriphilum* and *Acidithiobacillus caldus* indicated that attachment is stimulated in mixed cultures as compared to pure ones. Visualization showed that in mixed cultures *L. ferriphilum* is responsible for primary attachment, whereas *A. caldus* stimulates this process. Furthermore, *L. ferriphilum* responds to different AHLs. Depending on the type of AHL, attachment and leaching rates either increased or decreased. Thus, new strategies may be developed to control bioleaching.

Session 6: Structural dynamics and emergent properties of biofilms

Biofilm physical properties from the inside

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Many molecular factors associated with bacterial adhesion and biofilm development have been described using extensive molecular genetics analyses. This has significantly improved our understanding of these sessile bacterial organisations. Yet, much remain to be done and new approaches complementary to the ongoing molecular studies will certainly help making progress. We address in the group the question of the reciprocal influence of biofilm physical properties and biochemical molecular events. Up to now, no relationship has been established between the measured parameters and biofilm molecular properties. Previous studies in the field did not take into account the high heterogeneity of the bacterial architecture, providing averaged values, which probably strongly weakened the ability of the obtained data to report relevant biological functioning of the biofilm.

We will show here the first step of this investigation, i.e. the development of appropriate methodologies to measure the relevant parameters describing the biofilm physical properties. We propose here an original methodological approach based on the use of magnetic colloids to probe biofilm biophysics locally from the inside. The principle consists inserting exogenous magnetic micro-colloids into the 3-dimensional bacterial material either from the initial steps of the colonization or during the biofilm growth at various steps of the development. Thanks to their magnetic properties, these objects will be remotely actuated using adapted magnetic device. We will describe here the detail of the experiment and show the first measurements of the local rheological parameters of an E. coli biofilm.

We will then discuss how these data could help to better understand biofilm internal dynamics.

***Bacillus subtilis* biofilms on immersed surfaces: there is a life below the pellicle**

Romain Briandet[1] ,Stéphane Aymerich[1] ,Vincent Thomas[2] ,Florence Dubois-Brissonnet[3], Dominique Le Coq[1], Arnaud Bridier, [1]INRA, France, [2]Steris SA, France, [3]AgroParisTech, France

Bacillus subtilis is a Gram-positive bacterium which serves as a model for the study of biofilm formation and development since a decade. Most of the data on *B.subtilis* biofilm come from studies on the formation of a pellicle at the air-liquid interface or from complex macrocolonies developing on nutritive agar and only few studies have focused on surface-associated submerged biofilm models. In this work, we better characterized biofilm formation and structure on immersed surface by *B. subtilis*.
Biofilms of wild-type strains and strains carrying mutations known to affect pellicle and macrocolony formation

were grown for 48h in microtiter plate and observed using confocal laser scanning microscopy. Results revealed that some wild–type strains exhibited dramatic rising "beanstalk-like" structures that can reach more than hundred microns of thickness. Different mutations seemed to deeply affect biofilm architecture revealing the requirement of some genes in surface-associated biofilm formation. To determine the potential role of the three-dimensional structures in tolerance to disinfectants, the effectiveness of three biocides (peracetic acid, chloride benzalkonium and O-phthalaldehyde) was evaluated using time lapse confocal laser scanning microscopy and fluorescent viability markers on two strains showing different biofilm architecture. We found an overall resistance of biofilms in comparison to planktonic cells to the three disinfectants and that strain which produced important rising structure exhibited markedly resistance to peracetic acid compared to strain producing more flat biofilm.

Results obtained demonstrated the ability of *B. subtilis* to form biofilms on immersed surfaces and especially to form important 'beanstalk-like" structures may be involved in community resistance to disinfection.

Role of eDNA in biofilms of *Shewanella oneidensis* MR-1
Kai Thormann, Julia Gödeke, Kristina Paul

Shewanella oneidensis MR-1 is capable of forming highly structured surface-attached communities. By DNaseI treatments we demonstrate that extracellular DNA (eDNA) is required through all stages of biofilm formation and serves as a major structural component under static and hydrodynamic conditions. We determined whether eDNA might be released through lysis of a cellular subpopulation mediated by prophages of which *S. oneidensis* MR-1 harbors three: LambdaSO, MuSO1, and MuSO2. Mutant analyses and infection studies revealed that all three prophages individually lead to cell lysis. However, only LambdaSO and MuSO2 form infectious phage particles. Phage release and cell lysis already occurs during early stages of static incubation, a mutant devoid of the prophages was significantly less prone to lysis. A phage-less mutant was severely deficient in biofilm formation through all stages of development, and three-dimensional growth occurred independently of eDNA as a structural component. Thus, we suggest that in *S. oneidensis* MR-1 a release of crucial biofilm-promoting factors, in particular eDNA, occurs predominantly by prophage-mediated lysis of a cellular subpopulation. In addition, we identified a surface-associated nuclease that is involved in dispersal and spreading of the biofilm.

Session 7: Modulation of biofilm communities

Identification of Small Molecule Inhibitors of Biofilm Formation by Salmonella Typhimurium and Pseudomonsa aeruginosa and Investigation of Their Mode of Action
Hans Steenackers, Jeremy Levin, Sigrid De Keersmaecker, Dirk De Vos, Jozef Vanderleyden, Catholic University of Leuven, Belgium

A major difficulty in the control of *Salmonella* and *Pseudomonas* infections is the fact that these bacteria can form biofilms, in which they are protected against the influence of antibiotics, disinfectants and the immune system. Therefore, the prevention of biofilms could be an effective way to restrict the spread of *Salmonella* and *Pseudomonas.*

We chemically synthesized several libraries of natural product analogues and tested their influence on the biofilm formation of *Salmonella* Typhimurium and *Pseudomonas aeruginosa* by using a high throughput biofilm assay. In this way we identified different classes of potent biofilm inhibitors for which we delineated structure activity relationships. One of these classes are the brominated furanones, which were originally isolated from the seaweed *Delisea pulchra* and which are known to inhibit biofilm formation and quorum sensing in several other pathogens.[1] We investigated the mode of action of the different classes of biofilm inhibitors by using a broad scope of techniques such as gene reporter fusions, transcriptome analysis, qRT-PCR, mutant analysis and phenotypical assays, which has led to the identification of the target receptor of some of the compound classes. We are currently using our knowledge of the target receptor and the structure activity relationship of the biofilm inhibitors to identify new classes of biofilm inhibitors via a number of receptor-based and ligand-based computational techniques.

Conclusively, we identified several classes of potent *Salmonella* and *Pseudomonas* biofilm inhibitors and made progress in the elucidation of their mode of action.

Environmental triggers and intracellular signaling of biofilm dispersal
Nicolas Barraud[1], Staffan Kjelleberg[1], Alain Filloux[2], Joana Moscoso[2]
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Biofilm dispersal is a crucial event that determines the lifestyle of bacteria and strategies to induce dispersal are of interest for their potential to prevent biofilms and biofilm-related infections. Previous studies revealed an important role for the signaling molecule nitric oxide (NO), in inducing the switch from a sessile to a free-swimming, planktonic mode of growth in various microorganisms. Moreover, the dispersal response to low,

non-toxic concentrations of NO was found to involve the secondary messenger cyclic di-GMP in *Pseudomonas aeruginosa* biofilms, suggesting that NO signaling is part of a global regulatory network that controls lifestyle transitions in bacteria. To further investigate the regulation of biofilm dispersal, mature *P. aeruginosa* biofilms grown in continuous-flow culture microfermentors were exposed to a range of environmental stimuli, including NO and NO-related compounds, changes in oxygen and nutrient levels and other putative dispersal factors. The results show that, when induced biofilm dispersal was characterised by a rapid and massive release of bacterial cells in the bulk liquid flow. Addition of NO, nitrite at neutral pH but not at acidic pH, decreases in nutrient (glucose) and oxygen levels, as well as increases in temperature were found to be potent inducers of biofilm dispersal. Thus biofilm dispersal appears to be finely regulated in response to both physiological and environmental cues. These data suggest that modulation of environmental factors to affect key regulatory pathways may allow for novel and improved strategies to control biofilms in many industrial and clinical settings.

Analysis of Multiple Hypotheses for Antibiotic Tolerance of *Staphylococcus epidermidis* in Biofilms
Phil Stewart, Rani Surani, Frank Roe, William Davison, Montana State University, USA

Colony biofilms of *Staphylococcus epidermidis* were protected from killing by ampicillin, rifampin, or ciprofloxacin in comparison to planktonic cells of the same strain. This simple in vitro system was used to test, in parallel, five hypotheses to explain biofilm antibiotic tolerance: 1) poor antibiotic penetration, 2) poor killing in anoxic regions of the biofilm, 3) survival of infrequent persister cells, 4) poor killing of inactive cells, which rapidly initiate growth after treatment, and 5) poor killing of both active and inactive cells, the latter which mostly remain dormant after treatment. These mechanisms were tested experimentally by visualizing spatial patterns of DNA synthetic activity before and after treatment of biofilm with an antibiotic. Replicating cells were localized by 5´-bromodeoxyuridine labeling followed by immunofluorescent detection of brominated DNA. In biofilms not exposed to antibiotics, a distinct, heterogeneous pattern of DNA synthesis was observed in which two bands of activity, one along the air interface and the other along the nutrient interface, were separated by an interior stratum of low activity. In antibiotic-treated biofilms, the two bands of DNA synthetic activity were suppressed to a similar degree. A steep oxygen concentration gradient in the biofilm was measured using a microelectrode. All three antibiotics penetrated biofilm and acted as effectively on anaerobic as aerobic cells. The data did not support the persister hypothesis. The hypothesis most consistent with the experimental results was weak killing of both active and inactive cells in the biofilm followed by slow resuscitation of dormant bacteria.

Session 8: Signalling and communication in biofilms

A Novel Cell-To-Cell Communication Molecule, cis-2-Decenoic Acid, Acts To Induce Bacteria To Transition From A Biofilm Lifestyle To An Active Disseminating Lifestyle
David Davies, Binghamton University, USA

Cis-2-decenoic acid has been shown to act as a cell-to-cell signaling molecule responsible for inducing biofilm dispersion in Gram-negative bacteria, Gram-positive bacteria and fungi. Recently, we have shown that this signaling system is responsible for a number of effects that lead to the transition from a sessile mode of existence to an active disseminating lifestyle. Induction of dispersion of *P. aeruginosa* biofilms has been demonstrated to result in the release of degradative enzymes involved in hydrolysis of biofilm protein, nucleic acids and polysaccharides. Dispersed bacteria were observed to become motile and alter expression of virulence determinants, including elevated expression of pyochelin and exotoxin T. In addition, *cis*-2-decenoic acid led to increased dissemination of infection in a lettuce virulence model. The altered phenotype induced by *cis*-2-decenoic acid has also been demonstrated to result in enhanced susceptibility of bacteria to antimicrobial agents. Taken together, our results demonstrate that *cis*-2-decenoic acid acts to induce bacteria to transition from a biofilm phenotype typically associated with chronic infections (in which bacteria show a reduction in metabolic activity, growth and motility, and an enhanced resistance to antimicrobial agents), to bacteria with a phenotype more typically associated with planktonic growth or acute phase infections (as characterized by enhanced growth, activity and susceptibility to antimicrobial agents). These observations suggest that treatment with *cis*-2-decenoic acid alone or in combination with antibacterial agents, should have a significant enhancing effect on the killing of biofilm-associated bacteria.

Remote control of antibiotic resistance within bacterial communities
Jean-Marc Ghigo, Institut Pasteur, France

The development bacterial communities is associated with profound but ill-understood physiology changes, including a characteristic reversible increased tolerance to antibiotics. There are now compelling evidence that so-called secondary metabolites produced at late stages of microbial growth contribute to intra-or inter-species communication, a phenomenon suspected to be critical to how bacterial populations colonize their environment. Among the wealth of small molecules produced in bacterial communities, we identified a new widespread signaling system that modulates community level antibiotic resistance. The contribution of this

new type of chemical communication to transient, non-inherited bacterial antibiotic resistance displayed by high cell density bacterial populations such as biofilms will be discussed.

Denitrification and nitric oxide production in laboratory, *in vivo* and *ex vivo* biofilms of the oral cavity and nasopharynx

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In the context of pathogenicity *Pseudomonas aeruginosa* is generally considered an aerobic organism. However, It can also denitrify and indirect evidence suggests that *P. aeruginosa* was denitrifying in the anaerobic mucus of the CF lung (Yoon et al. 2002). Respiration of nitrate (or nitrite) is important because it allows *P. aeruginosa* to proliferate in the absence of oxygen and also because NO is produced in the denitrification pathway. NO is toxic in mM concentrations and is used by phagocytes to more effectively kill bacteria. NO is also a biofilm dispersal signal (Barraud et al. 2006). Our objectives were to determine the relative contribution of denitrification and oxygen respiration to the energy balance of *P. aeruginosa* microcolonies and to determine the concentration of NO that could accumulate within oral biofilms.

We used dissolved oxygen (DO), NO_3--, N_2O and NO microelectrodes to measure profiles in *P. aeruginosa* biofilm colonies grown from clinical and environmental isolates in aerated medium with nitrate. We also measured profiles within the *P. aeruginosa* infected middle ear of chinchillas in the otitis media model, as well as in human tonsiloliths. Finally, we measured denitrification and NO production in dental biofilms in response to stimulation with physiological concentrations of nitrate.

Denitrification can be physiologically important for *P. aeruginosa* biofilms, even in oxygenated environments, and was also demonstrated in the infected chinchilla ear and dental plaques. NO accumulated to 0.2 µM in human plaque which is in the signalling range for bacteria and human cells but below toxic levels.

Session 9: Biofilm development: A multidisciplinary approach

dATP/ATP in biofilm formation and pathogenesis

Chuanwu Xi, Jianfeng Wu, University of Michigan, USA

Signaling by extracellular adenosine 5'-triphosphase (eATP) is very common for cell-to-cell communication in many basic pathogenesis. Rapid release of ATP into extracellular from distressed or injured host cells due to pathogens or other etiological factors, being considered as a "danger signal", activates host innate immune system to remove pathogens. However, little is known about how bacteria respond to this "danger signal". Here we report that extracellular dATP/ATP can stimulate bacterial adhesion and biofilm formation via increased cell lysis and eDNA release. Furthermore, we confirmed that extracellular dATP/ATP also stimulates bacterial adherence *in vitro* to human bronchial epithelial cells. These data suggest that bacteria also sense extracellular dATP/ATP as a signal of "danger" and form biofilms to protect them for host innate immunity. This study reveals a very important and unrecognized phenomenon that both bacteria and host cells can respond to a common important signal molecule and fight against each other, which very likely lead to development of a novel will approach to prevent bacterial infections and to treat infection-related human diseases.

Regulatory systems required for biofilm development and the maintenance of the planktonic mode of growth

Karin Sauer, Olga Petrova, Binghamton University, USA

Biofilms are complex communities of microorganisms encased in a matrix and attached to surfaces. While biofilm formation has long been considered a developmental process, little is known about the underlying regulatory events that lead to the formation of biofilms. Furthermore, no biofilm-specific genes have been identified that are part of hierarchically ordered pathways dedicated to controlling transition through biofilm formation stages. Here we demonstrate that by mapping the phosphoproteome over the course of biofilm development, we identified three novel two-component regulatory systems (BfiSR, BfmSR, MifSR) that were required for the development and maturation of *P. aeruginosa* biofilms. Activation/phosphorylation of these regulatory systems occurred in a sequential manner. Inactivation arrested biofilm formation at three distinct developmental stages as indicated by analyses of biofilm architecture, and protein and phosphoprotein patterns, without affecting planktonic growth, motility, polysaccharide production, or initial attachment, while discontinuation of expression after biofilms had already matured, resulted in disaggregation/collapse of biofilms. Moreover, mapping of the phosphoproteome resulted in the identification of planktonic-specific regulatory proteins (PlkR) essential for the maintenance of the free swimming mode of growth. Inactivation did not affect growth but altered attachment, Psl production, and motility, while overexpression coincided with biofilms displaying dispersion events. Our data thus indicate the existence of a previously unidentified regulatory program dedicated to controlling stage-specific biofilm formation once *P. aeruginosa* cells have committed to a surface associated lifestyle. Our results further indicate that not only biofilm formation but also the free swimming mode of growth, is highly regulated.

Growth and development of 3-dimensional structures in biofilms and cancer - an integrated approach
Samuel Collins, Jeremy Blaydes, Jeremy Webb, University of Southampton, UK

Recently we have proposed that biofilm microcolonies can form through a process of clonal expansion involving a succession of mutational events. Microcolony-specific increases in mutation frequency have been detected in *Pseudomonas aeruginosa* biofilms and strains defective in DNA mismatch repair (MMR) exhibit enhanced microcolony growth. These data suggest that microcolonies may represent important foci for mutation and evolution within biofilms and that microcolony-based growth can involve mutation and subsequent selection of mutants better adapted for growth in crowded environments. This model for biofilm growth is analogous to mutation selection that occurs during neoplastic progression and it is argued that 3-dimensional structural development in bacterial biofilm and malignant tumour foci involve fundamentally similar processes.

Multiple cancer cell lines form microcolony-like structures - multicellular tumour spheroids (MCTS) when grown in surface-associated culture. Analogous to bacterial microcolonies, spheroids are formed by only a small subpopulation of cells. The processes responsible for this differentiation are poorly understood. Similar to microcolony growth genetic instability and mutation selection are proposed to be involved in spheroid formation. Using a murine neuroblastoma N2a model we report that the expression of the DNA MMR genes MLH1 and PMS2 are significantly reduced in spheroid structures and that siRNA silencing of these genes enhances both MCTS initiation and expansion. Thus in parallel with our biofilm microcolony experiments, impaired DNA MMR mechanisms can contribute to tumour initiation and progression in N2a. This integrated research aims to unify experimental approaches to the development of 3-dimensional structures in bacterial biofilms and malignant tumour foci.

Poster Abstracts

Theme: Microbial communities in disease

Adhesion abilities of Enterococcus faecium isolates under different physiological states
Clara Extremina, Ana Isabel Agular, Luisa Costa, Ana Freitas, Antonio Pedro Fonseca, and Luisa Peixe, University of Porto, Portugal

Number: 1
Significance and objectives: *Enterococcus faecium* (Efc) have become one of the most frequent causes of acquired nosocomial infections worldwide, mainly associated to CC17 clonal complex isolates. Ability of these clones to adhere to biotic and abiotic surfaces have been argue as advantage features, being studies conducted to evaluate these characteristics usually performed using stationary growth phase cells. *The aim of this work was to evaluate the interference in the enterococci adherence process of their growing phase (exponential and stationary) using a set of isolates that includes representatives epidemic clones.*

Methods and results: Adhesion (2h; under orbital shear stress) of 15 *Efc* isolates of different origins, being 7 of CC17 and presenting *esp* gene,was evaluated, under exponential (4h) and stationary (12h) phase using a microtitre-plate assay.Adhesion abilities were scored as non-adherent, weak, moderate and strong. The *E. faecalis* ATCC 29212 was used as control strain. In general there are no differences in adhesion abilities under different physiological states, although adhesion experiments show heterogeneity within the different *Efc* isolates.

Conclusions: Since there are no reduced efficiency in terms of adhesion abilities of stationary phase cells as compared with the exponential phase cells these group of isolates can persist more under starvation conditions, thus having an important effect on the outcome of the antimicrobial treatments. This work supports the need for further biofilm formation studies in order to understand the mechanisms that are behind the persistence of these *Efc* isolates.

Effect of Biomaterials surface properties on Candida albicans biofilm formation and growth patterns
Vishnu Agarwal, Priyanka Lal and Vikas Pruthi, India

Number: 2
Significance and objectives: Candida albicans biofilms are matter of serious concern especially in implant associated infections. These infections are believed to be caused by the introduction of yeast during the implantation or through blood stream infections onto the surface of the newly inserted device and are responsible for significant morbidity and mortality. The major problem associated with cure is complex nature of biofilm, uncertain and undefined growth pattern, exopolysaccharide production and drug resistance. The biofilm growth patterns depend not only to microbial factors but also on surface properties on which biofilm is growing. The present study gives a brief outline that how biofilm growth pattern affected with surfaces of different properties.

Methods and results: Different polymer surfaces like polyvinylchloride, polypropylene, silicone rubber and polystyrene was taken. Biofilm was developed by standard methods and quantification was done using XTT reduction assay. The biofilms was studied under CLSM after staining with FITC-ConA and PI for EPS production and sessile cell death.

The result showed difference in growth pattern of C. albicans biofilms in each polymer surface with difference surface properties in term of cell no and EPS production on different layers of biofilms. Further x-z analysis showed difference in biofilm-depth for each biomaterial. Results based on above studies, suggested dependency of biofilm on surface properties.

Conclusion: Surface properties play an important role in biofilm formation not only in terms of biofilm development but also towards biofilm growth pattern.

Novel Antimicrobials against *Pseudomonas aeruginosa*
Karla Mettrick[1]*and David Reid*[2]
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[2] *Queensland Institute of Medical Research, Australia*

Number: 3
The lungs of most cystic fibrosis (CF) patients become chronically infected with the pathogenic bacterium *Pseudomonas aeruginosa* leading to reduced life expectancy. Within the anaerobic/microaerophilic environment of the CF lung, *P. aeruginosa* forms biofilms that increase resistance to antibiotics making the bacterium impossible to eradicate. New therapeutics to combat *P. aeruginosa* infection are currently being investigated and there is evidence that iron chelation may disrupt biofilm formation and increase antibiotic efficacy. This disruption may be the key to successful treatment of *P. aeruginosa* infection. This study aimed to

further investigate the effects of an iron-chelator on *P. aeruginosa* growth, virulence and biofilm formation/ disruption.

The activity of the iron-chelator HBDA in combination with the antibiotics tobramycin, ceftazidime, azithromycin and colistin was studied for the effect on growth and biofilm formation/disruption. Assays were undertaken with a laboratory strain of *P. aeruginosa* (PAO1) and two CF clinical isolates; PA605, an isolate obtained from a child with recently acquired infection, and AES(III), an isolate from an adult with established long-term infection. qRT-PCR was used to determine the effect of the treatments on quorum sensing and virulence.

This research found that the iron chelator had variable effects on the three *P. aeruginosa* strains, but significantly decreased growth and biofilm formation through an iron-dependent manner, particularly under anaerobic conditions. The chelator significantly increased the efficacy of antibiotics with colistin and HBDA being the most efficacious combination. The effects on iron-related gene expression will be discussed further.

Assessment of Genes Associated with *Streptococcus mutans* Biofilm Morphology

Farzaneh Hosseini, Jamileh Nourozi, Maryam Ghavam Shirazi and Farzaneh Hosseni, Islamic Azad University, Iran

Number: 4

Significance and objectives

Streptococcus mutans, a biofilm-forming bacterium considered to be the primary etiological agent of human dental caries, possesses a variety of abilities to colonize tooth surfaces. *S. mutans*, under certain conditions, is numerically significant in cariogenic biofilms and forms biofilms with other organisms in the oral cavity. It seems differential assessment of biofilm-associated genes in clinical strains may provide useful information for understanding the morphological development of streptococcal biofilm, as well as molecular studying.

Methods and results

In the present study, 50 samples were collected from dental caries and they transferred to laboratory by transport medium. Different dilution of samples was prepared and inoculated them to specific medium TYCSB and non specific ETSA for isolation of *S.mutans*. Following cultivation in a tryptic soy broth with 0.25% sucrose, formation of biofilm was initiated. Evaluation of this formation was followed by Scanning Electron Microscope. Observations of maximum and minimum optical dansity of biofIms were selected for studying of *glrA* genes by PCR.

Conclusion

The *S. mutans* isolates showed different abilities to form biofilms on polystyrene surfaces in semidefined minimal medium cultures. Results showed that approximately most of the genes were differentially expressed in the strains. Analyses of the *glrA*-deficient strains and PCR confirmed the role of the gene in biofilm formation. Also the structure of biofilm has been quantified by SEM and differences in structure were seen. Studies of the cellular functions that are modified during the cellular transition from the planktonic to the biofilm state were considerable in progress.

In vitro activity of temocillin against planktonic and sessile Burkholderia cepacia complex bacteria

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Number: 5

Significance and objectives: Burkholderia cepacia complex (Bcc) bacteria are opportunistic pathogens which are difficult to eradicate because of their innate resistance and their capacity to form biofilms. The goal of the present study was to evaluate the bacteriostatic and bactericidal effects of temocillin on planktonic and sessile Bcc bacteria. Methods and results: 38 strains belonging to 17 Bcc species were tested. The determination of minimal inhibitory concentrations (MICs, planktonic) and minimal bactericidal concentrations (MBCs, planktonic) was based on the EUCAST broth microdilution method. Minimal biofilm inhibitory concentrations (MBICs) were determined using a resazurin-based viability staining. To determine the minimal biofilm eradicating concentration (MBEC), sessile cultures were exposed to temocillin (10 x MIC) for 24h. Results were obtained by plate count methods. 70.5% of the strains were susceptible when grown planktonically. B. pyrrocinia and B. arboris strains had the lowest MICs, while B. multivorans LMG 18822, B. cenocepacia LMG 16656 and B. latens LMG 24264 had MICs of 64 µg/ml, 256 µg/ml and >1064 µg/ml respectively. The MBCs were 2 to 16 times higher than the MICs but were still in the range of achievable plasma peak concentrations. No significant differences in resistance between exponentially grown planktonic cultures and young biofilms were observed. When grown in a biofilm, 61.7% of the strains were susceptible. After treatment with a concentration of 10 x MIC only a minor reduction was seen in the number of sessile cells, indicating a limited bactericidal effect on biofilms. **Conclusion:** Temocillin has a good bacteriostatic in vitro effect on planktonic and sessile cells, but seems of limited use to eradicate biofilms.

Analysis of the biofilm-associated microflora on endotracheal tubes and their role in ventilator-associated pneumonia

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Number: 6

Patients receiving mechanical ventilation are at increased risk for 'ventilator-associated pneumonia' (VAP). The placement of an endotracheal tube (ET) is considered an important risk factor as it allows the entry of bacteria in the lungs. The ET itself is also colonized by bacteria, forming a biofilm in the ET lumen.
In the present study, we characterized the microbial populations of ET biofilms and surveillance cultures (throat, nose and sputum samples) obtained from patients from the intensive care unit of the Ghent University Hospital by means of culture-dependent (differential growth media; Gram-staining; conventional microbiological tests; 16S rRNA gene sequencing) and culture-independent techniques (construction of clone libraries of the 16S rRNA genes). Also, the bacterial diversity was evaluated by pyrosequencing of the 16S rRNA genes using the Genome Sequencer FLX System (Roche). Finally, the antimicrobial resistance of isolated bacteria and consortia has been determined.

The first results confirmed the presence of several potentially pathogenic bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes*) but also indicate that the diversity is larger than initially thought, as e.g. *Empedobacter brevis*, *Photobacterium damselae*, *Bergeyella zoohelcum* were detected (organisms not thought to be associated with VAP). In addition, many ET biofilms contained MRSA and extended spectrum b-lactamase positive *Enterobacteriaceae*.

Effect of sanitizers on biofilm of soft rot causing *Erwinia carotovora subsp. carotovora*

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Number: 7

Erwinia carotovora subsp. carotovora (Ecc) is the most economically important bacterial soft rot causing plant pathogen of potato and various other vegetables and fruits which are responsible for severe losses in agricultural production during both vegetative period and storage. Contaminated surfaces of equipments, transport vehicles, and storage that come in contact with fresh produce is known to be an important means of spreading the bacteria among vegetables and fruits. Efficient sanitization of equipment and other contaminated surfaces may be compromised if the soft-rotting Ecc occur as adherent to them in the biofilm metabolic state. Such bacterial cells are usually less susceptible to antibiotics and biocides than those in planktonic state. Studies were initiated to assess the effect of sanitizers on cell adhesion, susceptibility and biofilm formation of Ecc. A static adhesion plate assay was used to quantify bacterial adhesion and biofilm formation to polystyrene surfaces, where Ecc was found to form biofilms efficiently. Ecc biofilms were less sensitive than planktonic cells to sanitizers such as analytical copper and different organic acids. Since recommendations for sanitization in eliminating harmful microorganisms from fresh produce is largely based on experiments using planktonic cell suspensions, our results suggest that the efficacy of sanitizers may need to be re-evaluated and in this context the studies on effect of sanitizers on biofilm of Ecc become significant.

Studying transport of nanoparticles in *Burkholderia* biofilms: towards improved drug delivery systems for the cystic fibrosis lung

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Number: 8

Increased morbidity and reduced survival is observed when biofilms of bacteria belonging to the *Burkholderia cepacia* complex (*Bcc*) are formed in the lungs of cystic fibrosis patients. Although *Bcc* bacteria are sensitive to tobramycin, treatment rarely leads to eradication of these infections. In this work, the transport of model nanospheres of different sizes and surface charges is investigated in *Burkholderia multivorans* biofilms with the aim of developing a suitable nanoscopic delivery system for delivering a local high dose of tobramycin into the biofilm clusters.

Transport of colloidal solutions in physiological water of 100 nm carboxylate-modified (-39.4 ± 8.2 mV), pluronic F-127 modified (-7.4 ± 8.9 mV) and dimethylamine-ethylamine modified (32.1 ± 9.4 mV) yellow-green fluorescent polystyrene nanospheres was studied in hydrated, SYTO 59 stained *B. multivorans* (LMG 18825) biofilms using confocal microscopy. After incubating one hour at room temperature, 3-D images of the biofilms were recorded. Similarly, the penetration of 40, 100 and 200 nm carboxylate-modified yellow-green fluorescent polystyrene nanospheres was evaluated.

The pluronic modified nanospheres interacted only slightly with biofilm material, in contrast to the other nanospheres. Furthermore, it was observed that the different types of nanospheres were all able to reach the biofilms substrate, indicating that net transport through the biofilm as a whole is very well possible. However, it was noted for all of the tested nanospheres that some biofilm clusters were accessible, while others were not. The cause of this is still under investigation but might be an important lead to the increased resistance of biofilms.

Endocarditis – A biofilm disease?

Annette Moter[1], Ulf B. Goebel[1], Roland Hetzer[2], Michele Musci[2], Kathrin Rugor[1], Christian Mallmann[1], Dinah Schmiedel[1], Annett Petrich[1] and Judith Schmiedel[1] [1] Institute for Microbiology and Hygiene, Charité University, Germany [2] Deutsches Herzzentrum, Klinik für Herz-, Thorax, Germany

Number: 9

Infective endocarditis (IE) is a microbial infection of the endocardium associated with significant morbidity and mortality. Bacteria colonize the heart valves leading to destructive vegetations that may culminate in heart valve replacement. It is assumed that IE is a biofilm infection; however, this view is based mainly on clinical observations and conventional histology.

Medical biofilms constitute a major diagnostic and therapeutic problem whereas clear definitions for biofilm infections are lacking. Several typical biofilm features summarized in the Parsek-Singh criteria and modified by Hall-Stoodley and Stoodley may help to classify infections as biofilm related. We discuss these criteria with reference to IE specimens investigated by Fluorescence in situ hybridization (FISH).
In 62 out of 110 IE patient samples examined by FISH we detected bacteria (predominantely Staphylococcus spp., Streptococcus spp., Enterococcus spp.). All of these cases were monospecies infections. Their spatial distribution within the tissue varied from single cells or microcolonies to highly organized, mature biofilms. We also found FISH positive bacteria in culture negative samples and samples from patients under antibiotic therapy. The high signal intensity of FISH correlates to a high ribosomal content of the bacteria indicating metabolic activity at the time of surgery.

In summary, biofilm features like spatial organization and limited growth in culture play an important role in endocarditis. We found that IE can readily be classified as biofilm infection based on the suggested criteria. This finding stresses the impact of in vitro experiments with monospecies biofilms and shows a correlate in the clinical in vivo situation.

Bacteriocin release profiles generated in static biofilms are heterogeneous amongst different strains of *Pseudomonas aeruginosa*

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Number: 10

Background

Pseudomonas aeruginosa is a versatile bacterium which survives and competes in biofilms in diverse environmental and clinical habitats where the availability of oxygen is variable. We have previously used microarrays to profile gene expression in static strain PAO1 biofilms (Waite *et al*., 2005; Waite & Curtis, 2009). These datasets revealed that the genes encoding the R-, F- and S-type pyocins (bacteriocins) are highly induced in all anaerobic biofilms studied but are only induced in developing microcolonies under aerobic conditions. This data was validated using bactericidal activity assays. The aim of this study was to determine whether the PAO1 profile of pyocin release is a phenotype shared by *P. aeruginosa* clinical isolates.

Results

The biofilm bactericidal activity release profiles generated for the burn wound isolate PA14 and several isolates with the Liverpool Epidemic strain (LES) genotype, were found to be very different to that of PAO1. For PA14, bactericidal activity was found under both aerobic and anaerobic conditions but only in developing biofilms. Whilst for LES bactericidal activity was found in all aerobic and anaerobic biofilms studied. In addition the number of indicator strains sensitive to biofilm supernatant from the three strains varied considerably (PAO1, 13/15; PA14, 7/15; LES, 1/15). The factors responsible for the different sensitivities are currently being investigated.

Conclusions

This study highlights the biological variability between different strains of *P. aeruginosa*; some strains are more aggressive bacteriocin producers than others and therefore the contribution of pyocin production to the colonization process will vary amongst strains.

Development of in vivo rat model of controlled biofilm infection in implantable port-access intravenous catheters

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Number: 11

Safe and easy-to-use port-access intravenous catheters (PAVCs) are integral part of daily clinical routine that may be subjected to major complications such as thrombosis and biofilm infections. The main objective of our study is to determine, using both classical microbiology and metagenomic approaches, the microbial population colonizing both infected and non infected clinically sampled PAVCs and to develop and validate an in vivo rat model of biofilm infection in PAVCs.

We were able to reproducibly establish and non-invasively monitor biofilm development in PAV catheterised rats using luminescent variants of biofilm-forming pathogens *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*. The bacterial colonization of catheters was associated with peripheral blood infection in about 50% of the catheterized rats. Metagenomics approach allowed us to identify pathogenic microbes in the infected PAVCs that otherwise could not be identified using regular culture based techniques as well as to detect several non-pathogenic environmental bacteria unique to non-infected clinical PAVCs. Mechanisms of cooperation, competition or interference are known to control the equilibrium of natural bacterial flora. Such mechanisms may also exist in PAVCs with environmental flora exerting some protection/interference against pathogen colonisation process, a hypothesis that would be assessed using our in vivo model and relevant identified bacteria.

This model will also allow evaluating antibacterial strategies as well as anti-adhesive and anti-thrombotic catheter coatings to prevent biofilm infections. Moreover, this controlled model of catheter infection opens the way for studies evaluating the influence of host immune response on the course of biofilm-associated infections.

The role of sphingolipids in the resistance of *Candida* albicans biofilms against miconazole

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Number: 12

Biofilms formed by *Candida* species consist of a network of cells, hyphae and pseudohyphae embedded in an extracellular matrix. Cells in these biofilms are highly resistant to antifungals. Previous research has shown that miconazole possesses fungicidal activity. Nevertheless, 1% - 10% of sessile *Candida* cells are tolerant to miconazole treatment. In the present study, the involvement of the sphingolipid biosynthesis in this tolerance has been investigated. A screening of *Saccharomyces cerevisiae* mutants in which genes involved in the sphingolipid biosynthesis are deleted was carried out. To this end, mature biofilms of these strains were cultured in 96-well microtiter plates and treated with miconazole (1 mg/ml) for 24h. The susceptibility of each mutant to miconazole was examined with a resazurin-based cell viability assay and compared to that of the wild type. Additionally, expression levels of genes involved in the sphingolipid biosynthesis were determined in *Candida albicans* biofilms using qPCR. The screening of *S. cerevisiae* mutants showed that the sphingolipid mannosyl-diinositol phosphorylceramide was associated with a higher susceptibility of sessile cells to miconazole. In contrast, the presence of dihydrosphingosine-1-phosphate led to a higher tolerance to miconazole. Addition of dihydrosphingosine-1-phosphate increased the miconazole tolerance of the hyper-susceptible *LCB4* mutant of *S. cerevisiae*, which is defective in the formation of dihydrosphingosine-1-phosphate. In conclusion, our results revealed a role for intermediates from the sphingolipid biosynthesis in the tolerance to miconazole. A higher tolerance of sessile cells to miconazole was observed in the presence of higher concentrations of dihydrosphingosine-1-phosphate.

Analysis of *Pseudomonas aeruginosa* mucoid-nonmucoid mixed biofilm: where do they exist in biofilm?

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Number: 13

Alginate-producing mucoid strains of *Pseudomonas aeruginosa* are the most threatening pathogen in Cystic Fibrosis (CF) patients. In CF focus, firstly asymptomatic colonization is occurred by nonmucoid strains, subsequently mucoid variants emerge and preferentially colonize during chronic infection. Although the colonization with nonmucoid strains is known to precede mucoid stains, the process and mechanism of the transition from nonmucoid to mucoid are not fully understood. We hypothesized that mucoid strains are more adaptive to the environment in CF lung sputum, which is known to be anaerobic condition. To address this hypothesis, we investigated the biofilm formations of mucoid and nonmucoid strain in aerobic and anaerobic conditions.

P. aeruginosa PAO1 (nonmucoid) and in-frame deletion mutant *mucA* (mucoid) were labeled with GFP and

DsRED, respectively. 96-well microtiter plate biofilm assay revealed that mucoid strain formed larger amount of biofilms than nonmucoid strain in anaerobic condition. Mucoid-nonmucoid mixed biofilm was formed by using flowcell system. To investigate the localization of each strain, mixed biofilm was analyzed by Confocal Laser Scanning Microscopy (CLSM). The result showed that mucoid strain could form mixed biofilm with nonmucoid strain, and it localized inside the biofilm. Interestingly, the addition of NO_3^-, the terminal electron acceptor of anaerobic denitrification, resulted in expansion of mucoid-localized area inside the biofilm. These results suggested that mucoid strains are more adaptive to the anaerobic environment than nonmucoid strains, and localize in anoxic region inside the biofilm. Therefore, mucoid strains anaerobic adaptation may make the transition from nonmucoid to mucoid in CF patients.

Effect of itraconazole on *Candida glabrata* biofilm matrix

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Number: 14

The emergence of non-*Candida albicans Candida* (NCAC) species as a common cause of fungal infection is often associated with the increasing number of immunocompromised patients, the widespread use of indwelling medical devices and the decreased susceptibility to azoles. The ability of *Candida* species to adapt to a variety of different habitats and to form biofilms is also of major contribution to this increased incidence. Thus, the aim of this work was to study the influence of the antifungal agent itraconazole on the matrix composition of *Candida glabrata* biofilms.

Biofilms of *Candida glabrata* vaginalstrain 534784 were formed in 6-well plates for 24h. Then, fresh RPMI1640/ MOPS medium (control biofilms) and itraconazole (256μg/mL) were added to the previously formed 24h biofilms. After 48h of exposure to these components, biofilms were scraped from the 6-well plates and the extracellular matrix extracted by sonication. The protein and carbohydrate content of the biofilm matrix were determined using a BCA kit and the Dubois method, respectively. The analysis of matrix composition of biofilms exposed to itraconazole showed an increase in both protein and carbohydrate content comparatively to the control.

The results indicate that the presence of itraconazole leads to an increase in the production of extracellular matrix components in *Candida glabrata* biofilms.

Live / dead discrimination of biofilm bacteria from a drinking water pilot distribution system

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Number: 15

Formation of biofilms in drinking water distribution networks, including pipelines of households and food industries, are of great concern. Biofilms are potential habitats for all kinds of bacteria, including pathogens, and may be responsible for contaminations of bulk water systems.

Nowadays, DNA-based methods are used for the detection and characterization of bacteria. One of the major disadvantages of these techniques is that they can not distinguish between DNA from live and dead cells. A battery of methods to face this problematic is presented in this work.

Conditioned surface water disinfected with ozone/ClO_2 flowed through a pilot scale built up with different pipe materials for biofilm formation. Bacterial population analysis was done by PCR-DGGE, comparing direct samples (total DNA) and samples pre-treated with Propidium monoazide or DNase I (DNA from live cells). Shifts in the DNA patterns observed after DGGE analysis, demonstrated: (i) the applicability of PMA and DNase I treatment in natural biofilm investigation; (ii) detection of DNA from dead bacteria and eDNA was blocked by pretreatment with PMA or DNase I; and (iii) DNase I treatment demonstrated a clearer effect on live/dead differentiation. Traditional cultivation methods and qPCR completed the biofilm analysis.

The results of the bacterial population analysis, and the results of the quantification methods that provide an overview of the different physiological states of bacteria: live cells, total amount of cells, and cultivable cells, are presented here.

A potential bacterial aetiology for pituitary apoplexy

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Number: 16

Pituitary apoplexy (PA) occurs when a pre-existing pituitary adenoma undergoes sudden haemorrhage. Despite the morbidity associated with this event, the aetiology of this condition remains unclear although inflammation of the sphenoid sinus and acute sinusitis have been reported in previous cases. We have investigated the possible involvement of bacteria in the onset of PA through the investigation of patients presenting with pituitary adenomas and PA. Sphenoid sinus specimens were explanted from subjects exhibiting non-functioning pituitary adenomas (NFPA) and PA and characterised using culture, PCR-denaturing gradient gel

electrophoresis (DGGE) and PCR-cloning using primers specific for the 16S rRNA gene. Whilst no bacteria were isolated by direct culture, 35% biopsies were positive following enrichment. Sequence analysis of cultures identified *Staphylococcus epidermidis, Staphylococcus lugdunensis* and *Corynebacterium fastidiosum* from sinus tissue. In contrast, DGGE suggested significant microbial diversity in all biopsies (mean 15 bands/biopsy) with apoplexy and NFPA groups clustering separately following UPGMA analysis. Following PCR cloning and insert sequencing, enterobacteria occurred from the sinuses of apoplexy subjects only, including *Citrobacter koseri* and *Escherichia coli,* whilst a single apoplexy subject also yielded *Pseudomonas aeruginosa.* In contrast, NFPA biopsies yielded predominantly lactobacilli (*L. delbrueckii, L. casei,* and *L. rhamnosus*), lactococci (*L. plantarum,* and *L. raffinolactis*), staphylococci (*S. epidermidis* and *S. lugdunensis*) and streptococci (*S. infantis and S. pneumoniae*). The occurrence of known respiratory tract pathogens associated with acute sinusitis from the sphenoid sinus of subjects exhibiting PA but not NFPA may suggest this condition to have a bacterial aetiology.

Detection of microorganisms involved in airway infection of cystic fibrosis patients by standard culturing and molecular methods

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Number: 17

Patients suffering from the genetic disease cystic fibrosis (CF), develop chronic lung infection. This infection persists due to highly viscous mucus occurring as a result of the disease, in which bacteria form biofilms. Diagnostic tools rely on culture based techniques performed on expectorated sputum samples, and most studies are centered on this sample type. It is however problematic to investigate biofilm-residing microorganisms, and further problems may occur since it is possible that the samples may be contaminated by oral flora during expectoration.

In this study tissue and sputum samples (n=24) from explanted lungs of four Danish CF patients were examined to circumvent possible oral flora contamination. Samples were examined by standard culturing techniques, including aerobic and anaerobic growth, at Rigshospitalet, Denmark. These findings were compared to results obtained by 16S rRNA gene analysis (16S rRNA gene amplification, cloning, sequencing and phylogenetic analysis) performed blinded of the growth results and quantification of the *oprL* gene of *Pseudomonas aeruginosa* by quantitative PCR at Aalborg University, Denmark.

The microorganism detected most often by 16S rRNA gene analysis in a sample was also detected by standard culturing techniques (which gave monomicrobial results). 16S rRNA gene analysis suggested that samples contained polymicrobial infection. A correspondence between the frequent detection of *P. aeruginosa* by the above methods and the number of *P. aeruginosa* cells in most samples were found by quantitative PCR. This suggests that detection of *P. aeruginosa* by culturing and 16S rRNA gene analysis is not caused by biases in the techniques.

Does antibiotic treatment help against catheter-associated urinary tract infections?

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Number: 18

Catheter associated urinary tract infections (CAUTIS) a form of biofilm-associated infections involve high mortality rates. An organism which is frequently isolated from CAUTIs is *Pseudomonas aeruginosa*. For this study different *P. aeruginosa*-isolates from urine or catheter samples where cultivated in an *in vitro* urinary tract catheter biofilm reactor, simulating the conditions of a catheterized urinary tract. This implies an artificial urine medium a flow rate of 1 mL/min as well as the temperature of 37 °C.

In a first step biofilms of *P. aeruginosa*-isolates grown in this catheter system were characterized. This involved the determination of different parameters like biofilm thickness, density, detachment rate as well as substrate consumptions. An optical biomass sensor allowed a non-invasive, on-line measurement of the growing biofilm in the system.

The second step comprised an analysis of a treatment strategy. The chosen antibiotic Ciprofloxacin was used in agreement with pharmacokinetic analysis. The biofilm has been cultivated under described standard conditions, afterwards Ciprofloxacin was added as if a urinary tract infection was treated.
Directly after the start of the treatment a reduced activity of the biofilm could be detected. Under standardized cultivation conditions the mean diameter of detached biofilm particles was around about 300 µm. Within two hours after the initiation of the antibiotic treatment the average maximal diameter of the detached particles reached 700 µm. At the end of the cultivation the results showed that a fraction of cells within the biofilm has survived the treatment.

Biofilm formation in *Acinetobacter haemolyticus* increases resistance to antibiotics and metal ions

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Number: 19
Acinetobacter is a potent nosocomial, multidrug resistant and biofilm forming pathogen. Biofilm formation enhances the property of resistance several fold in a wide range of bacterial pathogens. In present investigation, effect of biofilm formation on MICs of antibiotics and metals was evaluated. MIC for 2 *Acinetobacter haemolyticus* strains isolated from Indian hospitals, sensitive to various classes of antibiotics and metal ions, were determined by broth dilution technique. Minimum biofilm eradicating concentration (MBEC) was determined by calgary biofilm device (CBD). Amoxycilin/Clavulanic acid was ineffective on biofilm grown for 18 h and 72 h at concentration of >32750 µg/ml. Polymyxin B and rifampicin were effective at 18 h (2500 U and 16 µg/ml respectively) but were ineffective at 72 h (> 40,000 U and > 32750 µg/ml respectively), each of them leaving a fraction of essentially invulnerable persisters producing counts (74 X 10^7, 33 X 10^7 cfu/ml). Gentamycin and tobramycin were the only effective antibiotics on 18 h and 72 h grown biofilm. Isolates were resistant to metal ions present as normal media component such as Zn^{++}, Cu^{++},Co^{++}. Metal ions Ag^{+} and Hg^{++} were found to inhibit, planktonic, 18 h and 72 h grown biofilm, though resistance increased about 512 folds after 72 h. We confirm this as first ever report on biofilm formation by *Acinetobacter haemolyticus,* responsible for enhanced resistance against antibiotics and metals at later part of biofilm development, however warrants further evaluation, with larger sample size.

Mixed biofilms of *Staphylococcus epidermidis* and *Candida parapsilosis* as an insight in polymicrobial endocarditis
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Number: 20
Infective endocarditis is a serious clinical issue, often related to the prolonged use of intravenous catheters that can eventually become infected. Although polymicrobial endocarditis (PE) involving *Staphylococcus epidermidis* and *Candida* species is uncommon, it is generally associated with patients' high mortality due to the presence of *Candida* spp.. Additionally, investigation on the phenomenon of microbial competition in PE is still scarce. Thus, the main goal of this work is to deepen the knowledge of PE related to *Staphylococcus* and *Candida* spp., by studying the competition and the influence of each species on biofilm formation on silicone.

Both strains of *S. epidermidis* and *C. parapsilosis* were clinical isolates. Biofilm assays were performed on silicone coupons in 24-well plates, for 24, 48, 72h and 8 days. Total biomass was measured by crystal violet staining while cell viability was evaluated through colony forming units (CFU) enumeration. Species differentiation in mixed biofilms was achieved using selective mediums and also by Scanning Electronic Microscopy (SEM) observation. According to the results, and comparing to single spp. biofilms, it was noticed that mixed biofilms are not cumulative. Despite this, in all biofilm conditions an increase in the number of cells was observed after 72h. Moreover, an inhibitory effect of *S. epidermidis* on *C. parapsilosis* biofilms was noticed, especially at 72h.

In conclusion, this study provided an important approach for a better understanding of *S. epidermidis* and *C. parapsilosis* biofilms composition, structure and interactions, which can give new insights on PE caused by these microorganisms.

Identifying targets for inhibition of biofilm formation on the surface of urinary catheters
Nicola Tirelli, Mathew Upton, Michelle Webb and Nishal Govindji, University of Manchester, UK

Number: 21
Catheterisation is frequently used to treat urinary incontinence in older patients. Complications arise when bacteria attach to the surface of the catheter and develop into biofilms. To prevent biofilm formation, urinary catheters have been subject to many materials changes. One important development is the release of antimicrobial silver salts from hydrogel-coated catheters. Incorporation of silver ions or antibiotics may be disadvantageous in the long-term because heavy metal and antibiotic resistance genes are genetically linked, so their coexistence on the same genetic element potentially allows antibiotic resistance to be selected under heavy metal selective pressure. It would, therefore, be more desirable for future generations of materials to move towards incorporating novel inhibitors of functions vital to biofilm integrity.

Our research aims to identify proteins essential for biofilm development, with a view to recognising novel targets for biofilm inhibition. We will present proteomic data that shows that the outer membrane proteins, ompA and oprF, are upregulated during biofilm development by strains of uropathogenic bacteria. Furthermore, deletion of the ompA gene in *Escherichia coli* results in reduced biofilm formation. OmpA and its homologues are consequently potential targets for biofilm inhibition.

Natural polycationic polyamines and other cationic compounds are known to interact with the outer membrane proteins, including ompA, and affect their function. We have therefore investigated the effects such compounds have on biofilm development by isolates of *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Inhibition was demonstrated for some compounds and detailed results will be presented.

Genotypic and phenotypic characterization of biofilm production in *Pseudomonas aeroginosa* strains in the Bone Marrow Transplant Center of Tunis, Tunisia

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Number: 22

Introduction: *Pseudomonas aeruginosa* is a major opportunistic human pathogen. The aim of this work is to study the genotypic and phenotypic biofilm characteristics of *Pseudomonas aeruginosa* .

Methods: 62 non redundant *Pseudomonas aeroginosa* were isolated at the Bone Marrow Transplant Centre of Tunis (49 clinical and 13 hygienic control strains) and identified by conventional methods and Api 20NE (BioMerieux). Serotyping was done using agglutination test (Biorad). Detection and quantification of biofilm forming ability was done using 96 wells microtiter plate assay, then Biofilm Unit (BU) was calculated and producers strains was classified in three groups; week producers (0.142<BU<0.284), medium producers (0.284<BU<0.568) and strong producers (0.568<BU). The presence of *LasI, LasR, RhlI* and *RhlR* genes was determined by a simplex PCR.

Results: All clinical strains were able to produce biofilm, indeed, 55.10% (27/49) of those strains were moderate biofilm producer, 34.70 % (17/49) were weak biofilm producer. Only 8.10 % (5/49) of those isolates showed strong biofilm production. Likewise all environmental strains were biofilm producers exept of one strain. All strong biofilm producers were exclusively from clinical strain.

The study of serotyping showed of serotype O6 and O11in clinical strains than in environmental ones. In the current study, there was no significant difference in *LasI, LasR, RhlI* and *RhlR* distribution among clinical (respectively 83,67% 75,51% 85,57% and 89,79%) or environmental strains (respectively 84,61% 84,61% 76,92% and 84,61%) .

Conclusion: The data reported here indicate a more significant ability to produce biofilm in clinical strains than in environmental ones.

High-level antibiotic resistance in *Pseudomonas aeruginosa* biofilm: the ndvB gene is involved in the production of highly glycerol-phosphorylated b-(1,3)-glucans, which bind aminoglycosides

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Number: 23

Pseudomonas aeruginosa is an opportunistic pathogen which causes life-threatening infections in cystic fibrosis (CF) patients. Colonization of CF lung by *P. aeruginosa* involves a biofilm mode of growth, which is promoted by the production of exopolysaccharides. In the present study, we identified anionic cyclic glucans produced by *P. aeruginosa* strains PAKΔ*retS* and PA14. Their structure has been elucidated using chemical analysis, one- and two-dimensional NMR techniques, and mass spectrometry. They belong to a family of cyclic β-(1-3)-linked glucans of 12-16 glucose residues with 30-50% of glucose units substituted by 1-phosphoglycerol at *O*-6. The *ndvB* gene was predicted to be involved in the synthesis of perisplasmic glucans, capable of physically interacting with aminoglycoside antibiotics. We revealed that the glucans are lacking in the *ndvB* mutant, and we showed that these glucans are capable of direct binding with kanamycin. This observation fills a gap in our understanding of the relationship between biofilm, cyclic glucans and high-level antibiotic resistance.

Identification and Characterization of Biofilm-Deficient Mutants of *Moraxella catarrhalis*

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Number: 24

The Gram-negative bacterium *Moraxella catarrhalis* is a leading cause of both acute otitis media and infectious exacerbations of chronic obstructive pulmonary disease. Previous studies have demonstrated that this bacterium form biofilms both in vitro and in vivo. However, the mechanisms underlying biofilm formation by this organism are not well-understood. In this study, we performed random transposon-mediated mutagenesis and subsequent targeted mutagenesis on a *M. catarrhalis* strain that exhibits robust biofilm growth in a microtiter plate-based crystal violet biofilm assay system. Among the transposon insertion mutations that had a negative effect on biofilm formation was a gene encoding a predicted response regulator which we tentatively designated as *cpxR*. Disruption of this gene resulted in a biofilm growth deficiency and rendered the cells unable to grow in liquid media. Examination of the *M. catarrhalis* ATCC 43617 genome revealed the presence of another highly homologous predicted response regulator. Inactivation of this second gene, tentatively designated as *ompR,* resulted again in a biofilm-deficient phenotype. Inoculation of the *cpxR* mutant into a continuous flow biofilm system allowed recovery of variants that had regained both the ability to form biofilms and to grow in liquid media while retaining the original *cpxR* mutation. Efforts are currently underway

to characterize the *M. catarrhalis* CpxR regulon and to identify the gene(s) responsible for the restoration of the biofilm-positive phenotype in the *cpxR* mutants. While earlier analyses of *M. catarrhalis* biofilm formation concentrated on surface-exposed proteins, these studies will focus on the signaling pathway(s) involved in biofilm development.

***Candida glabrata* and *Candida albicans* single and co-colonization of acrylic in presence of saliva**

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Number: 25

Oral candidosis is a frequent problem in immunocompromised patients and *Candida albicans* is regarded as the leading cause of such infections. However, recently, *Candida glabrata* has emerged as an important pathogen, occurring both singly or in mixed species infections, often with *C. albicans*. Compared with *C. albicans*, few is known about the role of *C. glabrata* in oral infection. Furthermore the use of denture acrylic surfaces is a prerequisite for adhesion and biofilm formation by *Candida* species in oral environment. Thus, the aim of this study was to examine the adhesion and biofilm formation ability by single and mixed *C. glabrata* and C. albicans species on acrylic in the presence of artificial saliva.

Adhesion ability was quantified by colony forming units (CFUs) counting using CHROMagar medium and the total biomass quantified by crystal violet. Epifluorescence and confocal microscopy observations were used for examining the biofilm structure using species specific peptide nucleic acid (PNA) probe hybridisation.
The results showed that *C. glabrata* had higher ability to adhere and form biofilms on acrylic comparatively to *C. albicans*. Additionally, co-culture studies showed that the presence of *C. albicans* did not affect the ability of *C. glabrata* to adhere to acrylic surface. Microscopy images are in accordance with CFUs and crystal violet staining results.
The importance of recognising the occurrence of infections associated to mixed biofilms is highlighted by the results of this study and represents an area that should be considered by both diagnostic laboratories and clinicians.

Genetic Basis on Antibiotic Tolerance in *Pseudomonas aeruginosa* Biofilms

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Number: 26

Biofilms of *Pseudomonas aeruginosa*, in comparison to their planktonic complement, are more tolerant of antibiotics. In order to comprehensively characterize the genetic basis of this phenomenon, we generated a saturated transposon insertion library in the laboratory strain, PAO1, and subjected that library to different competitive selections in either biofilm or planktonic state, in the presence or absence of tobramycin. Through comparative microarray-based profiling of these selections, we identified several loci that are involved in biofilm-mediated tobramycin tolerance. Some of them belong to previously characterized functional categories like oxidative phosphorylation, LPS biosynthesis, quorum sensing, or membrane permeability, while many of them are novel. Using a combination of experimental and computational methods, we associated a subset of these novel genes to different functional and biological classes. The majority of the abovementioned loci also have minor growth advantage in the planktonic state in the presence of tobramycin. However, this effect is too small to be detected by traditional MIC (Minimal Inhibitory Concentration) determination assays. Furthermore, there are a few loci which have a biofilm-exclusive advantage. The acquired insight from this work could contribute to better understanding of this longstanding question and development of novel therapeutic strategies against microbial biofilms.

Microbial communities in different biofilm-related infections

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Number: 27

The objective of this study was to compare the microbial community in different biofilm-related diseases: endocarditis (n=18), chronical wounds (n=14), urinary catheter (n=24)-, central venous catheter (n=18)- and prosthesis-related (n=9) infections. The presence of microorganisms was investigated using traditional culture-dependent methods and a range of culture-independent molecular methods including construction of clone libraries, sequencing, phylogeny, fingerprinting, FISH and quantitative PCR. In general all species detected by cultivation were also found by molecular methods. *Staphylococcus* spp were identified in 50% of the infections included in this study (n=83), and were identified in all chronical wounds, most prosthesis samples and on few urinary catheters. *Pseudomonas* spp were associated with 15% of the samples but found in all infection types, while *Stenotrophomonas* spp were abundant on catheter- and prosthesis-biofilm. *Streptococcus* spp

were detected in endocarditis and prosthesis biofilms, whereas some species were primarily associated with one type of infection. Interestingly *Legionella* spp was detected in an infected heart valve by fingerprinting, specific q-PCR and in a clone library, but not by cultivation. In 75% of the investigated samples polymicrobial communities were detected and all urinary catheters, chronical wounds and prosthesis samples were polymicrobial as opposed to only 25% of endocarditis samples. FISH illustrated that microorganisms were often positioned locally in the biofilm. Some species generally appeared as microcolonies and other species as single cells in the same sample. In conclusion the significance of the findings needs further investigations, and future studies should focus on the development of optimal sampling, identification and treatment regimes.

Candida tropicalis biofilms: formation and virulence factors

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Number: 28

Significance and objectives: A substantial proportion of *Candida tropicalis* infections is associated with biofilm formation, especially on catheters. Thus, the aim of this study was to investigate *C. tropicalis* biofilm formation on silicone and its effect on epithelial cells and enzyme production (hemolysins and proteinases).

Methods and results: This study was performed with *C. tropicalis* (clinical isolate and reference strain ATCC 750). Biofilms formed on silicone coupons immersed in artificial urine, were quantified by crystal violet (CV) staining and by enumeration of colony forming units (CFU) and the matrix content in proteins and polysaccharides was also determined. Biofilm cells and matrix were assessed in terms of hemolysins and proteinases production and their effect on TCC-SUP urinary epithelial cells was evaluated as well. Biofilms of *C. tropicalis* ATCC 750 presented a higher number of cells than the clinical isolate although less biofilm biomass and less polysaccharides. Moreover *C. tropicalis* biofilm was able to express total hemolytic activity and higher proteinase but these factors were not detectable within the matrix. Additionally, *C. tropicalis* biofilm adhered in higher extent to epithelial cells than their planktonic counterparts. Moreover, epithelial cells showed low metabolic activity when in contact with biofilms.

Conclusions: Therefore, it is possible to conclude that enzyme production was detected in *C. tropicalis* biofilm cells, but not in its matrix and that biofilm cells can cause more damage to epithelial cells than their placktonic counterparts. This highlights the importance of biofilm formation, associated to the use of urinary catheters, on *C. tropicalis* virulence.

Changes of concentration and cultivability of *Escherichia coli* in biofilm of a drinking water distribution network

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Number: 29

Harbored in biofilm of oligotrophic environment *Escherichia coli* cells lose their ability to grow on conventional culture media. In this state *E.coli* has been found in several drinking water distribution networks, hence the rising concern about possible risk of recontamination. However, it is not possible to properly address this concern before the fate of these fecal bacteria in water supply systems is understood. Previous laboratory scale studies have shown that *E.coli* can grow in water in presence of native biota at concentration of assimilable organic carbon (AOC) and temperature typical for some of drinking water supplies.

In this study drinking water from a water supply having a temperature of 20°C containing about 400 µg-AOC/l was fed in a biofilm reactor (Propella™). Weekly samples from water and biofilm were analyzed using the culture based methods and fluoresce *in-situ* hybridization combined with Direct Viable Count. Results showed that no cultivable *E. coli* was found in the biofilm. However, the total concentration of *E.coli* in biofilm gradually increased, reaching the maximum after two weeks (460 cells/cm^2), after which it decreased below the detection limit (below 3 cells/cm^2).

The occurrence of *E.coli* has been previously linked with repair works. By examining the repair data and applying computer modeling of the flow it was concluded that this was not the case in this study. The possible origins of *E.coli* in the network could be from surrounding environment or regrowth.

The Pel and Psl polysaccharides in *Pseudomonas aeruginosa* display differential expression in both environmental and clinical isolates

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Number: 30

P. aeruginosa can produce three extracellular polysaccharides, Alginate, Psl and Pel, as part of its extracellular matrix. Although much work has revealed the individual function of these three polysaccharides, little work has uncovered how these polysaccharides may work together and if they have redundant or unique functions.

Here we explore the relationship between the Pel and Psl polysaccharides. PAO1 and PA14, two commonly used laboratory strains, drastically differ in the requirement for Pel or Psl for biofilm development. PA14 exclusively uses Pel in its biofilm EPS matrix since it is genetically incapable of producing Psl. Whereas in PAO1, Psl is the predominant polysaccharide. Using specific antiserum against Pel and Psl exopolysaccharides, we show that Pel is expressed at a much higher level in PA14 than in PAO1, while PAO1 produces more Psl than Pel for its matrix. We examined twenty *P. aeruginosa* isolates obtained from clinical (cystic fibrosis, blood, and UTI) and environmental (lake water and soil) settings and found much variety in the relative Pel and Psl expression profiles. These levels do not appear to be associated with where the isolate originated. Also, the relative levels of Pel and Psl do not appear to be predictive factors for wrinkly colony morphology, which has been demonstrated to be dependent on Pel and Psl production. Through mutagenesis studies, we are examining the contribution of each polysaccharide towards attachment, biofilm development and antibiotic resistance. These studies aspire to highlight the unique and redundant functions of Pel and Psl.

Cross-disciplinary research.

The aim of my work is to understand the role of surface carbohydrates in biofilm biology. My research bridges the fields of molecular biology, chemistry and biophysics. Using laser tweezers and biochemical methods, we have been able to access the involvement of surface carbohydrates to cell-to-cell adhesion in *Pseudomonas aeruginosa* biofilm matrices.

Formation of diverse morphotypes of *Erwinia carotovora ssp.* SCRI1043 during plant-microbe interaction

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Number: 31

Plant – pathogenic bacteria interaction includes several stages such as early latent period, acute infection, host death and preparation of microorganisms to non-vegetative period. Many prokaryotes were found to form specialized morphological types of the cells for example dormant cells (viable but non-culturable or cyst-like resting cells) and to arrange biofilms. This makes the bacteria to become more resistant to plant defense responses and to survive under starvation during non-vegetative period.

In this work we assessed the ultrastructural modification of plant (tobacco) and bacterial (*Erwinia carotovora*) cells from the fist hours of infection till the plant death. Diverse morphotypes such as dormant-like cells and biofilm communities were observed. Formation of these morphotypes occurred in a stage- and tissue-specific manner.

Biofilms are traditionally thought to be associated with both microbial resistance and virulence. However in our study biofilms formed in host-plant tissues seemed to be less pathogenic than planktonic cells. This means that the functions of biofilms in different model system are not universal and their ecological roles can be wider than is thought. After plant death, planktonic cells but not biofilms, transformed to diverse morphotypes, which resembled the dormant *Erwinia* cells that we found and characterized previously *in vitro* (Gorshkov *et al*., 2009, Gogolev *et al*., 2009). To the best of our knowledge we were the first to observe diverse bacterial morphotypes, including biofilms and dormant-like cells, *in planta*. Based on our data we propose the scheme of *Erwinia* attack strategy.

In terms of BICs and MBECs, the role of microbial biofilm in upper versus lower urinary tract infections: microbiological and clinical study

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Number: 32

Significance and objectives:- For detection the most common microorganisms isolated from infected JJ stents and infected calculi in upper urinary tract and their representative urine samples from lower urinary tract and their ability to produce biofilm quantitatively on these sites. Furthermore, depending on BICs and MBECs parameters, for detection the biofilm antibiogram for biofilm producer study isolates to three selected antimicrobial agents.

Methods and results:- One hundred and thirty (130) specimens obtained from Sixty five (65) patients admitted to Urology Department in Al-Ramadi Teaching Hospital and carried out during the period from April through December 2008. They include 94 specimens taken from 47 indwelling JJ ureteric stents and 18 renal stones were obtained from the renal pelvis during pyelolithotomy and at the same time a catheter collected bladder urine sample was taken for each patient. Quantitative biofilm formation assay and biofilm antimicrobial susceptibility test was achieved. Out of 47 upper end of JJ stent, 19 (40.4%) were positive for culture. Out of 24 microorganisms 19 (79.2%) were biofilm producer isolates. Out of 47 lower ends of JJ stent, 16 (34%) were positive for culture. Of these, 16 microorganisms were biofilm producer isolates. Among infected JJ stent, 10 (22.2%) *Klebsiella pneumonia* and 10 (22.2%) *Candida albicans* were the most common isolated microbes

from JJ stent. Also, 5 renal stones were positive for culture and all the yielded bacterial isolates were biofilm producers. The biofilm cells were required 50-100 times the MIC values for ciprofloxacin obtained for the same isolates in logarithmic phase of planktonic cells in each of JJ stent and infection stones while with cefotaxime, 50-500 X MIC values was required in JJ stent in comparison with infection stones (50-100 XMIC). Further, the biofilm producer isolates cells were required 10- 100 times the MIC values for amikacin in both of two specimens.

Conclusions:- The study concluded that *Klebsiella pneumonia* and *Candida albicans* were the most common microorganisms isolated from infected JJ stent while *Klebsiella pneumonia* was the most predominant microorganism in struvite stones followed by *Proteus mirabilis*. No significant difference observed between upper and lower urinary tract infections regarding biofilm formation. Further, Mixed biofilm species observed markedly on JJ stents of this study. Furthermore, the biofilm producer study isolates were required lower concentration of amikacin to remove bacterial biofilm from JJ stents

Glucorticoids improve or worsen antibacterial efficacy of aminoglycosides?

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Number: 33

Significance and objectives

Various commercially available antibacterial drugs are administered together with glucocorticoids to reduce inflammation. Some drugs topically administered even include both antibiotic and glucocorticoids in the same preparation (i.e. eye drops). It has been suggested that glucocorticoids could interfere with antibacterial properties of antibiotics but to date nobody could demonstrate it.

The goal of this study was to assess possible interferences of dexamethasone and betamethasone on antibacterial properties of tobramycin on Methicillin Sensible *Staphylococcus aureus* (MSSA) and Methicillin Resistant Staphylococcus aureus (MRSA).

Methods and results

We used 4 staphylococcal strains (2 MSSA: ATCC 6538P, and 1 clinical isolate; 2 MRSA: USA300 and 1 clinical isolate); grown in presence of tobramycin (3mg/ml) or dexamethasone (1mg/ml), or betamethasone (1mg/ml) or in presence of tobramycin plus dexamethasone and tobramycin plus betamethasone at the aforementioned concentration. Experiments were repeated in triplicate in Mueller-Hinton Broth (MHB, Oxoid, UK).

The 5 experimental conditions used showed: a) presence of both glucorticoids does not modify antibacterial activity of tobramycin; b) betamethasone improves the tobramycin antibacterial activity; c) only bethametasone alone act as potent inhibitor of the growth of all Staphylococcus strains (MSSA and MRSA).

Conclusions

These interesting results suggest that the presence of the two tested glucorticoids do not affect the efficacy of aminoglycosides on bacteria. On the contrary, surprisingly bethamethasone is a good inhibitor of bacterial growth. In particular it blocks the growth of MSSA and is very efficient against USA300 a major pathogen challenging the health system in the world.

Biofilms and catheters in intensive care service: Abidjan, Côte d'Ivoire

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Number: 34

Significance and objectives

In Africa and particularly in Côte d'Ivoire, there is very little data regarding studies on biofilm. Considering the link between biofilm and nosocomial infections, it appears very important to study biofilm in biomaterials, including catheters to better fight infections caught in hospitals. The aim of this study is to determine the biofilm bacteria in biomaterials and their susceptibility to antibiotics. Methods and results

This prospective study included 102 catheters collected from 69 patients admitted in intensive care services and the Cardiology Institute of Abidjan from April to July 2009. Catheters were cultivated using the method of Brown Bush. The test susceptibility was performed using the technique of agar diffusion. The results showed that 78 %(80) were peripheral catheters and 22% (22) were central venous catheters. Within the 102 catheters collected, positive culture represented 14.7% (15/102). In the 22 central catheters analyzed, 3/22(13.6%) had a positive culture. The peripheral catheter colonization rate was 15% (12/ 80) positive cultures. *Acinetobacter baumannii* are the principal strains isolated. Methicillin- resistant *S.epidermidis* was observed in 100% (1/1), 100% (1/1) of *Enterobacter aerogenes* were resistance of fluoroquinolone and 25% (1/4) of *A.baumannii* to imipenem.

Conclusion

This study showed the importance of biofilm study in intensive care particularly in Cardiology Institute. Therefore, it will be suitable to make molecular characterization of bacteria from the bioflim to study the mechanisms of resistance including resistance genes of strain To imipenem.

Biofilm formation by a mixed oral microflora on rough and smooth titanium

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Number: 35

The last twenty years of intensive research has provided titanium implant surfaces that stimulate a fast and strong osseointegration. Due to this the implant treatment can be provided to more, both fully and partly, edentulous patients and with a higher rate of success. However, the rising number of partly edentulous patients, often with a history of periodontitis, implant-related bacterial infection has become an issue of increasing concern. The aim with this study is to inquire more knowledge about the influence surface characteristics of dental implants have on biofilm formation. The bacterial biofilm formation on titanium discs with rough (TiUnite, Nobel Biocare) or smooth (turned) surface after 16 hours of incubation with a mixed bacterial culture of *Streptococcus sanguis, Actinomyces graevenitzii, Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans* and *Neisseria subflava* was investigated. Confocal laser scanning microscopy and image analysis was used to determine; the thickness of the formed biofilm, the mean area of the bacterial clusters in the biofilm, the total fraction of coverage by the biofilm and the ratio of dead bacteria in the biofilm. An extensive coverage of the smooth titanium surface by a thick biofilm with an average thickness of 27µm was found. In contrast the average thickness of the biofilm formed on the rough surface was 6 µm. In conclusion, the biofilm formed on the smooth surface was found to have nearly 5 times the thickness and 8 times the coverage than that formed on the rough. This indicates antibacterial properties of the rough surface restricting bacterial growth.

Nitric Oxide-Mediated Dispersal and Enhanced Antibiotic Sensitivity in *Pseudomonas aeruginosa* Biofilms from the Cystic Fibrosis Lung

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Number: 36

Biofilms present a major challenge in medicine due to their recalcitrance towards antimicrobials and ability to establish chronic and persistent infections. Cystic fibrosis (CF) is one condition where biofilms and their antibiotic resistance play an important role in disease progression. Biofilm dispersal of the opportunistic pathogen *P. aeruginosa* can be induced *in-vitro* with low, non-toxic concentrations of nitric oxide (NO). Our hypothesis is that delivery of low dose NO or NO donors to the CF lung will reduce carriage of *P. aeruginosa* significantly by inducing dispersal and reducing antibiotic tolerance of the biofilms. In this project, low-dose concentrations of nitric oxide donors, in the micromolar and nanomolar range, were used to induce dispersal of *P. aeruginosa* biofilms from CF patient sputum *in vitro*. The extent of biofilm dispersal was concentration dependent and could be significantly reduced by the addition of a nitric oxide scavenger. Moreover, biofilm dispersal was accompanied by an increased susceptibility of *P. aeruginosa* to clinically relevant antibiotics such as Tobramycin and Ceftazadime. This work provides strong evidence to suggest that NO-mediated dispersal can augment antibiotic sensitivity of biofilms and may offer a novel treatment for biofilm infection in the CF lung.

The role of enterococcal surface protein in pathogenicity of *Enterococcus faecium*

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Number: 37

Enterococci are usually harmless commensals in the human gut but, when host resistance is lowered, they can cause infections such as endocarditis and bacteraemia. They are clinically important due to their high levels of antibiotic resistance and their ability to form biofilms. One enterococcal protein shown to be involved in biofilm formation is the enterococcal surface protein Esp. Here we show that in *Enterococcus faecium* this protein has an effect on antibiotic resistance. Strikingly, this effect was only observed when using isothermal calorimetry, a method that is much more sensitive than classical methods to determine antibiotic resistance. Biofilm formation in *E. faecium* was tested in different environmental conditions. One compound encountered by enterococci in the gut is bile. We show here that biofilm formation is stimulated by bile, but only at physiological relevant concentrations. This was also shown to depend, in part, on the presence of Esp, suggesting that Esp may play a role in sensing the environment.

Biofilm resistance of the *Burkholderia cepacia* complex against fosmidomycin and fosmidomycin derivatives

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Number: 38

BACKGROUND: The *Burkholderia cepacia* complex (Bcc) is a group of 17 closely related species. Bcc bacteria are opportunistic pathogens able to infect the lungs of cystic fibrosis patients and due to their intrinsic resistance towards many antibiotics, they are very difficult to eradicate. Fosmidomycin blocks a key enzyme in the non-mevalonate pathway for isoprenoid synthesis. As this pathway is metabolically essential for Bcc bacteria, fosmidomycin could be considered a new potential antibiotic to treat Bcc infections. OBJECTIVES: To determine whether fosmidomycin and a number of fomsidomycin derivatives (including the acetyl derivative FR900098) have activity against plancktonic and sessile Bcc cells. RESULTS: The activity against planktonic cultures was tested for 38 Bcc strains. MICs of fosmidomycin were > 512 mg/l for all strains tested. FR900098 was capable of inhibiting the growth of 18 strains at concentrations ≤ 512 mg/l. It has previously been reported that glucose-6-phosphate lowers the MIC by inducing the hexose phosphate pathway by which fosmidomycin can be taken up into the cell. This was confirmed as the combination of glucose-6-phosphate and FR900098 significantly lowered MICs of *Burkholderia multivorans* LMG 13010 and *Burkholderia cepacia* LMG 1222. Because of these promising results, the bactericidal effect of FR900098, in concentrations of 10 x MIC (640 mg/l) and in the presence of glucose-6-phosphate was tested against biofilms. However, no effect was observed on biofilms. The mechanisms behind the increased resistance in sessile cells are currently being investigated using qPCR.

Phenotypic variation in the biofilm: A comparison of *in vitro* and *in vivo Pseudomonas aeruginosa* variants

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Number: 39

Numerous mucosal surface infections caused by *Pseudomonas aeruginosa* are characterized as biofilm infections. Within the biofilms that form at these surfaces, phenotypic variants often emerge in order to ensure survival of the bacteria. Studies have begun to elucidate the mechanisms by which these variants emerge *in vitro*; however, too little information exists on phenotypic variation *in vivo* to draw any links between variants generated *in vitro* and *in vivo*. Consequently, in this study, *P. aeruginosa* strains known to produce phenotypic variants with altered biofilm properties were studied in an *in vivo* mucosal surface infection model. For instance, we showed that the *P. aeruginosa gacS*$^{-}$ strain, a poor biofilm former that generates hyper-biofilm forming variants under *in vitro* stress, also generates variants under *in vivo* stress. Using the Calgary Biofilm Device, we showed that these *in vivo* variants possessed enhanced biofilm formation, akin to *in vitro* variants previously characterized. However, despite similarities in biofilm formation, the *in vivo* variants exhibited a less diverse resistance profile to antimicrobial agents than *in vitro* variants. We further characterized other variant strains that were deficient in biofilm formation *in vitro*. These variants were unable to sustain an infection *in vivo* and were very susceptible to antimicrobials. In all, these analyses showed that biofilm formation is important for *P. aeruginosa's* ability to cause infections at mucosal surfaces, and that variants generated *in vitro* and *in vivo* can be distinct.

ELISA detection of IgM against staphylococcal slime to diagnose prosthetic joint infection

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Number: 40

Significance and objectives

Staphylococci are the leading cause of infections related to implanted medical devices due to their capability to establish multilayered, highly structured biofilm on artificial surfaces. Delayed orthopaedic joint prosthesis infections (DOJP-Is) are relatively common and one of the leading cause of prosthetic revision. Specific and non-invasive diagnostic tests are unavailable and the detection of the DOJP-Is is commonly made at an advanced stage of disease. Aim of this study is the assessment of sensibility and specificity of an ELISA test capable to detect anti-staphylococcal biofilm circulating IgMs.

Methods and results

An ELISA assay developed to detect serum antibodies against staphylococcal slime polysaccharide antigens (SSPA) was tested to diagnose DOJP-Is in 90 subjects: 29 infected cases (staphylococcal DOJP-Is: 16 hip, 12 knee and one shoulder prosthesis) and 61 uninfected controls (with or without orthopaedic implants). The local ethics committee approved the study and all subjects provided written informed consent.

The mean titres of immunoglobulin M (IgM) against SSPA were 0.72±0.55 in the subjects with an ongoing staphylococcal DOJP-Is and 0.21±0.07 in controls ($p<0.001$). Using an antibody titre cut-off value of 0.35 ELISA

units, the test showed a specificity of 95.1 (95% CI: 85.4-98.7) and a sensitivity of 89.7 (CI:71.5- 97.3).

Conclusions
Anti-SSPA ELISA may represent a sensitive, specific and non-invasive diagnostic test for staphylococcal DOJP-Is, and its use should be considered for inclusion in the routine clinical monitoring of subjects with orthopaedic prostheses.

***Acinetobacter* biofilms; Protection against antibiotics and killing by hospital detergents**
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Number: 41
Acinetobacter baumannii is a major cause of nosocomial infections that lead to complications such as ventilator-associated pneumonia, septicemia, secondary meningitis and urinary tract infections. It has recently emerged as an important pathogen in Iraq where large numbers of US soldiers have contracted *Acinetobacter* infection following surgical procedures. Many of these strains are resistant to a high number of antibiotics making treatment of these infections a challenge. It has been well documented that *A. baumannii* can attach and survive for extended periods of time on solid surfaces. This is probably achieved through the production of bacterial biofilms. We have tested clinical isolate strains of *A. baumannii*, including those involved in specific hospital outbreaks, in their ability to form biofilms in complex and minimum media. Those that have been shown to be involved in hospital acquired infections are the most capable at forming biofilms and also are more resistant to a wide range of commonly used antibiotics e.g. kanamycin. The formation of bacterial biofilms also protects *A. baumannii* from higher concentrations of detergents and antibiotics with an increase in the bacterial MIC (Minimum Inhibitory Concentration) against these chemicals. *A. baumannii* can also remain in the environment by the production of persister cells with increased numbers linked to biofilm formation. This creates problems for the treatment and eradication for *A. baumannii*. Thus, these organisms through the production of biofilms can potentially survive for extended periods in hospital environments and pose a substantial challenge for the future.

A model of biofilm EPS for optimizing micro Raman spectroscopic analysis as related to antibiotic resistance.
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Number: 42
Staphylococcus epidermidis is an opportunistic pathogen known for its ability to cause "chronic polymer associated infections". Such infections arise when *S. epidermidis* colonise implanted medical devices in the form of multilayered biofilms.
Biofilms are single or multi species bacterial communities that are enclosed in an extracellular polymeric substance (EPS) matrix. The EPS matrix has two main functions which are 1) to promote the attachment of the biofilm to a substratum and 2) to protect the biofilm population from environmental stresses. A major problem with treating biofilm based infections is that bacteria in biofilms are 10s to 100s of times more resistant to antibiotics than their planktonic counterparts.
Since biofilms contain an EPS matrix while planktonic bacteria do not, it can be argued that the biofilm EPS is a common factor which mediates antibiotic resistance processes. The chemical, morphological and mechanical changes in *S. epidermidis* biofilm EPS at sub-MIC levels of antibiotics will be assessed using Micro-Raman Spectroscopy and Atomic Force Microscopy (AFM). To assess and optimize the sensitivity of Raman spectroscopy to detect EPS components we made "artificial biofilms" of poly N-acetyl glucosamine (P-NAG), a common component of *S. epidermidis* EPS. Several peaks were identified within two ranges of wavenumber between 200 and 800 cm^{-1} and 2500 – 2700 cm^{-1}. In addition we added protein (bovine serum albumin) and antibotics to increase the chemical complexity of the model biofilm EPS. These data will be used to interpret biofilms grown in *in vitro* systems at sub MIC concentrations.

Methods for preparing *in-vivo* oral biofilms for FISH and CLSM
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Number: 43
Unclear initial stages of periodontal diseases comprise a recruitment of bacterial biofilms, which might be facilitated by certain factors. So far the influence of orthodontic appliances is little explored. In a pilot project we assess the spatial biofilm organization and development of plaque on fixed orthodontic appliances with different culture-independent methods (FISH, cLSM, SSCP, Clone Libraries). Our test group consists of fifty adolescents carrying palatinal expanders for four months fixed in the oral cavity, thereby implanting an anaerobic docking station for early colonizers. After initial colonization we expect formation of a heterogeneous anaerobic biofilm. Our aim is to develop a method to get samples carrying the intact biofilm for FISH and CLSM. The samples need to be taken without disturbing the biofilm coherence. As the materials of these appliances such as acrylic and stainless steel are very hard, they cannot be easily cut with a cryotome. We are

experimenting with several methods to obtain the whole biofilm directly for FISH: (i) scratching it off directly from the appliance, (ii) enclosing a small metal grid in the material that can be broken out and (iii) using chips of the cement by which those appliances are fixed to the teeth. FISH experiments using EUB388 as a general proteobacterial probe show the complex structure with early colonizers, late colonizers, and further typical biofilm structures such as channels. Specific probes will be used for more detailed illustration of the biofilm composition. The applied methods will contribute to the knowledge about in-vivo oral biofilm formation.

Do anaerobic bacteria play a role in the pathogenesis of clinical exacerbations in chronic pseudomonal human pulmonary disease?

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Number: 44

Background and Objectives: Cystic Fibrosis (CF) is a genetic disease with reduced life expectancy. Chronic infection with exacerbations of symptoms (CFPEs) contribute to decreased quality and duration of life. CFPEs are typically treated with parenteral antimicrobial therapy targeted against *Pseudomonas aeruginosa (Pa)*. Here we present culture-independent microbiological data from before during and after CFPE.

Methods and Results: With ethical approval, 14 subjects (mean age 30.2 years; 6 female; median predicted FEV1 53%) with CF (genotype ΔF508 homozygote 8/14) provided sputum samples 3 times/ week for 12 months. Twelve subjects experienced at least one CFPE. One CFPE from each of these was selected. Sputum samples from before during and after the start of parenteral antibiotics were selected for analysis. All samples underwent PMA-treated 16S T-RFLP as previously described.

At least 42 distinct species were detected (median 12 per subject; IQR 9.5-15.25). On the day prior to start of antibiotic therapy, *Pa* contributed a median of 42 % (IQR 5-75%) rising to 88% (IQR 43-95%) 7-10 days after the end of treatment ($p>0.05$ by one way ANOVA). By comparison, anaerobic species contributed to a median of 10% (IQR 7-28%) of the detected bacteria on the day prior to antibiotic treatment, which fell to median of 0% (IQR 0-0%) 7-10 days after the end of treatment ($p=0.0002$ by one way ANOVA).

Conclusions: Antimicrobial therapy for CFPE may not be targeting *Pa* and anaerobic bacteria may be important both in the pathogenesis and response to treatment in this chronic biofilm-dominated disease.

Interfacial Control of Alloy Implants by Self-Assembled Monolayers and Surface-Initiated Polymerization

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Number: 45

Implant infections affect millions of people per year. The goal of this project is to develop thin, multifunctional films on alloy implant materials. The films are designed to resist the non-specific adhesion of protein, bacteria and cells while delivering biologically active moieties, such as cell adhesion peptides. Robust surface chemistrywas developedusing self-assembled monolayers (SAMs) which act as a synthetically flexible platform for controlling the interfacial region between implants and tissues. The SAMs were then characterized using IR, AFM, and MS demonstrating comprehensive, strongly adhered monolayer coverage on two different oxide surfaces: stainless steel 316L and Ti-6Al-4V, both of which are commonly used in orthopedic implants. SAMs of long alkyl chains were bound to the surface through organic acid head groups, including phosphonic, carboxylic and sulfonic acids. These molecules were then used to modify the interfacial properties of the substrate by presenting hydroxyl, carboxylic acid, methyl, tri-ethylene glycol and fluorinated hydrocarbons at the surface. Additionally, these SAMs were used in surface-initiated polymerization reactions resulting in the formation of polyfluorostyrene. *Staphylococcus aureus* was used as a model organism to test the ability of specific tail groups presented at the surface to modulate bacterial adhesion and subsequent biofilm formation. Biofilm development on SAM surfaces was assessed by CFUs and *in situ* confocal imaging and image analysis over short and long time periods to provide a sensitive, yet robust statistically-based comparison of different monolayers.

Inverse Gas Chromatography: a novel method to determine the surface properties of microbial cells

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Number: 46

Abstract: Biofilm formation involves several steps: the transport and conditioning phase, the initial adhesion of microorganisms, the attachment stage and the final step of colonization. In order to better control biofilm formation and biofouling, the understanding of the initial steps of microbial adhesion, which mainly consists in non-specific physico-chemical interactions, is of crucial importance. Therefore, it is necessary to offer new techniques for measuring surface properties of microorganisms, which determine the type and strength of these interactions.

To characterize microbial cell surfaces, we have developed a simple and accurate method by using Inverse Gas Chromatography (IGC). IGC is a vapor adsorption method based on the analysis of the stationary phase whose surface characteristics are studied. The method allows calculating parameters such as specific and dispersive components of the surface energy of microbial cells. By using this method, we have analyzed the surface properties of two bacterial strains: *Lactococcus lactis* and *Hafnia alvei*. After culture under well defined conditions, washing and freeze-drying, bacteria were used to make the stationary phase.

The accuracy and reproducibility of the IGC method were evaluated by comparing IGC results with those obtained by contact angle measurements, infrared spectroscopy and also by the MATS (microbial adhesion to solvents) method.

On the whole, the results showed that it was possible to use IGC to evaluate the surface properties of bacterial cells, the present technique offering some advantages compared to the existing methods.

Immobilization of N-acylhomoserine lacton lactonase AiiA and its influence on *Escherichia coli* biofilm formation

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Number: 47

Biofilm is an almost ubiquitous lifestyle of microorganisms widespread in natural and technical systems. Actually there are great financial losses and potential pollution problems in many industrial sectors due to biofilm formation. Once the biofilm is established in a technical system, it is hard to remove. It has been shown that biofilm formation is under the control of quorum sensing (qs). Using the qs system bacteria are able to detect the local cell density by secreting and detecting autoinducer molecules. Strategies to disrupt this communication are called quorum quenching. One strategy includes hydrolysis of signal molecules by enzymes e.g. AHL lactonase. The aim of this study is to prevent biofilm formation by disrupting molecular cell-to-cell interactions of Gram-negative microorganisms using agarose beads with covalently bound AHL lactonase.

The GST-tagged AHL lactonase AiiA, which was expressed in *Escherichia coli*, was covalently linked to S-hexylglutathione agarose beads. To determine the biochemical activity a pH-sensitive assay with the substrate N-hexylhomoserine lactone and further HPLC analysis was done. To analyse the effect on the qs system the b-lactamase activity, which was artificially connected to the qs system in *Agrobacterium tumefaciens* NTL4, was studied using ONPG and 5-Brom-4-chlor-3-indoxyl-b-*D*-galactopyranoside as substrates. Subsequently the effect of covalently bound AHL lactonase on an *Escherichia coli* biofilm grown under static conditions was highlighted.

Our results indicate that covalently bound GST-tagged AiiA is able to convert its substrate and can reduce *Escherichia coli* biofilm formation.

Combined epifluorescence- and Kelvin probe force microscopy (EFM-KPFM) for the study of microbial attachment to ferrous surfaces

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Number: 48

Initial bacterial attachment to ferrous surfaces was studied using EFM-KPFM for opaque substrata. By KPFM, relative surface potentials can be visualized with high spatial resolution. Different environments and surfaces were examined: first, attachment of sulfate-reducing *Desulfovibrio vulgaris* to steel and second, of acidophilic *Leptospirillum ferriphilum* to pyrite. The former is important for microbially influenced corrosion, the latter for bioleaching of sulfidic ores. Electrochemically active sites (where dissolution occurs) play a crucial role in the initial attachment to both surfaces. Furthermore, attached cells influence electrochemical processes and, thus, surface potentials. Aim of the study is to correlate active sites with microbial attachment and to investigate the bacterial influence on surface potentials.

A BioMAT Workstation equipped with a Kelvin force module (JPK Instruments) was used. Potential and topography were visualized in intermittent contact mode (trace: profile acquisition; retrace: potential mapping) using ElectriTap300 cantilevers (Budgetsensors). Fluorescence staining (DAPI) was used for identification of cells and fluorescently labeled Concanavalin A for EPS compounds.

First results indicate increasing surface potential in close proximity to cells of D. vulgaris on non-alloyed steel (+50 mV after 2h incubation). On stainless steel, the increase of the surface potential is significantly reduced (+5 to +10 mV). In contrast, with colonies of L. ferriphilum on pyrite a significant decrease of the surface potential was noted (approx. -150 mV). In all cases, the differences in surface potential between cells and the surrounding material continued to increase with time. Currently, a statistical analysis of the results is under way.

Understanding the impact of biofilms on substrates through microscopic and spectroscopic analyses

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Number: 49

Microbial biofilms are communities of bacteria that adhere to and live on a wide range of substrates. They are a constant nuisance in industrial settings, as they often degrade the material they colonize and can be extraordinarily difficult to remove. The military also constantly battles biofilms, particularly in fuel distribution systems, where they can degrade metal, paint, and the fuel itself. Understanding the biofilm-substrate interaction and mechanisms of degradation are critical to the development of new biofilm-resistant materials. Research in this area can be greatly enhanced by the application of materials characterization tools that seldom have been applied to the study of biofilm-exposed subtrates. To this end, we are using a suite of microscopic and spectroscopic characterization tools to investigate how model biofilms interact with and degrade a conductive polyurethane substrate. In minimal medium, *Pseudomonas fluorescens* rapidly colonizes the coating, utilizing it as a carbon source and forming intracellular inclusions. SEM, TEM and AFM microscopy analyses of the biofilm-substrate interface suggest that the biofilm is intimately associated with the substrate and may be causing physical damage. We are also using EELS, ATR-FTIR, and XPS spectroscopies to investigate the substrate-microbe interface and how the chemistry of the substrate changes upon exposure. Simultaneously we are testing the function of the substrate upon exposure, by measuring conductivity changes through electrochemical impedance spectroscopy (EIS). Thus, using combined methodologies, we are beginning to understand the impact of the biofilms on the physical, chemical, and functional characteristics of the substrate.

Smart antibacterial surfaces for soft biomaterials: mechanically switchable silver-containing coatings

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Number: 50

Functional antimicrobial coatings containing additives which could be released into the surrounding environment under external stimuli, also known as smart surfaces, has been actively developed in the past

few years. In these systems, the control of surface properties and/or of drug delivery is induced by various external stimuli such as chemical/biochemical, thermal, electrical or optical stimuli, but rarely mechanical ones. We propose new responsive antibacterial coatings based on mechanical stimuli and specifically dedicated to soft biomaterials. Antibacterial nanospecies (silver nanoparticles) are incorporated into an alternation of reservoirs and/or barriers of maleic anhydride plasma polymers multilayers attached onto biomaterial surfaces. Under specific conditions, the plasma treatment leads to the formation of brittle silica-like layer at the surface, inducing cracks under mechanical stress, thus allowing species to release under elongation of the coating. We have demonstrated that the versatile chemistry of the anhydride groups allows the loading of silver nanoparticles into the plasma polymer layer, and we have shown that, taking advantage of the open/close crack system, it was possible to control the fluxand then to prolong the lifetime of the antimicrobial agent. The ways of antibacterial action of the incorporated silver nanoparticles has also been investigated. We believe that the handling processes during surgery and the natural strains always present at the implantation sites will provide the necessary mechanical stimuli. Such systems open the door to a new generation of prosthesis for abdominal wall or genitourinary reconstruction

Impact of the surface functional group density on bacteria adhesion

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Number: 51

Functionalization of materials with biomolecules has a broad application spectrum in implants and biosensors. An essential parameter for controlling eukaryotic cell or bacteria response is the accessibility of surface functional groups, which is strongly related to their surface density. However, model surfaces suitable for addressing this topic are rare and usually incompletely characterised. Moreover, the understanding of functional group density impact on bacteria adhesion to modified surfaces is poorly understood. The first challenge here was to provide model surfaces with varying grafting densities. Mixed monolayers on silicon wafer and on glass slide were made from silanes with two different end groups, methyl and bromine. Different ratios of silanes were used to control the functional group density, and bromine groups could be converted into amino groups for further use for grafting biomolecules. Two series of samples were fabricated: surfaces with five densities (i) of NH_2 groups in CH_3 groups, and (ii) of mannose residues grafted on NH_2 groups. The surfaces were thoroughly characterised. The second goal is to understand the mechanisms of bacterial adhesion in response to the functional group densities. Bacterial adhesion was investigated under confocal microscope in static and dynamic conditions using two types of *E. coli* K12 strains which are able or not to produce curli, but are both able to produce mannose-receptor type 1 pili. Gene expression of membrane proteins by RT-PCR and proteomic approach are now under investigation.

Bioinspired antifouling using natural products against marine biofilms

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Number: 52

Biofouling is the accumulation of marine organisms on underwater surfaces, causing increased hydrodynamic drag, resulting in higher fuel consumption and decreased speed and range. Specifically, a 14 % increase in ship fuel cost resulted from diatom-dominated biofilms. Past solutions to antifouling have used toxic coatings that severely affected marine life. The prohibited use of these antifoulants has led to the search for bio-inspired antifouling strategies. Algal species have evolved mechanisms, such as natural products (NP) and surface texturing, to deter predation and epidermal fouling. Significant effort has previously been directed towards surface topography, however, ultimately a combination of surface features and chemistry will lead to greater antifouling performance.

This study assessed NP antifouling performance of crude extracts and isolated compounds, from marine sources (*Chondrus crispus*) and a furan derivative from a terrestrial source, against biofouling organisms which included marine bacteria (*Cobetia marina*, and *Marinobacter hydrocarbonoclasticus*) and diatoms (*Cylindrotheca closterium* and *Amphora coffeaeformis*). Biofilm growth and adhesion kinetics were quantified using a multidetection microplate reader utilising viability staining and natural bioluminescence. Bioassays were corroborated using a novel application of the imaging capability of the microplate reader, to quantify biofouling *in situ*. Confocal laser scanning microscopy was used to compare biofilm structures in the presence and absence of the NPs.

The furan compound gave the best inhibition of biofilm growth and attachment. We are developing these novel techniques in combination with traditional methods, such as EC50 and LD50, to gain greater insight into marine biofilms and assessment of NP antifouling efficacy.

Anti-biofilm betaine surface modification on polyurethane medical devices

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Number 53

Indwelling catheters put patients at risk for infections generated by biofilm formation and proliferation on the surfaces of the implanted device. Traditional biofilm prevention has largely focused on applying leaching antimicrobial coatings to devices with variable clinical success and drawbacks including short term duration, limited spectrum of activity, potential toxicity and generation of drug-resistant strains. We examined the performance of a potentially superior approach by using highly water-coordinating, nonfouling materials to prevent bacterial attachment and subsequent biofilm formation in a blood environment.

Polyurethane catheter substrates (14-French rods) were modified using betaine, zwitterionic structures. *Escherichia coli* ATCC 25922 was used in a modified CDC system. In order to mimic the clinical setting, samples were exposed to 50% fetal bovine serum for 18 hours before bacterial challenge. Rods were then exposed to a bacterial suspension of 10^6 cfu/ml in 1xPBS in batch mode for 2 hours at 37°C with agitation. Thereafter, the rods were transferred to a fresh reactor and exposed to modified M63 media under flow at 8 ml/min. Biofilm growth was monitored by plate counts and macroscopic visualization of biofilm surface coverage after 24 hours. Log reduction (LR) differences were calculated on surface modified rods and polyurethane controls. Betaine surface modified rods demonstrated a mean LR of 2.851 (Range 2.312-3.542 $p<0.001$) exhibiting strong antibiofilm properties. These structures have reduced thrombus formation in blood flow-loop studies, demonstrating dual anti-microbial and anti-thrombotic characteristics.

The impact of surface modification with fungal hydrophobins on microbial biofilm formation

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Number: 54

The characteristics of a material and its corresponding surface properties are discussed to affect the biocompatibility and consequently bacterial adhesion. In this approach hydrophobins are used as a novel modification of materials to change the surface properties and thus to influence microbial biofilm formation.

Hydrophobins are non-toxic fungal proteins which self-assemble on different surfaces into extremely stable monolayers in an amphiphilic manner. Recombinant hydrophobins provide the opportunity to use these highly surface-active proteins for large-scale surface modification and functionalization (with e.g. enzymes) of industrial and medical relevant materials.

Thus, hydrophobin coating protocols were developed for different materials. Quartz crystal microbalance measurements were used to analyse the adsorption behaviour and contact angle measurements, immunofluorescent labellings and atomic force microscopy were applied to characterize the protein coatings regarding hydrophobicity and homogeneity. The recombinant hydrophobins self-assembled on the surfaces depending on different parameters such as incubation temperature or incubation time.

The growth behaviour of various microorganisms was studied on hydrophobin modified versus unmodified surfaces. Single bacteria strains as well as natural bacterial communities were used to analyse biofilm formation. Apart from conventional plating experiments, fluorescent microscopy and molecular-biological methods such as denaturing gradient gel electrophoresis were applied. So far no significant differences in the biofilm formation on hydrophobin-coated versus uncoated surfaces were detected.

Recombinant hydrophobins could definitely be used for effective surface coating in monolayer manner. To stimulate the antibiofouling effect of the coating the hydrophobins need to be modified by e.g. antimicrobial peptides to influence the bacterial adhesion process.

Antimicrobial surface based on self-assembled nanoreactors: from bloc copolymer to bacterial adhesion study

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Number: 55

In order to prevent and treat biofilm formation, we aim at producing nanostructured bioactive surfaces resulting from the surface-immobilization of nanoreactors from self assembled amphiphilic polymer poly(isobutylene)-b-oligonucleotide, which was synthesized and characterized via various techniques, demonstrating the chemistry versatility and allowing the elucidation of its self-assembly properties. Self assembly into vesicles allows the encapsulation of enzymes like lactoperoxidase (LPO), used in a prodrug-drug system strategy to provide bioactive properties to the surface. LPO natural system is known to be highly biocompatible and not to be involved in any strain resistance. We demonstrated that LPO activity of encapsulated enzyme is preserved compared to the free enzyme and that encapsulation protects the enzyme from inactivation allowing to keep

activity for several weeks in the presence of Proteinase K. The production of antimicrobial surfaces was achieved through the specific immobilization of the nanoreactors onto surfaces via Crick-Watson base pairing using the oligonucleotide block of the copolymer.

Parallel to the analysis of the nanoreactor antibacterial efficiency, the impact of the chemical and topographical surface properties on the substrate-bacteria interactions is analysed both in static and in dynamic modes to determine the link between curli expression, oligonucleotide sequence onto surfaces and topographical feature during *E.coli* adhesion onto such surfaces. Using mutant strains of *E.coli* K12, convincing evidence was already obtained that, independently of the topographical feature used in this study, oligonucleotide-modified surfaces enhanced curli expression without increasing number of adherent bacteria [N.Cottenye et al. 2008].

Biofilms Growth on Nano-structured Surfaces
Jun F. Liang, Ridd Kharidia, Jinwoo Park

Number: 56
Significance and objectives: The properties of the substratum surfaces are crucial to bacterial adhesion and biofilm growth. Factors affecting bacterial adhesion and colonization have been extensively investigated. However, results from different studies are still controversy and very confused.

Methods and results: More and more evidence suggests that both the charge distribution and the receptor dispersion on bacterium surfaces are heterogeneous, and bacterial adhesion onto a surface is a nanoscale event. We have created surfaces with tunable nano-structures and chemical/biological properties to study bacterial adhesion and biofilm growth. Results show that surface nano-structures greatly affect cell growth, differentiation, and colonization.

Conclusions: Bacterial adhesion and growth can be controlled by modulating surface structures and chemical (or biological) properties at nanoscales.

Influence of subsurface composition on the adhesion of bacteria and the adsorption of proteins
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Number: 57
Biofilms are of special importance in various fields of the everyday life. Their initial formation is composed of two crucial steps: the adsorption of proteins and the adhesion of bacteria. These are complicated processes that depend on many factors.

So far, most studies focused on surface chemistry, hydrophobicity and surface roughness - factors which influence mainly the short range interactions.

Our studies concentrate on the impact of long range interactions, in particular van der Waals forces, which can be tuned by the use of tailored substrates. To characterize the processes, we follow two pathways: One way is to characterize protein adsorption on a fundamental level via ellipsometry. Another is to directly probe bacterial adhesion by AFM - force spectroscopy. As model systems we use *Staphylococcus aureus* bacteria and proteins like amylase, lysozyme and bovine serum albumin.

The results of our experiments show that protein adsorption kinetics as well as bacterial adhesion are dependent on the subsurface composition of the substrate [1,2]. Hence it is of great importance for the design of anti-adhesive surfaces to consider not only the lateral but also the vertical composition of the substrate.

[1] A. Quinn et al., *Europhysics Lett.* **81** (2008) 56003

[2] M. Bellion et al., *J. Phys.: Condens. Matter* **20** (2008) 404226

Micro-beads as models and probes for studying the interactions between a surface and microbes
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Number: 58
Interactions between a surface and microbes (and single-cells in general) are the basis of infection and contamination properties of microbial strains and key in engineered innovative surfaces preventing or promoting microbial adhesion and biofilm formation. For contributing to this science, we suggest observing model micro-beads mimicking the bulk physical-chemistry of microbes and using them as probes to evaluate bulk surface properties. Here we apply this approach for deciphering key interactions modulating microbial adhesion in order to identify routes to prevent such events.

We use paramagnetic micro-beads with defined size, density and surface properties as model micro-objects. Their interaction with a surface is evaluated semi-quantitatively through the resulting impact on their magnetic mobility when placed into a magnetic field gradient with ad-hoc geometry using the BioFilm Ring Test (R) system. Physical-chemistry parameters of the solution and surface coating strongly modulate the observed signal reflecting beads magnetic mobility. Using diversified conditions led to identify the contributions of

sources of attractive and repulsive interactions and also to identify one unexpected route for efficient anti-adhesion coating. Using the same setup, it is possible to follow microbial adhesion and biofilm formation and evaluate the biological efficiency of such coatings.

In conclusion, paramagnetic microbead magnetic mobility measurement is a useful tool for fast and easy evaluation of surface coatings and for following microbial colonization of a surface and initiation of biofilm formation. With this approach a key interaction between surface and micro-objects could be identified and engineered into a promising anti-adhesive coating.

Attachment of Campylobacter jejuni to stainless steel is enhanced by increased cell surface hydrophobicity and agrees with XDLVO predictions

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[3]Monash University, Australia

Number: 59

The ability of *Campylobacter jejuni* to attach to abiotic surfaces is a major concern for food industries. This work aimed to investigate the influence of bacterial surface physicochemical properties on attachment of three *C. jejuni* strains to, and their probability of detachment from, stainless steel (SS). Cell surface hydrophobicity and charge were determined by water contact angle and zeta potential measurements, respectively. The numbers of cells attaching to, and their probability of detachment from, SS were quantified by epifluorescence microscopy enumeration and a successive blotting technique, respectively. The results revealed the three strains tested varied significantly ($P < 0.05$) with respect to cell surface hydrophobicity but had comparable surface charge. The more hydrophobic strains attached at higher numbers to, and had lower probability of detachment from, SS suggesting that increased cell surface hydrophobicity enhances the level and strength of attachment of *C. jejuni* to SS. The more adherent strains were found to have significantly ($P < 0.05$) greater interaction energies calculated using the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory, suggesting *C. jejuni* attachment behavior to SS can be interpreted by this theory. However, cell electron donor/acceptor properties obtained from contact angle measurement (CAM) to calculate acid-base (AB) interactions in the XDLVO theory did not significantly ($P < 0.05$) correlate with the ones obtained from microbial adhesion to solvents (MATS) also determined in a related study. This indicates that AB interactions may not be accurately quantified using CAM data only and its usefulness should be verified using the outcomes from the MATS method.

Theme: Global scale biofilm systems

In situ monitoring of biofilm growth and disinfection using a thermal analysis measurement technique

Céline Bouteleux, Olivier Horner, EDF Research and Development, France

Number: 60

Biofilms are heterogeneous bacterial formations, which can grow on the inner surface of loop water circuits. They provide several advantages to bacteria, in particular they offer better protection against toxic agents (*i.e.* disinfectant solutions). In some cases, biofilms can function as a reservoir of pathogenic organisms such as *Legionella*. In an industrial cooling circuit, the development of biofilm on the walls of circuits can have various harmful consequences, such as loss of thermic performance and present health and environmental risks. To control this bacterial development, a better understanding of the growth and behaviour of biofilm is necessary.

With this objective in mind, we have used a thermal analysis measurement technique (a fouling sensor) in order to measure in real time and *in situ* the biofilm thickness in an industrial cooling circuit pilot. Indeed, a number of non-invasive and non-destructive techniques have been put forward and investigated in order to obtain information about the dynamic properties of biofilms.

The thermal analysis measurement technique was sound to be reliable in our conditions and sufficiently sensitive to follow the behaviour of a biofilm in raw river water circuit, and to evaluate the efficiency of two biocide treatments (*i.e.* a monochloramination and a chlorine cleaning treatment) which were applied. However, the data show that there is no correlation between biofilm thickness measurements and microbiological counts (especially the pathogenic *Legionella* bacteria and *Naegleria fowleri* amoeba), either in recirculating cooling water or in biofilm, as already shown in the literature.

The EPS matrix as an external digestion system

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Number: 61

The extracellular polymeric substances (EPS) provide a complex matrix in which microorganisms organize the biofilm mode of life. It consists of polysaccharides, proteins, nucleic acids and lipids. Most proteins in the matrix are enzymes, with examples of strong interaction to polysaccharides retaining the enzymes, forming an activated matrix. A wide variety of extracellular enzymes has been identified in biofilms from many different sources. Most are involved in the degradation of biopolymers. Natural substrates of extracellular enzymes can be water-soluble polymers (many polysaccharides, proteins and nucleic acids) and water-insoluble compounds. The latter include, e.g., cellulose, chitin, and lipids as well as organic particles trapped in biofilms. This is why the EPS matrix can be regarded as an external digestion system, breaking down biopolymers to low-molecular-weight products which then can be taken up as carbon and energy sources**.** Enzyme-mediated degradation of polymers represents the rate-limiting step in microbial utilization of dissolved and particulate organic matter in all terrestrial and aquatic environments. Since biofilms represent the common mode of microbial life, it can be plausibly assumed that matrix-associated enzyme activities are critical for the global turnover of biopolymers. Extracellular enzymes carry out the self-purification processes in soils, sediments and water. The quantitative contribution of extracellular enzymatic activity of the biofilm matrix in the carbon cycle has global dimensions but it has not yet been quantified on that scale.

Variations in Extracellular Polysaccharides content and microbial community in grassland soils

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Number: 62

A study of the variation of Extracellular Polysaccharides (EPS) in grassland soils is reported with a view to understanding the relationship to soil properties such as soil stability or aggregation. EPS which is the larger fraction of the biofilm from the biomass in soils, also has the capacity to retain moisture during dry periods. The relationship between EPS concentration and the microbial community and seasonal variation was also determined. Different strength acid was used to extraction microbial or labile EPS and total EPS that may contain vegetative polysaccharides. Changes in microbial community were assessed using phospholipids fatty acid (PLFA) analysis, PLFAs that originate from cell walls of active gram positive, gram negative, fungal and actinomyces organisms in the soil can be quantified. Further fractionation of polysaccharides was performed after enzymatic hydrolysis of the labile fraction using HPLC methods. HPLC analysis of sugars can be used to distinguish between microbial and plant carbohydrates in soils.

Results demonstrated that a variation in the labile EPS (with a range of 0.3mg to 0.9mg g^{-1} dry mass and total EPS fractions with a range of 0.4mg to 0.9mg g^{-1} dry mass for different soil locations in the grassland soil

samples collected at North Wyke research station. The HPLC results confirmed the different fractions identity from the EPS extracted from the samples. Soil aggregation was also influenced by the quality and quantity of EPS present.

A review of biofilm decontamination in space module habitats and on space suits

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Number: 63

Significance and objectives: Biofilms in the MIR space station caused material biodegradation that may have led to safety issues and there are concerns that biofilms on planetary missions may forward contaminate the target planet breaching international planetary protection treaties. Therefore there is a requirement to decontaminate space flight module interiors and also Extra Vehicular Activity (EVA) space-suits whilst on Mars to reduce forwards or backwards contamination. Methods: A literature review of decontamination technologies that could be employed singularly or in combination for habitat module interiors on missions < 6 months and > 6 months was carried out using a scoring criteria and trade-off matrix to select appropriate technologies. Results: For missions < 6 months disinfectant wipes were recommended in combination with air disinfection/filtration systems. Missions > 6 months would also require gaseous decontamination systems, to periodically reduce the inaccessible bioburden. In addition, decontamination technologies for the inside and outside surfaces of EVA space-suits (on Lunar and Martian surfaces) were also reviewed. Only physical decontamination of the exterior EVA suit surface is required for Lunar missions, but for Martian missions this would be enhanced with gaseous disinfection. The interior of the suit could be decontaminated using passive antimicrobial fabrics and active cleaning using disinfectant wipes. **Conclusions:** As a result of this review a range of different decontamination technologies have been recommended to prevent the colonisation of surfaces in the habitat module and on the EVA suits but further research is required to develop these technologies for use in actual missions.

Biofouling monitoring in semi-open cooling water circuits

Bart Verhasselt, Laborec, Belgium

Number: 64

Fouling monitoring in semi-open cooling water circuits of power plants is an interesting tool for the set up, the follow up and the control of the disinfection and the anti-scaling treatments of the cooling water. Laborelec tested in 2009 the Neosens FS-900 fouling monitor in its own "MERADES II" cooling water pilot installation. The working principle of this fouling monitor is based on a patented thermal process. This enables to characterize the fouling thickness in the circuit. During the tests, the influence of a continuous/shock injection of chlorine dioxide on the biofilm layer was observed by the FS-900 fouling monitor. Tests showed out that this type of fouling monitor detect well the growth of biofilm and the impact of a biocide injection on the biofilm thickness in a semi-open cooling water circuit. However, with the thermal system developed by Neosens, it is sometimes difficult to conclude if the fouling comes from a biofilm accumulation or a scale formation on the sensor. It has also been evidenced that the system is quite sensitive to non constant hydrodynamic conditions at the sensor level. This problem should be partly solved by the new generation of FS-1000 monitors. In 2010, Laborelec is executing tests with this new type of fouling monitor on a full scale cooling water circuit.

Theme: Community ecology and evolution

Succession and interaction of bacterial and protozoan communities within semi-natural river biofilms

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Number: 65

Grazing by protozoans is considered to be one major sink for bacterial production in both planktonic and benthic environments. Our aim is to develop and apply tools to investigate the effects of protozoan grazing on semi-natural river biofilms, and to identify the mechanisms underlying the observed effects. Therefore we cultivated bacteria in flow cells fed by natural river water at the Ecological Rhine Station of the University of Cologne (Germany) and manipulated the presence of flagellated grazers by size fractionation. Previous experiments with this setup have shown that the natural bacterial assemblage in the Rhine forms biofilms faster and with a higher microcolony/single cell ratio when HF are present, compared to setups without HF. Our hypothesis for the present experiment was that these morphological differences are also accompanied by changes in the bacterial community composition (BCC). We examined morphological and taxonomical changes during biofilm succession by light microscopy, Confocal Laser Scanning Microscopy and Denaturing Gradient Gel Electrophoresis over a period of five days. HF abundances rose from 0 to ca. 2,500 ind. cm^{-2} within this period of time, with *Planomonas* sp., *Rhynchomonas nasuta* and *Neobodo designis* as the dominating species. Although we could again detect the above mentioned changes in biofilm morphology due to the presence of HF, the BCC did not differ between the two setups. Still more surprising, we could not detect a succession of the bacterial community in both setups, i.e., BCC was the same for both treatments on all days.

Biofilms in metalworking fluids

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Number: 66

Metalworking fluids play a critical role whenever metal is shaped in modern tool machines. The cooling and lubrication provided by these oil-water emulsions are important factors in improving the quality of the product and the lifetime of the tools.

As these machines are open systems, the metalworking fluids are colonized and eventually degraded by microorganisms. Consequently industrial research has a considerable interest in the microbiology of these systems. However, most previous studies have focused on the planctonic organisms of the liquid phase, with little work done on biofilms.

In this CTI-funded project an existing industrial test reactor for metalworking fluids (designed by Blaser Swisslube AG) was adapted for biofilm cultivation. Biofilms were cultivated in different Blasocut®Bioconcept formulations, a metalworking fluid designed to maintain stability by allowing a dominant population of *P. pseudoalcaligenes* that prevents growth of other species.

The reactors were monitored for up to eight weeks for planctonic and biofilm growth, which was quantified using classical cultivation methods and total mass. Growth was reproducible independent of the test coupons material (stainless steel or aluminium). Different biofilm quantities were shown for different Blasocut®Bioconcept formulations, strongly corresponding with planctonic bacterial densities.

In order to study the microbial diversity of the biofilm and determine how it differed from the planctonic phase, samples were also analysed by mass spectroscopy (MALDI-TOF), quantitative PCR and denaturing gradient gel electrophoresis.

Qualitative proteomic comparison between planktonic and biofilm-forming cells of Acidithiobacillus ferrooxidans ATCC 23270

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Number: 67

Bioleaching is the extraction of metals, such as copper or gold, from sulfidic ores by microorganisms. Their energy for growth is obtained by oxidation of ferrous iron and/or reduced inorganic sulphur compounds. Bacterial attachment increases leaching activities since a special microenvironment is formed between the bacterium and the metal sulfide surface, filled by extracellular polymeric substances (EPS). Planktonic and sessile cells should significantly differ in their gene expression and proteomic patterns. We started proteomic analyses of Acidithiobacillus ferrooxidans ATCC 23270 biofilm cells, attached to pyrite, and planktonic cells. A protocol for protein extraction from both subpopulations was developed. After separation by SDS page, gel lanes

excision and tryptic protein digestion of gel slices, the resulting peptides were analyzed by high resolution mass spectrometry (reverse-phase nano-UHPLC column coupled to an LTQ-Orbitrap XL). More than 500 proteins were reliably identified in both samples after 24 h of biofilm formation. Transport functions, lipoproteins, membrane related functions and unknown proteins were found to be enhanced in biofilm cells. When possible, functional categories and probable subcellular location were assigned. This first global proteomic study will provide new insights into At. ferrooxidans biofilm formation process and lifestyle, improving also its genome sequence annotation.

Effect of arginine dosing on salivary microcosms in continuous culture

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Number: 68

Objectives: To evaluate the effects of arginine dosing on salivary microcosms in continuous culture.

Methods: Constant depth film fermenters (CDFFs), were inoculated with fresh saliva and fed with artificial saliva. Separate CDFFs were supplemented with sterile water or 5% sucrose. Separate CDFFs were dosed with arginine (ARG; 8%) or with a dentifrice with (DA) and without (DN) arginine (8%). for 40d, after which fermenters were maintained for an additional 8d without dosing. Plaque samples were removed at regular intervals for viable counting, pH monitoring and eubacterial PCR-DGGE.

Results: Significant pH increases ($p < 0.01$) occurred within plaques following dosing with ARG and DA but not with DN. This effect was maintained after dosing ceased only for DA. Whilst numbers of total anaerobes, aerobes, Gram negative anaerobes, acidoduric, acidogenic or arginine utilising species, bifidobacteria, lactobacilli and streptococci were significantly higher ($p < 0.01$) in sucrose-supplemented fermenters, c. 40d dosing regimes did not markedly alter the relative densities of these organisms. Principal components and cluster analysis of DGGE fingerprints indicated that DA and DN-dosed plaques were not markedly different from undosed plaques.

Conclusions: Prolonged exposure of established oral microcosms to arginine resulted in increases in plaque pH without markedly influencing eubacterial composition.

Multidisciplinary approach using Confocal Laser Scanning Microscopy-Image analysis and fluorochromes to determine the effect of heavy metals in viability and biomass of *Micrococcus luteus* DE2008, isolated from microbial mats

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Number: 69

Significance and objectives

In the past few years our working group has developed a method to determine the mass of microorganisms, individually and to micrometric scale using Confocal Laser Scanning Microscopy and an image analysis system (CLSM-IA). We have recently improved this method by including fluorochromes (FLU) for analyzing the samples and the percentage of living and dead cells. The aim of this work, by using this method, is to evaluate the effects of the heavy metals, lead (Pb) and copper (Cu) on *Micrococcus luteus* DE2008, isolated from Ebro Delta microbial mats.

Methods and results *Micrococcus luteus* DE2008 was grown at different concentrations of lead and copper. Culture aliquots were analysed by CLSM-IA-FLU, using the following fluorochromes: SYTOX Green (dead cells) and Hoechst 33342 (live cells).

The viability and biomass from *Micrococcus luteus* DE2008 is influenced negatively by the presence of heavy metals. The results show a 95% decreasing viability at 60% to 2 mM Pb^{2+}, and a 95% decreasing viability at 40% to 2 mM Cu^{2+} and these data are well correlated in both cases with biomass, which decreased from 92.04 to 48.61 and 80.93 to 5.34 mgC/cm^3 in Pb and in Cu-polluted cultures, respectively.

Conclusions From these results, we conclude that CLSM-IA-FLU is a feasible method for analyzing the physiological state of cells and their biomass, not only in cultures, but also in aggregates and biofilms.

Diversity of bacterial biofilm communities growing on γ-Hexachlorocyclohexane

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Number: 70

γ-Hexachlorocyclohexane (HCH) is an organochlorine pollutant that persist in the environment for a long time. This compound is very difficult for a bacteria to degrade it. However, bacteria can help each other within a community making the degradation process easier. The purpose of this work was to determine the extent of microbial potential for the degradation of γ-HCH in soils. This study addressed the huge diversity of bacteria

from 13 soil samples collected around insecticide and pesticide producing factories from (Egypt). From γ-HCH enrichment cultures, all samples yielded high biodiversity as revealed by the analyses of the16S rRNA genes of the isolates. γ-HCH-degrading bacteria were found to fall within the genera *Acetobacter, Achromobacter, Bacillus, Brevundimonas, Cupriavidus, Novosphingobium*, *Paenibacillus, Pseudomonas* and *Rhodococcus*. Soil and sediment samples were used to inoculate γ-HCH microcrystals on a substratum (Permanox™) in microcosms to grow complex biofilm communities on γ-HCH. The biofilms were monitored for about 42 days by community fingerprinting using single strand conformational polymorphism (SSCP) of 16S rRNA gene amplicons. All soil samples yielded biofilms on γ-HCH and SSCP analyses of the biofilms revealed rather diverse bacterial communities with species of genera closely related to *Pseudomonas*, *Sphingomonas* (as dominant members), *Burkholderia, Caulobacter, Comamonas, Nitrosospira, Ochrobactrum, Planococcus, Parvibaculum* and *Sterolibacterium*. Samples were also analysed by confocal and scanning microscopy. From the soil samples, multispecies were obtained and most of them could use γ-HCH as sole source of carbon in a minimal medium. The majority of these isolates belonged to the genus *Bacillus* but *Paenibacillus* or *Achromobacter* species were also among them. Bacteria in microbial communities play different roles and together they are able to form biofilms using γ-HCH as a carbon source: (a functional diversity cooperation).

Seasonal stability of microbial population of the rock biofilm from ancient gold and arsenic mine
Karolina Tomczyk-Zak, Urszula Zielenkiewicz, Szymon Kaczanowski, IBB PAS, Poland

Number: 71
Unusual rock biofilm developed in the closed mine in Złoty Stok (Poland) with a very high concentration of As. The biofilm's matrix is a heterogeneous, lattice structure, composed mainly of silica, with embedded bacteria and mineral particles. Analysis of the species content using culture independent techniques revealed a high level of diversity. The best represented classes were *α-Proteobacteria*, *Verrucomicrobia* and *Planctomycetes*. The remaining sequences were closely related to *β-*, *ε/δ-*, *γ-Proteobacteria*, *Chloroflexi*, *Bacteroides*, *Bacilli* and *Actinobacteria*.

Due to seasonal washing of the biofilm by ground water and a high probability that it is a recent formation which occurs in a small part of the mine, it was important to investigate whether this population is already stable.

We compared two pools of unique 16S rRNA sequences of the biofilm taken from the mine at a six month interval. To accomplish this we isolated the total DNA, amplified and cloned 16S rRNA genes, which were sequenced following the selection of the unique V3 region by MSSCP method. Two sets of 16S rRNA sequences were investigated using the LIBSHUFF program and by phylogenetic analysis. The probability that both pools were identical was above 0.05. Phylogenetic analysis revealed similar location of sequences from the two pools in the phylogenetic tree and a similar percentage number of sequences belonging to the same class.

In conclusion, the microbial population of the analyzed biofilm seems to be seasonally stable with respect to quality and quantity. Statistical analysis confirms these results.

Activity and structure of microbial biofilm in river sediments with focus on methane production
Lenka Brablcova, Palacky University in Olomouc, Czech Republic

Number: 72
Methane production in river sediments is primarily dependent on the methanogenic archaea presence. We studied five localities alongside a stream and two sediment layers by means of following methods.
Total numbers of bacteria were counted, phylogenetic composition of main domains and selected groups (methanogenic archaea, methanotrophic bacteria) were identified by fluorescence in situ hybridization (FISH) and enzymatic activity and methane production of the river sediments were measured. The research was supported by a grant GACR 526/09/1639

Characterization of microbial populations of the biofilm developed on carbon steel immersed in seawater
Mikaël Langumier, Benoît Fernandez, Isabelle Lanneluc, Marc Jeannin, René Sabot, Sophie Sable, Phillippe Refait, Université de La Rochelle

Number: 73
Since several years, the influence of micro-organisms on carbon steel corrosion has been studied. It is now clearly demonstrated that in some cases micro-organisms can accelerate corrosion processes and lead to significant damages, even to a structure breakdown after several years. This phenomenon called biocorrosion or microbiologically influenced corrosion (MIC) is not completely understood yet. Various bacteria communities grow on metal structures and form a biofilm in which these communities interact and can affect corrosion rates. Sulphate-reducing bacteria are micro-organisms commonly associated with MIC, but other species are supposed to be involved. The purpose of this study was to characterize accurately the bacteria microflora of the "rust/bacteria" composite biofilm formed on steel in marine environments.

Bacterial diversity was studied on biofilm samples collected on carbon steel at various immersion times. The quantitative variation with time of total population was monitored by epifluorescence microscopy. Then, the study was separated in two parts. On the one hand, cultured bacteria were isolated in aerobic and micro-aerobic conditions and identified by molecular biology. On the other hand, total bacterial diversity of the same biofilm samples were studied by TTGE (Temporal Temperature Gradient Gel Electrophoresis) focusing on a polymorphic region of 16SrDNA. The results obtained by culture and TTGE will be compared to determine the representativeness of the cultured bacteria in the total bacterial diversity in the biofilm and we will study their effect in the steel corrosion.

Community interactions promote *Legionella pneumophila* survival in drinking water biofilms

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Number: 74

Legionella pneumophila is a waterborne pathogen that can cause Pontiac Fever or Legionnaires' disease, a type of pneumonia that can be fatal. Although *L. pneumophila* is not able to replicate in low nutrient environments, such as drinking water, it is known that heterotrophic biofilms have a crucial role in the survival of this pathogen in drinking water distribution systems. The aim of this work is to study the community interactions that influence the survival of *L. pneumophila* in biofilms. For that, mono and dual-species biofilms of *L. pneumophila* and the predominant biofilm isolates *Variovorax paradoxus*, *Mycobacterium chelonae*, *Acidovorax* spp., *Sphingomonas* spp., were formed on PVC surfaces and sessile cells quantified for total cells, viable and cultivable *L. pneumophila* and cultivable non-*Legionellae*. Results demonstrated that *Acidovorax* spp. and *Sphingomonas* spp. appear to have an antagonistic effect on *L. pneumophila* cultivability but not in the viability, leading to the formation of viable but noncultivable (VBNC) cells, while *M. chelonae* increased the cultivability of this pathogen. *M. chelonae* is one of the microorganisms commonly found in drinking water and this work demonstrates that this strain is able to promote *L. pneumophila* survival in these systems. It is also demonstrated that other species might stimulate this pathogen to enter a VBNC state and consequently be underestimated in the drinking water quality control, as drinking water safety assessment still relies on standard culture techniques. It is essential for future work to study other biofilm community members to understand their ecological interactions with *L. pneumophila*.

The Inclusion of Anaerobic Isolates in Multispecies Continuous Culture Biofilm Model Systems Grown Under Aerobic Conditions for Evaluation of Treatment Efficacy

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Number: 75

Naturally occurring biofilms usually contain multiple species. Single species models have provided crucial data regarding the nature and treatment of biofilms. However, interactions within multispecies biofilms affect community physiology and metabolism which may impact treatment efficacy. Anaerobic species have been shown to be present in biofilms on infected devices and in chronic wounds. The development of a multispecies in vitro model which includes anaerobes can be complicated, requiring the detection of all test species in order to confirm their presence within the biofilm and to evaluate treatment efficacies. We have developed continuous culture multispecies models which include anaerobic species grown in biofilms under aerobic conditions. The nine species dental model, grown in the Drip Flow Reactor (DFR) at 37°C, is inoculated with five facultative anaerobic species first followed by inoculation with four strict anaerobic species three days later. The three species wound model, grown in the ColonyDFR, is inoculated simultaneously with *Clostridium perfringens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The two species device infection model, grown in Sorbarod filters, is inoculated simultaneously with *Propionebacterium acnes* and *Staphylococcus epidermidis*. In all three model systems, anaerobic species are grown anaerobically but are inoculated into the model system under aerobic conditions. Confirmation of species present in these model biofilms was performed using selective plating; counts ranged from 10^5 to 10^{10} CFU/cm^2 with run to run repeatability. Whole and cyrosectioned biofilms were microscopically imaged. Treatments showed different efficacy based on species indicating that these models can provide valuable information regarding treatment efficacy in multispecies biofilms.

Effect of salinity on the biological activities and the biodiversity of a summerged filter bioreactor for the treatment of urban waste waters

Carmen Cortes, Spain

Number: 76

The presence of salinity in urban waste-waters may affect in a significant manner the good function of the summerged-filter biological reactors due to the effect of high salt concentrations on the structure and biodiversity of the microbial biofilms responsible of the depurative process.

The wastewater treatment plant (laboratory scale) consisted of one aerated column with a plastic filling on which the biofilm was formed. The spatial diversity of the bacterial communities in the plant biofilms was analysed by taking samples of the system when it was subjected at four different salinity concentrations.

The bacterial diversity of the submerged filter was studied by a cultivation-independent approach based on PCR/TGGE (temperature-gradient gel electrophoresis) and Fluorescence in situ hybridizacion (FISH) was applied to follow the evolucion of de microbial composition of a submerged filter. In addition, the biological activities of microorganisms forming the biofilm were studied by performing enzymatic activities assays, e.i. glucosidase, phosphatases, proteases and esterases.

The TGGE profiles of PCR-amplified sequences of the 16 S rRNA gene (V3-hypervariable region) showed significant variations of the bacterial diversity, mainly depending on the concentration of salt in the system.

Overall, the results obtained in our study suggest that in spite of changes induced by the different salinities, the fix-biofilm systems represent an important adaptation capability to these environmental changes referring to their depurative activity and their bacterial communities. This fact makes these systems specially useful for the treatment of waste-waters subjected to variable salt concentrations.

Combined high-resolution microscopic techniques to determine the capacity of cyanobacteria and a microalga DE2009 to remove heavy metals in natural ecosystems

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Number: 77

Significance and objectives

Little is known about the role of intracellular inclusions in microorganisms living in natural ecosystems and also if they can be used as bioindicators of pollution by heavy metals.

The aim of this work is to evaluate by high-resolution microscopic techniques, the capacity of different cyanobacteria and a microalga (DE2009) living in microbial mats, to bioadsorb and/or bioaccumulate heavy metals.

Methods and results

Microbial mat samples were grown in microcosms and then polluted with lead or copper. To demonstrate the capacity of different microorganisms to capture heavy metals, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) coupled to Energy dispersive X-ray spectroscopy (EDX) were applied to all the samples. Similar experiments were performed in axenic cultures of cyanobacteria and in the microalga.

Among all the cyanobacteria tested (*Microcoleus* sp., *Oscillatoria* sp., *Spirulina* sp. and *Chroococcus* sp.), *Microcoleus* sp. was the most efficient for heavy metals adsorption. In polluted cultures and microcosms, this cyanobacterium showed peaks of lead and copper in cell envelopes and in polyphosphate inclusions. No peaks of metals were detected in unpolluted conditions. The microalga DE2009 also showed a great affinity for both heavy metals, but it was found in lower numbers than *Microcoleus* sp. (the most abundant) in microcosm samples and in Ebro delta microbial mats.

Conclusions

From these results, we conclude that *Microcoleus* sp., and in particular, analysis of its polyphosphate inclusions, could be considered as a good bioindicator to predict whether an ecosystem is polluted by heavy metals and also to indentify them.

***In vitro* adaptation of *P. aeruginosa*: colony morphology variants selection and virulence characterization**

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Number: 78

One of the bacterial strategies to respond to environmental pressures is the switch of their phenotypic traits by a mechanism called phase variation. This reversible process provides the generation of varied bacterial phenotypes, leading to a mixed population and colony diversity.

In this work it was aimed to isolate and characterize the virulence of colony morphology variants selected by environmental pressures from planktonic and sessile *P.aeruginosa*, in order to understand the biological significance of phase variation in virulent-bacteria selection.

Bacteria were *in vitro* stressed by continuous exposure to increased concentrations of benzalkonium chloride (BZK) and by heat and peroxide hydrogen shock. The stressed bacteria were suspended, serial diluted and plated onto TSA to inspect and collect colony morphology variants.

It was observed, for planktonic and biofilm states, that adaptation and heat and chemical shocks selected different colony morphologies. These differences were in colony circumference and outer edge (smooth or irregular), surface texture and surface shape (craters). All the colony morphotypes were collected and used to evaluate their biofilm formation ability and its susceptibility to some antimicrobials. Data revealed that some morphotypes shown less ability to form biofilms but were more tolerant to BZK. Other morphotypes, with more ability to form biofilms, were resistant to Ciprofloxacin, and others were susceptible to Ciprofloxacin but resistant to Erytromicine.

It can be concluded that *P.aeruginosa* is capable to undertake phenotypic changes when facing stress pressure. These different morphology variants may play a significant role in *P.aeruginosa* antimicrobial resistance, contributing its increased pathogenicity.

Cyanobacterial activity and composition in modern conical photosynthetic biofilms

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Number: 79

The shapes, dimensions and textures of modern biogenic conical stromatolites, cm-sized laminated and lithified structures formed by filamentous cyanobacteria, resemble conical stromatolites as old as ~ 2.8 billion years. Nevertheless, processes responsible for this morphological similarity remain poorly understood. In particular, it has been unclear whether the various morphologies present in stromatolites (cones and surrounding mat structures) reflect different bacterial communities or different activities of similar communities. It has also been unclear the characteristic conical shape of some modern small stromatolites is a product of phototaxis or perhaps other microbial behaviors. By integrating morphological and molecular data with high-resolution mapping of labeled carbon taken up in cm-scale conical biofilms in the hot springs of Yellowstone National Park, here we relate the morphology of coniform mats to the spatial differences in the metabolic activity of thin filamentous cyanobacteria (Oscillatoria). All analyzed samples show similar diversity patterns among morphologically different cones from different pools that contain a shared "background-community" of filamentous cyanobacteria and more diverse non-filamentous cyanobacteria. Our findings do not support a significant role for microbial phototaxis in the formation of analyzed structures, but show that cyanobacteria that grow on the topographic highs incorporate more inorganic carbon than the morphologically and molecularly similar microbes in the topographic lows. These topography-dependent differences in microbial activity are consistent with microbial growth in diffusive gradients, a mechanism that may have controlled the growth of coniform photosynthetic biofilms throughout geologic history.

Role of bacterial hypermutation in the development of a multi-species drinking water biofilm

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Number: 80

The survival of bacteria in nature is greatly enhanced by their ability to grow within surface-associated communities called biofilms. Biofilms provide a protective stronghold for harmful pathogenic bacteria within the environment, for example biofilms in drinking water distribution pipelines can harbour bacteria such as the opportunistic pathogen *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Much research has been carried out on *P. aeruginosa* single species biofilm formation and its role in human infections, but little is known about the interactions that can occur within complex and mixed-species bacterial communities within the environment.

A recent finding is that strains of *P. aeruginosa* obtained from natural biofilms are often hypermutable due to defective DNA error repair systems. However, the role of mutation frequency in determining survival and fitness of *P. aeruginosa* within the environment has not been explored. This research is therefore investigating interactions between *P. aeruginosa* wild-type, mutator and environmental strains and natural mixed-species biofilm communities derived from drinking water and the mutability of *P. aeruginosa* within biofilm cultures generated by growth in a drinking water system, and the role of mutation frequency in their survival.

Our results show *Pseudomonas aeruginosa* mutator strains are better able to integrate into drinking water biofilms compared to non-mutator strains and also can persist longer within these multi-species biofilms. Moreover, the mutation frequency of wild type of *P. aeruginosa* was found to increase by an average of 5-fold when integrated within mixed-species biofilms compared to pure-culture *P. aeruginosa* biofilms and planktonic cultures. Our data suggest that elevated mutation frequencies may contribute to enhanced pathogen survival within drinking water biofilms.

Q-PCR as quantification and detection tool for *Pseudomonas* spp. in biofilms reverse osmosis membranes grown on

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Number: 81

In water treatment plants reverse osmosis (RO) membrane filtration enables the production of biologically stable, very clean drinking water. High quality water is obtained by removal of microorganisms and chemical compounds. However, biofouling may degrade system performance and increase operational costs. Therefore the study of biofilms grown in these systems is of importance for the development of e.g. operational procedures, cleaning agents, and cleaning strategies. Quantitative PCR techniques are widely applied in microbial ecology to quantify gene or transcript numbers within environmental samples. This study shows the implication of Q-PCR for the detection and quantification of *Pseudomonas sp.* in biofilms grown in a lab scale RO membrane system.

Q-PCR analysis was carried out on the bacterial 16S rDNA isolated from the biofilm. As for Q-PCR analysis a standard curve is needed for each target 16S rDNA, two standard curves were generated during this experiment: again one for the 16S rDNA specific for *Pseudomonas* spp., one for the 16S rDNA of all bacterial species. At the end of each Q-PCR reaction the program provides automatically generated Q-PCR data sets, together with the automatically calculated initial amounts of target genes. Considering the PCR efficiencies and the length of the products, the ending value was determined. Bacteria from the genus *Pseudomonas* constitute 25% of the whole bacterial population within the studied membrane biofilm.

The present study shows the applicability of quantitative PCR for the detection and the quantification of *Pseudomonas* spp. in biofilm communities. Future studies will focus on the possibility of detection and quantification of single species e.g. for hygienic reasons.

Isolation and phylogenetic analysis of extracellular polymeric substances producing bacterial strains from a biogas plant

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Number: 82

Biofilms provide protection for microbial life in natural environment. Besides cellular constituents, extracellular polymeric substances (EPS) are the important fraction of the biofilm matrix in biogas reactors. EPS is important in a biogas reactor because of involvement in the formation of bioflocculation of methanogenic organisms with other symbiotic species. This study was performed to isolate EPS producing organisms for phylogenetic analysis to better understand EPS formation within the microbial population. Twenty four EPS producing strains were isolated from the sludge of biogas plant samples collected from methanogenesis stage of a commercial biogas plant in UK. EPS producing bacteria were selected based on their production properties on solid agar sucrose medium. Three types of EPS producing strains were identified based on 16srRNA gene sequence. Based on sequence similarities all the strains were assigned a taxonomic position belonging to four different genera viz., *Sporosarcina*, *Bacillus* of the phylum *Firmicutes and Paenalcaligenes*, *Alcaliliqenes* of the phylum *Proteobacteria.* Phylogenetic analysis was performed for the strain 10c that showed 95.09 % 16S rRNA gene sequence similarity with *Sporosarcina sp*, after multiple alignments of the data by CLASTAL W and using the neighbour joining algorithm of MEGA software and results supports that this strain belongs to a previously unidentified EPS producing species. Results demonstrated that EPS producing novel species were present in the biogas plant and may have a vital role in EPS formation that stabilizes aggregate formation and improve biogas production. Current literate also suggests that these novel species will also produce novel EPS structures.

Ecological influences on antimicrobial tolerance in *Pseudomonas aeruginosa* biofilm development

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Number: 83

Persister cells within a bacterial population are extremely tolerant to high concentrations of antibiotics. Despite recent progress in understanding molecular determinants of persister cell formation, few studies have examined in-vitro the evolutionary and ecological drivers that sustain their presence. Sessile bacterial biofilm communities present an excellent model system for studying selection on bacterial persister allocation, because they exhibit rapid mutation and genetic diversification. We test a recent theoretical model for the evolution of senescent ageing by hypothesising a functional role for persister cells in deferring replication within biofilm populations that are too crowded to sustain rapid growth. In support of this model for evolution of negligible senescence, variant Pseudomonas aeruginosa strains from PA01 biofilms showed stable, heritable changes in growth rate, with slow-growing isolates having higher persister levels than both fast-growth and wild-type isolates. We observed that frequent exposure of biofilms to both lethal and non-lethal antibiotics gave rise

to isolates with raised levels of persister cells, consistent with a bet-hedging role for these cells. Our results are the first to demonstrate that biofilm-specific ecological and evolutionary processes can impact on the formation of persister cells *in-vitro*.

Legionella pneumophila is an autochthonous species of potable water biofilms

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Number: 84

Legionella pneumophila is the causative organism of Legionnaires' disease and Pontiac fever. Outbreaks of Legionnaires' disease occur worldwide and there are increasing travel-associated outbreaks. These usually arise from poorly maintained water systems in the built environment, leading to contamination of hot water supplies, jacuzzi baths, spas, indoor fountains and air handling systems. While focus has concentrated on the maintenance of such systems, little consideration has been given as to the source or ecophysiology of the pathogen. Here we present ecological data collected from across Europe which clearly shows the high numbers of *L. pneumophila* present in high species diversity biofilms forming on the surface of mains supply drinking water pipes. Using a specific peptide nucleic acid probe for 16S rRNA FISH analysis and episcopic differential interference contrast/epifluorescence microscopy we have quantified the population of *L. pneumophila* on the surface of highly corroded cast iron pipes and the population surviving within the corrosion deposits at various depths. We found between 10^3 and 10^4 cells cm^{-2} on all pipe surfaces examined, and up to 10^7 cm^{-2} when the corrosion deposits were scraped from surfaces. Such high numbers were found on all samples from all locations. The high fluorescence intensity of labelled cells indicated a high 16S rRNA content, suggestive of viable cells. This suggests that microaerophilic legionellae may be autochthonous rather than transient allochthonous species of established potable water biofilms. This work has implications for *Legionella* and biofilm ecophysiology, the efficacy of disinfectant procedures and public health.

Theme: Novel biotechnology and bioengineering

Control of bioleaching or acid mine drainage by understanding biofilm formation

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Number: 85

Bioleaching is the dissolution of metal sulfides by bacterial oxidation processes. These are used for the winning of metals such as Cu, Zn, or Ni from low grade ores, like chalcopyrite. On the other hand bioleaching causes acid mine drainage/acid rock drainage as a natural process.In leaching processes the bacteria form a biofilm on the surface of a metal sulfide, their substrate and substratum. Consequently, the attachment of microorganisms as the first step in biofilm formation is the critical one. To develop methods to enhance or reduce bioleaching, our investigations were focused on the initial processes of attachment and biofilm formation. Interactions of pure and mixed cultures with respect to initial attachment processes are still unknown. Therefore, we quantified and visualized initial colonization and biofilm formation on pyrite by strains of the genera *Acidithiobacillus* and *Leptospirillum* using DAPI-, Lectin or FISH- staining in combination with atomic force microscopy. Microcalorimetric measurements were used to determine metabolic activity of cells. As a result it was shown that interactions of different bacterial species resulted in increased production of extracellular polymeric substances, increased attachment and leaching rates. Another finding is that large areas of minerals remain uncolonized, whereas at some places bacteria attach in clusters. Obviously, bacteria prefer specific areas such as cracks, holes or other irregularities for attachment. Based on these findings an improved control of bioleaching processes seems to become feasible.

A novel surface attached acidophilic gamma-proteobacterium for bioleaching

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Number: 86

Bioleaching is the dissolution of metal sulfides by bacterial oxidation processes. The main part of the biooxidation takes place within a biofilm formed on the metal sulfide surface. The excreted EPS act as an increased reaction space. Bioleaching is an alternative method for cost efficient winning of metals out of low grade ores and environmentally friendly. Consequently, the aim is to enhance the bioleaching rate. This can be achieved by the use of highly effective microorganisms.

A novel strain, designated SP III/3 was isolated from an open cast mining area in the Iberian Pyrite Belt near to La Esperanza in Murcia, Spain. The attachment behaviour and biofilm formation on pyrite surfaces have been investigated using atomic force microscopy and bright field microscopy. Additionally, the morphology and distribution were visualized by fluorescence microscopy using different staining methods. For classification of the bacterium, cloning and subsequently sequencing of 16S rDNA was done.

The phylogenetic study of 16S rDNA revealed that SPIII/3 is not a member of the genus *Acidithiobacillus*, but is a new genus within the line of gamma-proteobacteria and affiliated distantly with the genus *Nevskia*. It is an aerobic, chemolithotrophic, sulfur- and iron-oxidizing bacterium, which is also able to grow on pyrite and chalcopyrite. It is acidophilic and grows within a temperature range of 15 up to 35°C. Morphological analysis shows a pleomorphic, 0,3-0,5 µm wide and 1-10µm long rod-shaped cell with EPS excretion.

Silica-nanoprobes for the study of bacterial biofilm

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Number: 87

The study is a collaboration at Chemistry-Microbiology interface in order to develop innovative tools for biofilms' exploration. A complete inhibition of the biofilm formation is currently not possible. So, it's essential to control their development. New tools for studies *in situ* without biofilm destruction are required. Studies of W.J. Drury [1] *et al.* and D.de Beer [2] *et al.*, had shown that luminescent latex-particles (µm) were able to circulate inside the biofilm through channels.

By analogy to latex-particles, we use luminescent silica-nanoparticles having different surface properties as a new tool of exploration.

These characteristics making them multifonctionnal nanoparticles and permitting to up-date favourable particle–biofilm interactions for exploration.

We'll present the synthesis of luminescent silica-nanoparticles ranging from 20 to 200 nm. The influence of their physico-chemical properties (size, hydrophobicity and ionic charge) on their interaction with *Pseudomonas aeruginosa* biofilm is in progress, the first results will be presented.

Evaluation of different extraction methods for the capsular EPS fraction of Desulfovibrio vulgaris

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Number: 88

Abstract: In recent years extracellular polymeric substances (EPS) attracted the attention of scientists and industry because of their possible biotechnological potential. Industry is constantly looking for EPS with novel functional properties to satisfy the need for modern technology. A particular objective of this study was to evaluate three different methods for the extraction of capsular EPS from *Desulfovibrio vulgaris* in order to establish a standard extraction method. Until now almost nothing is known about the capsular EPS of *D. vulgaris*. Therefore the cell pellets were treated with one chemical (EDTA) and two physical methods (Dowex 50 x 8 and Crown Ether cation exchange resins).

Results show that the amount and composition of capsular EPS was dependent upon the extraction method. Depending on the method 2% up to 20% (w/w) of total-weight of the extracts were identified. The analysis revealed that capsular EPS of D. vulgaris are mainly composed of carbohydrates and proteins. Additionally, the EDTA- and Dowex- extract contained high amounts of iron ions. The Crown ether method was proven to be superior for extracting lipids.

A frequently recommended method for determination of DNA in EPS is the Burton method (1956). In this study the measurement was performed with Quant-iT PicoGreen dsDNA reagent. This method was proven to be functional for DNA quantification analyses and had several advantages like easy performance and time saving. Furthermore, the results indicate that the EPS content of the capsular fraction was affected, if the culture medium was either centrifuged or filtered.

Characterization of multispecies biofilms by peptide nucleic acid fluorescence in situ hybridization (PNA-FISH)

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Number: 89

Abstract: Our current understanding of biofilms in the environment and in health indicates that these structures are typically composed of many different microbial species. However, the lack of reliable techniques for the quantification, visualization and discrimination of each population has meant that studies assessing multi-species interactions between sessile microorganisms are scarce and low-throughput.
Employing novel peptide nucleic acid fluorescence *in situ* hybridization (PNA-FISH) methods, we present here a characterization of *Salmonella enterica*/*Listeria monocytogenes*/*Escherichia coli* single, dual and tri-species biofilms in seven support materials. Ex-situ, we were able to relate quantitatively the populations of ~56 mixed species biofilms up to 48h, regardless of the support material.*In situ* a correct quantification remained more elusive, but a qualitative understanding of biofilm structure and composition is clearly possible for most support materials. Regarding biological behavior, composition of mixed-culture biofilm seems to be the final result of competition between microorganisms, both for available nutrients and for free surface to colonize. It is also suggested that the ability to form biofilm is mostly a species-dependent phenomenon rather than surface-dependent, as six of the materials maintained both the species profile and had similar total cell numbers. The exception was copper, that inhibited the biofilm formation for the species tested.

Our findings concluded that, using a single method, such as PNA-FISH, to confidently discriminate multispecies early-stage biofilms, researchers can infer about spatial organization, intra- or inter-specie interaction and also assess viable but not cultivable states.

Speedy Removal of petroleum Pollutants by a novel *Rhodococcus* Strain with the aid of its biofloccule formation

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Number: 90

]A novel strain, *Rhodococcus erythropolis* NTU-1, could efficiently remove some hydrocarbon pollutants by combining biological and physical traits. For the viewpoint of practical application, 10000 ppmv diesel oil or crude oil were investigated with NTU-1. During the incubation, both biodegradation and biofloccules' formation were confirmed. It was found that NTU-1 could degrade *n*-alkanes ranging from C10 to C32, and the most constitutes of diesel or crude oil could be tapped in biofloccules, though some might not be biodegraded by NTU-1. The total removal of diesel oil or crude oil could achieve around 90% out of 10000 ppmv (about 30% of biodegradation and 60% of biosorption) within 4 days batchwisely. In addition, the concept was further extended to a bioreactor with intermittent feed (n-hexadecane, diesel or crude oil), aeration and pH adjustment. Within 4 weeks successful operation, about 87% of 42000 ppmv total *n*-hexadecane addition was removed by this novel strain (24% of biodegradation and 63% of biosorption) and more than 90% removal of 35000 ppmv disesel or crude oil addition could be achieved (about 20% of biodegradation and 74% of biosorption) within 2 weeks. The results suggested the feasibility of strain NTU-1 for bioremediation of petroleum pollutants in environment.

Application of the Resazurin Metabolism Assay for Evaluation of Root Canal Disinfection Treatments

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Number: 91

Endodontic infections are caused by polymicrobial biofilms. Novel root canal treatments therefore should be evaluated not only on single species biofilms but also on dual or mixed species biofilms. A simple, high throughput assay is urgently needed for this. In this study we investigated the efficacy of disinfection using the resazurin metabolism assay in dual-species biofilms. We grew *Enterococcus faecalis* with or without *Streptococcus mutans* in biofilms in an active attachment biofilm model for 24h. Subsequently we treated the biofilms with various concentrations of NaOCl for 1 min. After confirming resazurin metabolism by both organisms we evaluated treatment efficacies using 0.0016% resazurin. We showed that during NaOCl treatments, resazurin metabolism displays a clear dose response, not only in single species *E. faecalis* (and *S. mutans)* biofilms, but also in dual species biofilms. Notably the assay revealed that the resistance of dual species biofilms to NaOCl was 30 fold higher than in a single species *E. faecalis* biofilm. Viability counts on a selected NaOCl treatment (0.004%) confirmed this result and showed the increased resistance of *E. faecalis* in dual species biofilms. Clearly the high throughput and low cost resazurin metabolism assay has a great potential for testing novel root canal antimicrobial treatments in mixed species biofilms.

Characterization of *Pseudomonas* fluorescens Biofilm using DB-FIB, SEM and STEM

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Number: 92

Confocal microscopy is a useful tool for three-dimensionally (3D) characterizing large areas of bacteria but does not have the spatial resolution of transmission electron microscopy (TEM) or Dual Beam Focused Ion Beam (DB-FIB). Recently, DB-FIB technology has automated 3-D serial sectioning to expedite data collection. This technique has been applied to Pseudomonas fluorescens, which forms a biofilm on a polyurethane substrate. Characterization of the interface between biotic and abiotic material is of interest for surface adherence properties. Biofilms were coated with gold to minimize degradation by high-energy ion bombardment. Platinum was deposited to protect the area of interest from Ga^+ ion damage while milling was performed on both sides of the section to be excised. New processes are being developed to reduce the damage to the biofilm when milled in the DB-FIB. For example, secondary electron (SE) imaging at 350 volts, revealed different surface properties than when SE imaging at higher voltages.

Serial sectioning of the biofilm provided information about the bacteria polyurethane interface. Using 2-D images acquired with FEI Slice and View software, a 3-D volume has been reconstructed using algorithms developed by CAMM at OSU. The 3-D volume allowed for evaluation of surface adherence mechanisms throughout the entire sample and structure of the bacteria in a given volume. The TEM characterization offered similar information as well as the spatial variation of the Extracellular Polymeric Substance (EPS) as a function of density and has shown promise with detailed information about the bacteria polyurethane interface.

Study of the initial phase of biofilm formation using a biofomic approach

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Number: 93

Studies on biofilms are impaired by the lack of a rapid, reliable method with a high-throughput. Such a method would greatly facilitate the testing of antibiotics on bacteria inside a biofilm, an absolute requirement to implement an efficient treatment in chronic infections like pulmonary infections such as those occurring in cystic fibrosis. The purpose of our work was to compare a new method for the study of biofilms, the biofilm ring test, with the classical crystal violet staining. Eight clinical and reference strains of *Pseudomonas aeruginosa* to form a biofilm were studied.

The two methods revealed that four strains formed a rapid biofilm. The biofilm formed by these strains was detected after only 30 minutes with the biofilm ring test. The enumeration of bacteria of the PA01 strain (one of the four strains forming rapidly a biofilm) confirmed that, after 30 minutes, a significant amount of bacteria had attached on the bottom of the culture wells. After 48 hours the crystal violet method detected a biofilm with all strains. The four strains which rapidly formed a biofilm could not be detected by their mucoid character or their swarming motility or their synthesis of rhamnose. They showed higher swimming mobility.

In conclusion our results show that the biofilm ring test is a method specially suited for the study of the initial phase of the formation of a biofilm. It seems to be as efficient as the much more tedious enumeration method.

Casbane diterpene as novel and natural antimicrobial agent against biofilm infections

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Number: 94

Croton nepetaefolius is a plant native from northeastern Brazil and belongs to Euphorbiaceae family. The essential oil of this plant is widely used in folk medicine from the treatment of gastrointestinal disorders to the use as an antiseptic agent, with an antifungal action scientifically proven. The action of this plant has been extensively explored by the scientific community, being the secondary metabolites, which are responsible for their properties, alkaloids, diterpenes, and triterpenes. The aim of this study was to evaluate the ability of the secondary metabolite, casbane diterpene (CD) (isolated of the species mentioned above), to inhibit microbial growth and biofilm formation of several clinical relevant species (about 15 species among bacteria and fungi). Minimal inhibitory concentration was assessed by the standard technique of microdilution and biofilm inhibition was tested using microtiter plates with biomass quantification by crystal violet staining method. It was found that the CD possessed biocidal and biostatic activity for the majority of the species screened, with the minimal concentration active between 125 and 500 mg/L. Moreover, CD anti-biofilm action was also observed for some of the species. In addition, it was noticed some biofilm formation inhibition even when the planktonic growth was not significantly affected. In conclusion it can be speculated that casbane diterpene shows potential to be a natural tool for the treatment of diseases caused by different infectious microorganisms.

Inhibition of Biofilm Formation by Extracts of Sphagnum Moss

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Number: 95

The accumulation of biofilm in man-made water systems creates numerous and significant problems. Depending on the specific system, these problems include health and infection issues, increased maintenance expenses, and significant operating inefficiencies. Mitigation or removal of biofilm from within these systems is difficult and typically requires the use of harsh and toxic chemicals.

Previous studies in our laboratory have demonstrated that extracts of sphagnum moss significantly inhibit the growth of a number of planktonic bacteria including Pseudomonas aeruginosa. Studies further indicated that this inhibitory effect was bacteriostatic, and not bactericidal. These results led us to investigate the effects of sphagnum moss extracts on biofilm formation in a standard biofilm assay.

Extracts were created in aqueous solution utilizing the Sphagnum cristatum species of sphagnum moss.. A standardized inoculum of P. aeruginosa (ATCC 700888) was prepared and used to create biofilm on the pegs of 96-well MBEC plates. Various concentrations of filter-sterilized extract were prepared and added to the MBEC assay. Biofilm determinations were made 24 and 48 hours after inoculation using crystal violet staining with subsequent methanol extraction and quantification of staining on a plate reader. The results demonstrated significant inhibition of biofilm formation at both the 24 and 48-hour time point. Sphagnum moss extracts were capable of maximally inhibiting the accumulation of biofilm up to 61% and 84% at 24 and 48 hours

respectively. This inhibition was dose dependent with the inhibitory effect maintained over the 48-hour time-period. These results demonstrate the ability of a non-cidal, natural plant material to mitigate biofilm formation and provide support for the development of non-toxic alternatives for biofilm control.

Hydrodynamic forces to detach *Listeria monocytogenes* biofilms from stainless steel surfaces
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Number: 96
Listeria monocytogenes is a foodborne pathogen that causes listeriosis, a mortal disease that affects mainly pregnant women, immunocompromised people, newborns and elderly people. This pathogen is ubiquitous in natural environments but one of the routes of transmission is the consumption of contaminated processed food that has been in contact with contaminated surfaces, where *L. monocytogenes* may attach and develop into a biofilm resilient to disinfectants. The efficient cleaning of industrial surfaces is therefore essential to the safety of processed food. The aim of this work was to study the shear forces needed to detach *L. monocytogenes* cells from stainless steel (SS) surfaces, a material commonly used in food industry. For that, 0.5, 4 and 24 hours-old biofilms were formed on SS discs and then moved to a Radial Flow Cell (RFC) were a water flow rate of 94 $ml.s^{-1}$ (laminar flow) and 400 $ml.s^{-1}$ (turbulent flow) was applied for 1 and 5 minutes. For 24 hours-old biofilms both flows were also applied for 10 minutes. Results showed that the application of a low flow rate, corresponding to the application of shear forces between 1.1 and 34 $N.m^{-2}$, did not significantly remove the cells from the SS surface. In contrast the application of shear forces between 24 and 144 $N.m^{-2}$, was able to reduce the concentration of cells on the SS surfaces. This demonstrates that *L. monocytogenes* forms strongly adherent biofilms that require the application of a high cleaning pressure to ensure the safety of food processing surfaces.

Theme: Structural dynamics and emergent properties of biofilms

Ca and Mg - induced biofilm differs architecturally in *Paracoccus sp.*
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Number: 97
Variety of environmental factors specifically the divalent cations, Ca^{++} and Mg^{++} are known to enhance bacterial biofilm formation. This study reports the spatio-temporal structural dynamics in the calcium and magnesium induced biofilm of a denitrifying bacterium *Paracoccus* sp. W1b isolated form a dentification reactor. Ca^{++} and Mg^{++} individually increased biofilm by inducing cohesion as visualized by environmental scanning electron microscope (ESEM). CSLM images of biofilm grown with minimal levels of Ca^{++} and Mg^{++} in the medium showed well networked mosaic architecture and distinct voids. Though Ca^{++} and Mg^{++} enhanced biofilm by inducing cohesion, the biofilm architecture was different. Dense and confluent biofilm was observed with 10mM Ca^{++} whereas, monolayer cells with mosaic skeletal structure and dense protruding subpopulation distributed over it was observed in 10mM Mg^{++} -grown biofilm. Vertical distribution pattern of live to dead cell ratio in the biofilm formed in presence of high calcium and magnesium was also be studied. Significantly, Ca^{++} and Mg^{++} - induced biofilm showed increased denitrification activity. Chelator treatment on biofilm of various ages revealed importance of divalent cations in initial stages of biofilm development

Production and Degradation of Extracellular DNA in Oral Bacterial Biofilms
Nicholas Jakubovics, Lesley Old and Claire Graham, Newcastle University, UK

Number: 98
The accumulation of bacteria in dental plaque occurs in a reproducible spatiotemporal pattern, initiated by the adhesion of primary colonisers such as streptococci and actinomyces. Subsequent expansion of the biofilm results in a 3-dimensional structure bound together by an extracellular matrix. Here, the role of extracellular DNA (eDNA) in the matrix of model biofilms formed by two common primary oral colonisers was investigated. Extracellular DNA was purified from *Actinomyces oris* and *Streptococcus gordonii* biofilms and analysed by agarose gel electrophoresis. A sharp band comparable to intact chromosomal DNA was observed in the matrix of *A. oris* biofilms. By contrast, *S. gordonii* eDNA consisted of relatively small fragments. Addition of DNase I during the formation of *A. oris* biofilms reduced the adherent biomass by 50%, whereas *S. gordonii* biofilms were insensitive to DNase I. *S. gordonii*, but not *A. oris*, produced an extracellular nuclease capable of degrading DNA during growth in rich medium. *In silico* analysis identified a gene, *ssnA*, in the genome of *S. gordonii* with ~65% identity to the cell surface nuclease of *Streptococcus suis*. Disruption of *ssnA* in *S. gordonii* ablated extracellular DNase activity. Further, biofilms formed by an *S. gordonii ssnA* mutant were sensitive to DNase I, indicating that eDNA was an important structural component of these biofilms. Together, these data demonstrate that large fragments of eDNA help to stabilise the structure of biofilms formed by oral bacteria. However, eDNA may be a target for competitive strategies by DNase-producing organisms such as *S. gordonii*.

Impact of sessile growth state on *Pseudomonas aeruginosa* lipidome
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Number: 99
Pseudomonas aeruginosa is a common opportunistic and nosocomial pathogen and the leading cause of morbidity and mortality in cystic fibrosis patients. Many previous studies using genetic and proteomic approaches have revealed physiological differences between planktonic and sessile *P. aeruginosa*. One of these proteomic studies revealed that sessile *P. aeruginosa* cells accumulated some enzymes involved for fatty acid and phospholipids biosynthesis. These data question about the impact of the sessile growth state on the bacterial lipidome.

After inner membrane extraction of planktonic or sessile cells, lipid extraction was done according to Bligh and Dyer protocol. Lipids were analysed using Electrospray Ionization Mass Spectrometry. The impact of the biofilm growth mode on phospholipid organisation has been studied by reconstitution in monolayers and thus visualized by Brewster Angle Microscopy (BAM) and Atomic Force Microscopy (AFM).

The results obtained by mass spectrometry show a drastic decrease of the uneven-numbered chain phospholipids and a relative accumulation of long chain lipids in organisms grown in biofilms, suggesting better lipid stability in the bilayer and a decrease in membrane fluidity. The images taken by BAM and AFM showed that inner membrane lipids of *P. aeruginosa* could form domains (larger in biofilm bacteria) when the pressure is near to the physiological conditions. This observation is coherent with mass spectrometry analysis. This reflects a bacterial adaptation to the sessile mode of growth and might play a key role in the particular physiology of biofilm cells. This study is an innovative approach that could allow to a better understanding of the mechanism of biofilm formation and the switch between the two growth states.

OMP Proteomic analysis of Benzalkonium Chloride and Ciprofloxacin adapted Biofilm cells
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Number: 100
Adaptive resistance to antimicrobials has been widely reported in planktonic studied trough phenotypic characterization and proteomic analysis. Concerning biofilm adaptation, the response of biofilm-entrapped cells to chemical stress conditions is not yet well studied. There is evidence that proteins involved in oxidative stress response, cell envelope synthesis, as well as in synthesis of EPS become up- or down-regulated in biofilms, indicating that these altered phenotypes might contribute to antimicrobial tolerance. This work aimed to examine whether exposure of *Pseudomonas aeruginosa* biofilms to benzalkonium chloride (BC) and ciprofloxacin (CIP) could induce an adaptive response in bacteria. This was attained by inspection of proteome alterations of the outer membrane (OMP) in biofilm cells. Biofilms were formed in 6-well plates for 24h being after submitted to the presence of 0.9 mM BC and 6.0 ug/ml CIP, during 13 days. The obtained biofilm-cells were separated and the OMP extracted. Protein patterns were analysed by 2-DE and gels by the SameSpot software. Biofilm-proteome showed that *P. aeruginosa* adaptation to BC promoted the down-regulation of 36 OMP and the up-regulation of only one. OMP 2DE of *P. aeruginosa* adapted to CIP revealed the down-regulation of 29 OMP. Six OMPs were changed in common by both antimicrobials, revealing a possible similar stress response. Proteins identification is in progress. This study highlighted that there might be an OMP regulation when bacteria within biofilms are submitted to chemical adaptation. This particular response to the environment can be one of the causes of the well-known biofilm resistance phenotype. Acknowledgments:IBB-CEB, FCT(PTDC/SAUESA/64609/2006;SFRH/BD/31065/2006)

Impact of physical disturbance on the evolution of A-L interface biofilm structure
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Number: 101
Bacterial growth results in assemblages ranging from micro-colonies to slimes and biofilms, with varying structure, rheology and ecological advantage. Growth at the meniscus of static liquid microcosms often produces air-liquid (A-L) interface biofilms in which oxygen and nutrient gradients are opposing, and maintenance at the surface involves meniscus attachment, hydrophobic structures and surface tension effects. A-L biofilm formation is a deep-rooted ability amongst environmental Pseudomonads, but as yet, the relationship between biofilm properties and ecological advantage is poorly understood. In order to investigate this, we have characterized three distinct A-L biofilms formed by the soil and plant-associated *Pseudomonas fluorescens* SBW25. Two of these, the Wrinkly Spreader (WS) and CBFS biofilms are the result of mutations, whilst the Viscous Mass (VM) biofilm is non-specifically induced by iron. These differ significantly in terms of biofilm strength, attachment and maintenance at the A-L interface, rheometry, cell hydrophobicity, stickiness, and surface recruitment. Each of the biofilms are resistant to low-level physical disturbance but not to extreme events, suggesting that there is a trade-off between physical resilience and the minimal structural requirements needed to colonise the A-L interface. Fitness assays confirm the advantage of biofilm formation in static liquid microcosms though

the cost involved in producing these structures are not similar. It appears that the ability to resist physical disturbance at the A-L interface is the most significant challenge in the microbial landscape faced by SBW25 when colonizing this favourable environment.

Biofilm and colony morphology of *Escherichia coli* is correlated with inherent molecular structure
Selina Akter, Kazi Sabrina Afreen, Mazhurul Islam, Nafisa Azmuda, Nils Kare Birkeland, Sirajul Islam Khan, University of Dhaka, Bangladesh

Number: 102
Colony or biofilm morphology is a characteristic property of a bacterium which has been shown to depend on the expression of certain surface antigens. The synthesis of O-antigen, Antigen 43 and Type 1 fimbriae are reported to be responsible for smooth and rough colony forms of *Escherichia coli*. The surface matrix also seems to influence the biofilm morphology. The present study analyses the relation of biofilm or colony morphology to inherent biochemical and genetic makeup.

One hundred and forty environmental *E. coli* strains, isolated from different aquatic sources of Bangladesh, were divided into six subgroups, e.g. A, B, C, D, E and F, based on surface characteristics, size, shape and pigmentation. Repetitive-element PCR (rep-PCR) fingerprinting analysis revealed that group A, D and F formed three different clusters, whereas group B, C and E were placed in the same cluster. The isolates belonging to a specific group share common biochemical properties like sugar utilization and gas production patterns. Motility was one of the distinctive properties of the isolates among the subgroups.

The rep-PCR fingerprinting method as well as physicochemical properties revealed that the isolates of a particular subgroup are similar, while subgroups clearly differ in their repetitive intergenic DNA sequences and genetic makeup. It may be concluded that a major factor determining colony and biofilm morphology is linked to the inherent molecular and biochemical features of the cells rather than phase or growth-specific expression of surface antigens.

Phenotypic variants arising in *Staphylococcus aureus* biofilms may promote dissemination during infection
Victoria Ryder, Ian Chopra, Alex O'Neill, University of Leeds, UK

Number: 103
The final stage of bacterial biofilm development usually involves dissemination of organisms. Here, we report the identification and preliminary characterisation of morphological variants of *S. aureus* that arise in biofilms, and which may promote dissemination, in the form of white variants (WV) and large-pale variants (LPV). Using a cellulose filter model of biofilm formation, it was found that of the total biofilm population, 2% were WVs and 15% were LPVs. In the shed planktonic population, 26% and 52% of the population were WVs and LPVs, respectively. Both variants were stable upon subculture. WV strains were defective in biofilm formation. LPVs, however, had equivalent biofilm forming capacity to the parental strain, as determined by viable counting. The lack of pigmentation in WVs suggested reduced staphyloxanthin production, and therefore decreased protection against oxidative damage, which was observed as reduced survival in the presence of hydrogen peroxide. Genetic analysis of the WVs revealed mutations in the alternative sigma factor, SigB, although the genetic basis of the LPV morphology remains unknown. In conclusion, we have identified WVs and LPVs emerging from *S. aureus* biofilms. Based on their high numbers in the shed planktonic phase, and the inability of WVs to form biofilms, we hypothesise a function for these variants as a mechanism to promote *S. aureus* dissemination from biofilms during infection.

Subinhibitory concentrations of ethanol stimulates biofilm formation by the *Pseudomonas aeruginosa* PAO1
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Number: 104
Pseudomonas aeruginosa is a gram-negative bacterium ubiquitously distributing in various environments. *P. aeruginosa* is well recognized as opportunistic pathogens. The contamination of medical instruments such as implants or contact lenses is known to leads to establishment of *P. aeruginosa* infections.

Ethanol have been utilized for disinfecting skins and instruments. Preceding study has demonstrated that low-concentrations of alcohol increase biofilm formation of *Staphylococcus epidermidis*. However there have been no study addressing the effect of low-concentrations of ethanol on *P. aeruginosa* biofilm. Here, we studied the effect of ethanol on *P. aeruginosa* PAO1 biofilm.

P. aeruginosa PAO1 were grown with or without various concentrations of ethanol. The result showed that, although high-concentrations of ethanol killed or inhibited growth of *P. aeruginosa*, relatively low (0.1% - 4%) concentrations of ethanol stimulated biofilm formation. The confocal microscopic observation revealed that,

the low-concentrations of ethanol induced the formation biofilms witch is thicker and more rigid than that formed without ethanol. The stimulation of *P. aeruginosa* biofilm formation by ethanol might be due to, in part, increase in initial attachment. Our result showed that, supplement of low-concentrations of ethanol in medium increased the numbers of cells attached on surface. Ethanol enhanced the initial attachments of living cells, but not of chloramphenicol-treated resting cells. This result indicated that enhancement of initial attachment by ethanol requires active cellular response.

In conclusion, we propose that the ethanol-inducible biofilm phenotype of *P. aeruginosa* could add to the development of bacterial contaminations.

Coupled Multiphysics Study of Biofilm Streamers: Reverse Engineering Nature
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Number: 105
A decade ago, it was shown experimentally that under fast flows, biofilm streamers form and oscillate with large amplitudes. The current work is a first theoretical attempt to answer the questions on the mechanisms behind the oscillatory movement of the streamers, and whether this movement together with the special streamlined form of the streamers, have both physical and biological benefits for biofilms. In this study, a state of the art two-dimensional fluid–structure interaction model of biofilm streamers coupled with mass transfer of solutes was developed. The model implements a transient coupling between the fluid flow, biofilm mechanics, and substrate transport. It is shown that the formation of biofilm streamers reduces the fluid forces that the biofilm surface experiences. In addition, simulations showed that the lateral oscillatory movement of the streamer tail enhances the substrate transport into the biofilm significantly. While the frequency of streamer vibration and mass transfer coefficients increase with increasing flow rates, the drag coefficient decreases. On the other hand, streamer elasticity coefficient also affects substantially the substrate mass transfer rate. In conclusion, model simulations suggest that the development of streamer-like structures oscillating in the flow is a good strategy for microorganisms to obtain the highest possible supply of nutrients out of the bulk phase, while reducing the detachment chance.

A Small angle X-Ray scattering (SAXS) approach to study complex extracelulluar bacterial matrices
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Number: 106
The bacterial matrices are composed of a variety of exopolymeric substances (EPS): polysaccharides, proteins, nucleic acids and lipids. Many of the EPS physicochemical properties are closely linked to three-dimensional structure of the biopolymer network. A Small angle X-Ray scattering (SAXS) is one of the few experimental techniques that allows a non-destructive study of conformational properties of biopolymers on a size scale of 1-100 nm. The interpretation of SAXS scattering curves in terms of underlying molecular conformations is not straightforward and a suitable structural model is needed. We have developed a SAXS structural model, which can simulate various conformational shapes of polymers and generate 3D structures of EPS from SAXS experimental data. The model can simulate single helices with different pitch values, number of monomers per pitch, and cross sectional radius of gyration, as well as different random coils with excluded volume taken into account. We successfully tested our model on polysaccharides (i.e. gellan, pollulan). To study more complex systems native and TCA/ethanol purified bacterial matrices were analyzed. The analysis showed that EPS in native bacterial matrices is more helical and heterogeneous, compared to its purified form. By decreasing the pH of the solvent, the chain local density in the polymer matrix of purified sample decreased. In contrast to the purified sample, the native sample did not show increased susceptibility to alkaline pH. The new SAXS data analysis proves to be a very powerful tool to structurally characterize complex native EPS matrices in solution on the scale from 1 to 100 nm.

A new method of producing *Legionella pneumophila* biofilms
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Number: 107
Little is currently known about *L. pneumophila* surface colonization and biofilm formation. Some authors argue that *L. pneumophila* is unable to produce biofilms without another prokaryote or eukaryote species, while some studies show that *L. pneumophila* biofilms can be produced in a rich medium, depending on planktonic growth. This study introduces a method of producing mono-species *L. pneumophila* biofilms independent of planktonic growth.

Three environmental isolates of *L. pneumophila* and reference strain ATCC 33152 were inoculated in 24-well microplates containing the tested growth media. To eliminate planktonic bacteria, the wells were frequently rinsed and the medium renewed. Biofilm formation kinetics were monitored by culture counts or quantitative PCR. Confocal laser scanning microscopy (CLSM) was used to determine the 3-D structure of biofilms.

Among 9 growth media tested, a medium consisting of a mix of mineral salts and a low-concentration carbon source supplemented with iron and cystein was selected. This medium supported sessile growth but not planktonic growth: after 6 days of incubation, biofilms consisted of 5.36±0.40 log CFU cm^{-2} or 5.34±0.33 log GU cm^{-2}. The adhered population remained stable for up to 3 weeks after inoculation.

In situ CLSM observations of adhered *L. pneumophila* revealed a typical biofilm structure, comprising cell clusters ranging up to 300 µm. The *L. pneumophila* biofilm matrix had a specific makeup, with clusters or sheets of carbohydrates scattered between cells.

High reproducibility and the absence of other bacterial or protozoan species make this model an innovative protocol in the study of genes specifically involved in biofilm development.

An *alg*-like gene cluster is required for formation of biofilm, circular floating bodies at the air-liquid interface and multicellular aerial structures

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Number: 108

In biofilms, bacteria are encased and protected by the extracellular matrix where exopolysaccharide (EPS) is a key component. A genus *Pseudomonas* is found in diverse environments and performs a diverse array of metabolisms for global cycling of materials. Although their habitat diversity may imply many kinds of EPS for biofilm formation, the genes related to biofilm EPS formation have been limitedly reported. *Pseudomonas alkylphenolia* KL28 is known to form specialized aerial structures during growing on the vaporized *p*-cresol and unusual circular floating bodies at the air-liquid interface. A group of mutants having Tn*5* insertions at a delineated region was isolated due to defects of those structure formations. The mutants showed reduced abilities in forming biofilms on glass, circular floating bodies, aerial structures, and spreading on surface. The mutated gene cluster contains eleven ORFs in a same transcriptional orientation and named as *epm* for abbreviation of extracellular polymer for matrix. The same gene organization with unknown functions has been identified from the *P. mendocina* ymp and *P. entomophila* L48 genomes with deduced amino acid sequence identities of 41-87%. The genes in the cluster also showed amino acid sequence similarities to those found in the gene cluster for synthesis of alginate. Mutational analysis showed that the genes encoding acetylase and epimerse are required for functioning. Our results demonstrate that the *epm* gene cluster is involved in the formation of a polymer similar to alginate, which is important for formation of multicellular structures in strain KL28.

Correlated development of morphology and bacterial communities in monochloramine-exposed biofilms?

Kim Milferstedt, Jean-Jacques Godon, Renaud Escudié, Nicolas Bernet INRA, France

Number: 109

Mixed-culture biofilms have been referred to as “microbial landscapes”. As for their full-scale counterparts, in microbial landscapes, the community compositions of their microbial inhabitants can been quantified and followed over time. In the literature, models begin to emerge that describe the succession of these biofilm communities.

As implied in the term landscape, biofilms also evolve spatially. The resulting three dimensional morphology of a biofilm impacts its nutrient supply by influencing e.g. diffusion-limited mass transfer.

How spatial organization and community composition are linked in mixed culture biofilms is not known to date. In our research, we use multivariate data analysis tools to correlate results from molecular fingerprinting of bacterial communities to the results from automated and quantitative analysis of images at the scale of centimeters.

Currently, we follow morphology and community composition in biofilms over periods of 2 months on coupons placed in three parallel bubble column reactors. While one reactor is operated under constant environmental conditions, the remaining two reactors receive daily and weekly pulses of monochloramine.

We hypothesize that biofilms subjected to (1) controlled disturbances at a high frequency or (2) no disturbance will reach stable, but different bacterial communities and morphology. The less-frequently disturbed system will develop a dynamic community that responds to the biocide additions with concurrent dynamics of biofilm morphology.

In our analysis, the onset of detachment and its effect on community structure deserve special attention as localized detachment may initialize new succession cycles. At what frequency of biocide addition autogenic succession overrides the biocide effect is evaluated.

Cultivation of defined photoautotrophic/heterotrophic biofilm communities

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Number: 110

Biofilms that colonize surfaces in the euphotic zone of freshwater and marine habitats are represented by a photoautotrophic and heterotrophic contingent of microorganisms: phototrophs such as diatoms, green algae and cyanobacteria produce organic carbon, and thereby fuel the life of heterotrophic bacteria. Such 'photoheterotrophic biofilm communities' have received little attention in biofilm research, presumably due to the lack of suitable laboratory model systems. We currently develop a continuous-flow system for the sterile incubation and monitoring of phototrophic biofilms, and evaluate this setup in growth experiments with axenic, pure culture diatoms and cyanobacteria isolated from Lake Konstanz (Bodensee) in comparison to biofilms co-inoculated with defined heterotrophic bacteria, also isolated from Lake Konstanz. Another option explored is the work with genome-sequenced model organisms, e.g. diatom *Phaeodactylum tricornutum* and bacterium *Silicibacter pomeroyi*. The system has been designed to simulate incubation conditions similar to the euphotic, littoral zone of lakes. It comprises sterilely aerated incubation chambers into which trays can be inserted with a microscope cover slide as base, illuminated, and overlayed by a continuous flow of sterile culture medium. Other features of the system include: the overall biofilm density can be monitored photometrically; the macroscopic appearance of biofilms can be followed by time-laps photography; the tray can be removed for confocal microcopy and harvesting of the biofilms; control of irradiance, temperature, flow velocity, and composition of the culture fluid. On the conference, we will present the design of the setup, and our first result from work with defined photoheterotrophic biofilm communities.

Detection of biofilm-forming coagulase-negative *staphylococci*

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Number: 111

Objective: Property investigation in coagulase-negative staphylococci (CNS) that favor displaying of their sorption activity and biofilm forming. *Methods:* Thirty CNS clinic strains were analyzed. Human erythrocytes were used to detect adhesins. Secretion of CNS mucosal matrix was examined on agarized medium containing the Congo red indicator. Biofilm formation was realized on polystyrene plates using nutrient media - LB and enrichment medium being developed by authors. *Results:* There was no correlation between the properties under investigation being found while comparing methods of CNS adhesive properties' assessment. Specifically, bacterial ability to erythrocyte agglutination demonstrated for 93,3% strains did not evidence for their manifestation of intensive biofilm formation. However, slime-forming bacteria were indeed good biofilm formers. In addition, apparent biofilms were also formed by strains that did not possess the ability to slime formation that was detected on agarized medium with Congo red. Efficient detection of biofilm-formers among staphylococci was achieved while using authorial nutrient medium. In this case 85% strains were found to form highly apparent biofilms. LB-based cultivation demonstrated that this could be poorly applied to detect biofilm formers among coagulase-negative staphylococci, as under those conditions only 45% strains analyzed appeared to form biofilms.

EPS composition and calcification potential of filamentous cyanobacteria

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Number: 112

Tufa deposits in freshwater habitats are often the result of calcium carbonate precipitation within interfacial microbial ecosystems. Calcite precipitation is influenced by the saturation index and the occurrence of extracellular polymeric substances (EPS), which are produced by a variety of microorganisms. STXM has been used at the C K-edge to map the major biomolecules (proteins, lipids, and polysaccharides) in complex microbial biofilms. Furthermore, by means of STXM it is possible to differentiate between different species and calcium carbonate phases at the Ca L-edge. The purpose of this study is to determine if there are differences in calcium adsorption dependent on the specific composition of the EPS produced by filamentous cyanobacteria isolated from a hard water creek (Westerhöfer Bach, Harz Mountains, Germany). The sheaths of the cyanobacteria contained mainly polysaccharides and proteins, and a small amount of lipids. Both cyanobacterial sheaths contained spectral signatures of Ca^{2+} adsorbed to EPS. In *Pseudanabaena* sp. sheaths, the adsorbed Ca was distributed more homogeneously. Aragonite-like $CaCO_3$ in close association with the cyanobacterial cell surface was detected in *Leptolyngbya* sp. only. In the same region, polysaccharides were detectable. In the case of *Pseudanabaena* sp., only very small aragonite-like spots were recorded in association with accompaning cells

of heterotrophic bacteria. In conclusion, EPS produced by cyanobacteria were shown to be a component for calcium adsorption. Depending on the spatial composition of EPS, different components potentially participate to various extents in nucleation processes of calcium carbonate in close association with the cells.

Applying homogenization techniques to determine macroscopic mechanical properties of bacterial biofilms

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Number: 113

Understanding the complex dynamics of bacterial biofilms constitutes an important scientific and economic issue. Understanding biofilm mechanics is a key-issue to explain its physical characteristics. However, biofilm properties change drastically from one point to another. Indeed biofilms are composed of bacteria and EPS matrix, which present different mechanical properties. We can thus consider a biofilm as a heterogeneous material whose properties depend on the scale where they are calculated. Homogenization techniques seem particularly appropriate to compute their mechanical properties. In this work, we apply homogenization techniques which quantify macroscopic mechanical properties of heterogeneous materials to bacterial biofilms. In this first approach, we distinguish two entities in the bacterial biofilm: bacteria and EPS matrix particles. We observe the structure of their mixing both in an individual based model and on images from freeze-substitution transmission electron microscopy. In both cases, we consider a representative volume element (RVE) and derive its macroscopic mechanical properties in the case of the elasticity framework. We particularly focus on the asymmetry of the results (anisotropy). The oral presentation will summarise the main basis of homogenization techniques. We shall address the issue of the RVE size and analyze the obtained mechanical properties.

In situ Vizualization of Biofilm Structure be means of Optical Coherence Tomography

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Number: 114

Optical Coherence Tomography (OCT) was succesfully applied to visualize the meso-scale structure of three different heterotrophic biolms. Therefore, biofilm volumes of 4 x 4 x 1.6 mm^3 were scanned with spatial resolution in the lower µm-range within a short acquisition time of two minutes. A heterogenous structure was detected for biolms cultivated at laminar and transient ow conditions, respectively. The structure was more homogeneous for the biolm grown at turbulent ow condition. This biofilm structure was characterized by a volumetric porosity of 0.36 whereas the porosity calculated for biolms grown at laminar and transient conditions was 0.65. These results were directly generated from the distribution of porosity within OCT B-scans and can be linked to structural properties affecting transport processes of the whole biofilm. Major benefit of OCT is the determination of the biofilm structure at meso-scale. Up to now the meso-scale biolm structure was only observable by time consuming and expensive studies with Magnetic Resonance Microscopy. OCT will surely be helpful for improved understanding and prediction of the behaviour of biofilms with respect to mass transfer and detachment as the information about meso-scale is easily accessible. Nevertheless, to determine the local distribution of biofilm constituents, microscopic methodes like Confocal Laser Scanning Microscopy are still required.

Respiratory Activity within *Escherichia coli* Biofilms

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Number: 115

Abstract: The variety of electron transport pathways that can be represented in a biofilm surely contributes to the special ecology and antimicrobial resistance facilitated by biofilms. Given the fundamental significance of respiratory system, it is surprising that there have been very few investigations in which the electron transport chains in biofilms have been elucidated. In this study, changes in the *E. coli* respiratory- and central metabolic-transcripts during biofilm formation were monitors relative to the planktonic aerobic steady-state. We found that the bacteria respond to changes in electron transport pathways during the development to mature biofilms. In order to investigate the respiratory chains in mature biofilms we examined the effects of various inhibitors of electron transport with well descript sites of action. We showed that the terminal oxidases and reductases are branched, modular and inducible during *E. coli* biofilm formation. The biofilms switched from high oxygen to low oxygen conditions during the development and maturation. ArcA is an important transcriptional regulator of adaptation to changes in O_2 availability inside biofilms. Furthermore, inhibitors of electron transport are able to block the bacterial biofilm formation. The results of this feasibility study might pave the way for new treatments for biofilm-related infections and may be exploited for prevention of biofilms in general.

Long-term stability of aerobic granules and microbial community dynamics in a sequencing batch reactor

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Number: 116

Long-term stability of aerobic granules is one of the main concerns for its practical use to treat wastewater. So far, very little research has been done in this area. This study aims to investigate the stability of aerobic granules and it microbial community dynamics in a granular sequencing batch reactor (GSBR) operated over 1100 days. The granule characteristics were generally stable over the 425-day monitoring period started since the operation day 698. The COD removal efficiency remained high (Ave. 93%). The system was able to recover from small partial disintegration with the operating condition unchanged. During the fluctuations, marked changes in the abundance and spatial distribution of the main populations were revealed by Fluorescence *in situ* hybridization analysis. The high proportion of *Thauera* (74%) in the re-formed granules at the initial stage of the recovery process after the first time partial disintegration suggested *Thauera* likely facilitated the granule re-formation during this recovery process. Interestingly, the most dominant population was changed after the system recovered from the second time fluctuation. Some other *Betaproteobacteria*, instead of *Thauera* spp. dominated (69% of the total bacterial community) the recovered community which has being functionally stable for more than 60 days till now. Our results showed the adequate flexibility of granular microbial community was important to maintain the long-term functional stability of a GSBR. The general stable long-term performance and its recovery ability showed a potential practical application of GSBR for treating wastewater.

Analysis and modeling of growth and sloughing of phototrophic biofilms

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Number: 117

The growth of microbial biofilms often deviates from classical, deterministic models (e.g. logistic or Gompertz) because of sloughing events, i.e. the random detachment of large portions of the biofilm biomass. The random nature of these events calls for new approaches to the quantitative description of biofilm growth, going beyond standard techniques to represent sloughing, e.g. through average net detachment rates, manually predefined sloughing times and sizes, or by derivation from very complex and computation intensive multidimensional biofilm models.

In the present work, we present results from analyzing and modeling experimental growth curves from the former EU-project PHOBIA on phototrophic biofilms. Statistical analyses suggest that marine biofilms have higher sloughing frequencies, durations and sizes than freshwater biofilms. Elevated flow velocities of the bulk medium elicit the same effect. Higher light intensities yield less frequent sloughing of increased size, while higher temperatures diminish the size and increase the duration of the events. By fitting classical growth models of the logistic type, we obtained information on the growth parameters before and after sloughing, suggesting that the intrinsic growth processes generally are not affected by sloughing.

We furthermore present a stochastic model for biofilm growth, which incorporates the accumulated information from the statistical and time series analyses, and discuss possible ways of integrating the prediction of sloughing events by this purely temporal model with other, more detailed spatio-temporal models of phototrophic biofilm dynamics, e.g. based on the AQUASIM software.

Theme: Modulation of biofilm communities

Effect of single versus antibiotic combinations on Staphylococcus epidermidis biofilm viability and on genetic expression of some virulence genes

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Number: 118

In this study five clinical isolates strains were used, and nine antibiotics at breakpoint concentrations: vancomycin, tetracycline, rifampicin, gentamicin, cefazolin, cephalothin, levofloxacine, daptomycin and clindamycin were tested. 48 hours biofilms were grown on Calgary Biofilm Device (CBD) and challenged overnight with antibiotics alone and in combination. Biofilm cells viability was determined by colony forming units (cfu). Afterwards, the effect of the most active antibiotics combinations against *S. epidermidis* biofilm on genetic expression of some genes of interest such as: icaA, icaR, sarA and rsbU was determined by real-time PCR.

Although biofilms were generally insensitive to individual antibiotics, they were more susceptible to combinations. Levofloxacine was a constituent of almost all the combinations active against *S. epidermidis* biofilm pointing to be part of any antibiotic therapy directed against biofilms of these organisms.

Reduction of marine biofouling by complex communities of micro-organisms on organic polymer surfaces

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Number: 119

The oceans play a key role in the regulation of the Earth's climate. However, much of the oceans' biogeochemical processes remain undersampled, making future predictions on the Earth's climate unreliable. Oceanographic surveys are limited to a relatively small scale due to the high costs of ship based water sampling (~£15k per ship, per day) or the opacity of deep seawater to electromagnetic radiation used for remote sensing. For this reason in situ sensors have been identified as a solution to providing large scale data on biogeochemical processes in the world's oceans.

Sensors that are deployed in the ocean for long periods are prone to biofouling, a process by which microorganisms colonise immersed surfaces, producing a slime or biofilm on the sensor equipment. These biofilms can have a detrimental effect on the functioning of sensors and so need to be limited to ensure accurate, long-term measurements by the sensors.

We are developing methods of biofilm remediation using a combination of biocompatible polymers and controlled release of chemical effectors of cell physiology. One such chemical that has been tested for its efficacy for reducing biofilm formation is nitric oxide, which has been shown to reduce marine biofilm formation by up to 75% on hydrophobic, abiotic surfaces. Further experiments will study the effects of other chemical agents and their effects on biofilm community structure, using DNA based community analysis techniques

Novel chemical countermeasures against *staphylococcal* biofilms

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Number: 120

Some natural and synthetic related pyrrolomycins, a family of halogenated pyrrole antibiotics, showed anti-biofilm properties *in vitro* at low concentration against preformed staphylococcal biofilms. Moreover, considering the human cell toxicity, the selectivity indexes (ratio of cytotoxicity to antibiofilm activity) of some of them was very interesting.

The present study aims to investigate if the pyrrolomycins could also prevent staphylococcal biofilm formation. The evaluation of *S.aureus* ATCC 25923 biofilm formation inhibition was conducted by safranin staining method. At tested concentrations of 0.18, 0.09, 0.045 μg/mL the novel pyrrolomycin derivative IV resulted effective as biofilm inhibitor showing inhibition percentages ranging from 56.5 to 29% against *S.aureus* ATCC 25923. We are investigating if sortase A which is responsible for the anchoring of surface proteins to Gram positive cell wall, could be the rational target of pyrrolomycins. The surface proteins play pivotal roles in the adhesion to host's tissues, and the evasion of host-immune responses, moreover they facilitate attachment on biological and abiotic surfaces in the first steps of biofilm formation. It has been observed that inhibition of sortase A by different chemicals resulted in decrease of virulence and staphylococcal biofilm formation.

A molecular modeling study conducted by using a crystal structure of sortase A, recovered from protein data bank, and by studying docking properties of a known sortase A inhibitor and of pyrrolomycins, is in progress. *In vitro* experiments on sortase inhibition activity are needed to confirm the computational results.

A novel signaling network, exhibiting differential activity during planktonic and biofilm growth, coordinates the dispersion response and in vivo virulence of the opportunistic pathogen *P. aeruginosa*

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Number: 121

Dispersion, a process allowing biofilm cells to respond to changing stimuli and to disseminate to new environments, has been shown to be positively regulated by BdlA and six GGDEF and/or EAL domain-containing proteins, and to be limited by the sensor/regulator hybrid SagS. The present work assessed the activity of this putative dispersion regulatory network during the dispersion response and different modes of growth, and investigated its role in *in vivo* pathogenesis. qPCR analysis revealed that the expression of the dispersion-promoting regulators is elevated during motile compared to surface-attached growth, with the highest levels observed in dispersed cells released from biofilms, and lowest in biofilms remaining post-dispersion. Pull-down assays, confirmed by Far-Westerns, revealed the sensory protein BdlA to exhibit differential protein-protein interactions and processing depending on growth conditions, underscoring the dynamic nature of signaling by the dispersion regulatory network. Lastly, considering an established link between biofilm-planktonic transitions and virulence capabilities, the role of the dispersion regulatory network during *in vivo* infections was assessed. All of the dispersion-deficient strains demonstrated reduced infection spread and killing compared to the wild type. Similarly, site-directed mutations in *bdlA* previously shown to prevent dispersion were presently found to result in reduced virulence. In contrast, inactivation of the dispersion-limiting SagS accelerated infection spread and killing, suggesting a positive correlation between dispersion levels and *in vivo* virulence. Overall, we demonstrate that the signaling network coordinating biofilm dispersion exhibits growth mode dependent expression and protein interactions and is required for pathogenesis by regulating dispersion and dissemination within the host.

Inhibition of quorum sensing and biofilm formation by synthetic quorum signal analogues in *Pseudomonas aeruginosa*

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Number: 122

Pseudomonas aeruginosa is an opportunistic pathogen that mainly relies on quorum sensing (QS) and biofilm formation for its virulence. As central control of virulence, QS regulates the expression of many genes related to virulence factor production and biofilm enhances the persistence against challenge by host immunity and antibiotic medication. In *P. aeruginosa*, QS is mediated by two small diffusible acly-homoserine lactones (acyl-HSLs), N-3-oxododecanoyl-HSL (3OC12-HSL) and N-butyl-HSL (C4-HSL) which are synthesized by two signal synthases, LasI and RhlI, respectively. These signals are recognized by three QS signal receptors, LasR, QscR, and RhlR. To control the virulence of *P. aeruginosa*, we tried to develop inhibitors against QS and biofilm formation. A series of QS signal analogues that have modifications on the head, body, or tail parts of acyl-HSLs were synthesized based on *in silico* modeling analysis of QS receptor-ligand bindings and screened for anti-QS and anti-biofilm activities. Some had a significant inhibition on either QS or biofilm formation, or both. To test the potential for an anti-*Pseudomonas* agent we investigated whether these compounds could alleviate the virulence of *P. aeruginosa*. Some of our candidate compounds showed significant correlation among *in silico* modeling, QS inhibition, biofilm inhibition, and virulence, but others did just limited correlation. We suggest that the modeling studies can provide insight into the binding pose and affinity of ligand compounds, and help in the design of new inhibitors, at least until a better method that offers a full and easy prediction of the tertiary structure of the ligand-receptor binding can be developed.

The Rcs and Pvr two-component systems are novel players in the switch between the planktonic and sessile lifestyles of *Pseudomonas aeruginosa*

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Number: 123

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that causes acute and chronic infections in immuno-compromised individuals. Its ability to form biofilms has been linked to persistent infections, and regulatory systems have been identified that govern the switch between planktonic and biofilm lifestyles. Extracellular appendages produced by the Chaperone Usher Pathway (Cup) play an important role in biofilm formation, and *P. aeruginosa* PAO1 encodes at least four Cup systems (CupA, CupB, CupC and CupE). The more virulent *P. aeruginosa* isolate PA14 encodes a fifth Cup cluster (CupD) on the PAPI-1 pathogenicity island.

In this study, we show that the *cupD* gene cluster is inversely regulated by the PAPI-1 encoded Rcs and Pvr two-component systems. Expression of the RcsB response regulator leads to *cupD* gene expression and the production of CupD fimbriae. This leads to clinically relevant phenotypes, such as the formation of small colony variants and increased biofilm formation. Furthermore, increased *cupD* expression coincides with decreased expression of other surface appendages and reduced motility. The intracellular concentration of cyclic di-GMP (c-di-GMP) has also been shown to be a major factor in the switch between planktonic and biofilm lifestyles. Expression of the EAL-containing response regulator PvrR counteracts the effects of RcsB in a process that probably involves c-di-GMP degradation. The Rcs and Pvr regulatory systems are therefore novel players in the switch between planktonic and sessile lifestyles, and they may play a role in the increased virulence of the PA14 strain.

Repair potential in natural drinking water biofilms after water treatment
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Number: 124
Biofilms are present in drinking water distribution systems despite water treatment and disinfection at waterworks. They are a possible niche for hygienic relevant bacteria, and therefore a main concern for water industries. Up to now knowledge about survival strategies of bacteria during their regeneration process in biofilms after disinfection treatments has been limited. To get a deeper understanding of this problem, biofilms of ground water and surface water were investigated in different waterworks.

In each waterworks the same pilot scale, built up with different standard pipe materials, was used to simulate a household water distribution system. The water that flowed through the pilot scale was exposed to disinfection methods such as UV and chlorine dioxide. Three month old biofilms were compared using RNA and DNA based methods.

When stress markers on RNA level were investigated, UV disinfection was found to be responsible for the up-regulation of *recA*-mediated dark repair in natural biofilms. The highest *recA* induction in biofilms was associated with copper, confirming previous investigations from other waterworks. No or only low *recA* expression was found in biofilms gained from the waterworks in which drinking water was not disinfected or treated with ClO_2.

The total amount of bacteria present in the biofilms did not depend on the different materials or disinfection processes. But DGGE analysis showed a significant shift in the bacterial population when different materials and disinfection treatments were used, showing e.g. an interesting species selection when grown on copper.

Ecological fate of *Lactobacillus reuteri* in continuous culture salivary biofilm ecosystems
Tejal Madhwani, Andrew McBain, University of Manchester, UK

Number: 125
We have investigated the ecological fate and bacteriological effects of a proprietary dental probiotic formulation using previously validated continuous culture plaque simulator. Salivary biofilm microcosms were maintained in constant depth film fermenters (CDFFs) for 36 d. Following the attainment of dynamic steady-states, plaques in test fermenters were dosed daily for 10 d with *c.* 9.4 $\log_{10}$ cfu *L. reuteri.* Biofilm composition was monitored using differential culture, eubacterial-specific PCR-DGGE, and quantitative-PCR targeting *L. reuteri,* lactobacilli and total eubacteria. *L. reuteri* addition caused significant decreases in streptococci ($p<0.05$) and Gram-negative anaerobes. PCR-DGGE analyses indicated that the exogenous lactobacillus displaced endogenous *L. delbrueckii*. Morphological and qPCR tracking indicated *c.* $4\log_{10}$ cfu/mm^2 and $4\log_{10}$ copies/mm^2 of *L. reuteri* respectively three weeks after cessation of treatment. In summary, dosing of *in-vitro* plaques with a probiotic formulation of *L. reuteri* probiotic suppressed streptococci and Gram-negative anaerobes. The probiotic bacterium persisted within plaques following treatment.

Continuous biofilm annular reactor: fingerprint investigation of microbial competition diversity
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Number: 126
Microbial communities in mixed biofilms are frequently composed of several types of different bacteria. In a continuous aerobic reactor, the competition between biofilm and planktonic cells is promoted by the hydraulic residence time (HRT).

The aim of this study was to determine the influence of the HRT on the microbial diversity of biofilm during the growth.

Three different values of HRT (0.3, 1, 8 hours) were tested in a rotating annular reactor at a constant substrate loading rate. Total biofilm DNA and suspended biomass DNA were extracted weekly over the period of 42 days. PCR-SSCP was used for fingerprinting the bacterial communities for calculation of log Simpson Diversity Index (SI).

The resulting profiles show that, for the dynamics over time, the bacterial community did not change in the

suspension and were similar in the three conditions tested. The SI was around 2.5. Biofilm communities evolved and diversified at lower HRT. The SI increased from 1.9 to 3. In contrast, at higher HRT, communities of biofilms did not change over the period of experiment, with a SI of 3.

The increase of HRT increased the number of peaks in the communities of biofilms and thus increased the SI. We observed this aspect during the first weeks of the experiments. Towards the end of experiments, HRT lost its influence on diversity.

In search of Broad Applicable, Small Molecule Inhibitors of Salmonella Biofilm Formation.
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Number: 127
Salmonella is an important cause of foodborne infections worldwide. A major difficulty in the battle against *Salmonella* is the fact that *Salmonella* can form biofilms on various biotic and abiotic surfaces, both in- and outside the host. Therefore, the inhibition of these biofilms could be an effective way to combat *Salmonella*.

We are currently conducting a high-throughput screening (using the 'Calgary Biofilm device') of a compound library consisting of > 20.000 small molecules, in search of *Salmonella* biofilm inhibitors which are active at a broad temperature range and therefore have potential to be used both in- and outside the host.

The compounds have a molecular weight between 200 and 500 dalton and are selected on their possible drug ability. The screening is executed both at 16 °C and 37 °C. We aim at identifying compounds that inhibit biofilm formation, without killing the bacteria. This way, the development of resistance is less likely.

After screening of 16.000 compounds (80%), we identified 133 possible biofilm inhibitors. Subsequently the dose-response relationship of the 'hits' was determined, as well as the growth-influences of the compounds. The compounds with maximum biofilm inhibitory capacities and minimal growth influences, were studied further, both on prevention and destruction of biofilms from *Salmonella* and *Pseudomonas*. Using these results we identified 8 'lead' families off which the "structure-activity relationship" will be determined aswell as the activity in different *in vitro* and *in vivo* testsystems. Finally the 'Mode of Action' of will be determined using reportergene studies and/or microarray analysis.

A comparison of the antimicrobial and anti-biofilm potency of apolipoprotein E- derived antimicrobial peptides and cationic biocides.
Sarah Forbes, Andrew J McBain, Bridie A. Kelly, Curtis B. Dobson, University of Manchester, UK

Number: 128
A series of cationic peptide derivatives of human apolipoprotein E (apo-E peptides) have been developed that demonstrate potent antimicrobial activity and low mammalian cytotoxicity. The aim of this investigation was to evaluate the effectiveness of an apoE-derived antimicrobial peptide for the ability to inhibit 18 microorganisms associated with medical device infections (including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis),* as well as assessing ability to inactivate biofilms. The apo-E peptides performance was compared to that of the commonly used antiseptics polyhexamethylene biguanide (PHMB), triclosan (TCS) and chlorhexidine (CHX). Susceptibilities of planktonic organisms (Minimum Bactericidal Concentrations) compared to those of the same organisms in their biofilm state (Minimum Biofilm Eradication Concentrations) ranged from 0-20 fold higher for PHMB, 0-35 for CHX, 0-40 for the apo-E peptide and 0-430 for TCS. Whilst the ability of each antimicrobial to eradicate single species biofilms varied widely between test bacteria, the apo-E peptide performed equal to, or better than PHMB and CHX for 7 of the 18 organisms tested and TCS for 6. The apo-E peptide demonstrates broad-range antimicrobial activity and indicates an ability to inactivate certain single species biofilms comparable to that of commonly used biocides.

Analysis of biofilm and aggregation in rhodococcus sp. Sd-74
Kikuko Takuma, Tomohiro Inaba, Hiroo Uchiyama, Nobuhiko Nomura, Toshiaki Nakajima, University of Tsukuba, Japan

Number: 129
Microbes make a community, called "biofilm", or aggregation. Bacterial aggregation is microbes form a groupe, and large chunks, which precipitates. Aggregate form, such as to facilitate the industry in terms of solid-liquid separation process, there are many benefits. Therefore to control the aggregation is very important in industry. However, there are some theories of aggregation mechanism. So, it is difficult to control the aggregation. We have investigated the mechanisms of the aggregate morphogenesis and biofilm formation in *Rhodococcus* sp. SD-74.

Rhodococcus sp. SD-74 was found to abundantly produce biosurfactant from *n*-alkanes. This biosurfactant , succinoyl trehalose lipid (STL) consisted of trehalose, fatty acids, and succinic acids. *Rhodococcus* sp. SD-74 forms strong aggregates in liquid culture. To investigate the mechanisms of aggregation and biofilm formation,

a transposon mutagenesis system was adapted for SD-74, and apporoximately 1900 mutants were generated. 4 mutants lacking the ability to form biofilm and aggregation were obtained. The hydrophobicity of the microbial cell surface is generally accepted to be a major factor in adhesion. We measured cell hydrophobicity of wild-type and 4 mutants. As a result, cell hydrophobicity and aggregate ability are not related. To investigate other factor, we have been analyzing some aggregation factors of the 4 mutants.

Effect of N-Acetylcysteine alone and in combination with rifampicin on *Staphylococcus epidermidis* biofilms

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Number: 130

Medical device-associated infections caused by pathogens such as *Staphylococcus epidermidis* might involve biofilm formation and those are particularly challenging. The involvement of antibiotic resistant Staphylococci, exacerbates the problem. Rifampicin cannot be used as a single agent to treat infections because of the rapid selection of resistant mutants. However, combinations of rifampicin with other anti-staphylococcal agents could prevent the emergence of rifampicin resistance during therapy. N-acetylcisteine (NAC) decreases biofilm formation by a variety of bacteria and reduces the production of extracellular polysaccharide matrix. The goal of this study was to assess the antimicrobial activity of NAC in combination with rifampicin against biofilm of *S. epidermidis*. Two *S. epidermidis* strains biofilm-producing (9142 and 1457) were used in this study. 1xMIC (4mg/ml) and 10xMIC (40mg/ml) of NAC and 10mg/l of rifampicin, based on preliminary *in vitro* data, were added to 24h biofilm cells. Biofilm susceptibility to tested antimicrobial agents was assessed through scanning electron microscopy, crystal violet staining (total biofilm biomass) and cellular viability through XTT and colony forming units (CFU). The effect of NAC 1xMIC was similar to that of the control. Rifampicin, NAC 10xMIC alone and NAC-rifampicin combination (independently of NAC concentration used) showed significant bactericidal effect, promoting a 3-4 $\log_{10}$ decrease in biofilm cells. In conclusion, the results didn't point to any synergistic effect between the two agents. Nevertheless, NAC seems to be a possible alternative to antibiotics in the treatment of infections associated to *S. epidermidis* biofilm.

Particle sizes and disinfection susceptibilities of detached biofilm and planktonic cells in single species and co-cultures of *Burkholderia cepacia* and *Pseudomonas aeruginosa*

Sabrina Behnke, Anne Camper, Montana State University

Number: 131

The disinfection susceptibilities of suspended planktonic cells have been well-studied for a large variety of biocides. These biocides have been found to be much less effective against cells in biofilms, requiring biocide concentrations orders of magnitude higher than those necessary to kill suspended planktonic cells. Although the detachment of aggregated cells from biofilms is of fundamental importance to the dissemination of organisms in both public health and clinical settings, the disinfection efficacies of commonly used biocides on detached biofilm particles has not been investigated. Therefore, the question arises whether cells in detached aggregates can be killed with disinfectant concentrations sufficient to inactivate planktonic cells. *Burkholderia cepacia* and *Pseudomonas aeruginosa* as model organisms were grown in standardized laboratory reactors either as single species or in co-culture. With the help of fluorescent microscopy and computer image analysis the cluster size distributions in the chemostat and the biofilm effluent were determined. Chlorine susceptibility has been assessed for planktonic cultures, attached biofilm, and particles and cells detached from the biofilm.

Disinfection tolerance highly depends on the occurrence of larger cell clusters in the samples. Cells and clusters that detached from the biofilm were generally less susceptible to disinfection when samples contained a higher number of large clusters than the planktonic culture. The disinfection efficacy was also dependent on species composition; co-culture of both species was advantageous to their survival when grown as a biofilm but surprisingly resulted in a lower disinfection tolerance when grown as a mixed planktonic culture.

Activation of autolytic degradation of *Staphylococcus epidermidis* 33 biofilms

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Number: 132

S. epidermidis appears to be major cause of nosocomial diseases with biofilm formation. This determines the necessity in the effective methods for biofilm degradation. *Objectives.* The aim of current investigation was the examination of staphylococcal biofilm sensitivity to small cationic peptide warnerin and antibacterial proteins lysozyme and lysostaphin. *Methods. S. epidermidis* 33 biofilms were formed on polystyrene plates at 37°C for 24 h (LB, 0.2% glucose, log-phase bacteria,10^7 CFU/ml). Biofilm mass was judged by the gentian violet binding; amount of living cells was determined with Cell Proliferation Assay (Promega). Warnerin, lysozyme

(Sigma) and lysostaphin (Sigma) in phosphate buffer (pH 7.2) were applied to biofilms. After 24 h the biofilm supernatants were used for renaturing PAGE with gels containing autoclaved cells *S. epidermidis* 33. *Results.* Effect of all those antibacterial compounds resulted in film biomass and number of living bacteria reduction. Electrophoresis results demonstrated that the action of all cationic factors used here was conditioned by non-specific activation of biofilm autolysins. The range of activated autolytic systems of bacterial cell walls within biofilms was similar to that observed under the warnerin action on planktonic cells [1]. *Conclusions.* Cationic peptide compounds are able to suppress the *S. epidermidis* 33 biofilm growth via activation of autolytic systems in their cell components. [1]. Korobov et al., 2010, Microbiology, 2010, 79(1), 125-127.

In vitro antimicrobial activity of _Pseudomonas aeruginosa_ by-products against single and mixed biofilms: the role of Gram- bacteria in the biofilm consortium

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Number: 133

Since bacteria are permanently acquiring resistance to chemicals, the development of novel strategies for biofilm control is needed. Certain microorganisms represent an important source of novel bioactive compounds with marked antibacterial activity, as the secondary metabolites. This work aimed to investigate the antimicrobial effect of *P.aeruginosa* by-products on planktonic and sessile growth of several pathogenic bacteria.

Supernatants from *P.aeruginosa* planktonic cultures (isolated: PaI and from collection: Pa) were recovered, filtered and tested on their own and on *S.aureus*, *S.epidermidis, E.coli* lawns. Their antimicrobial action was also assessed in single *Staphylococcus* biofilm formation and in polymicrobial biofilms formed by *Staphylococcus* species together with Gram- bacteria. Supernatants were applied as biofilm growth media complement or as biofilm disruption agents. Both supernatants inhibited only Gram+ species lawns, being the more remarkable inhibition halos obtained with PaI supernatant. Concerning biofilms, PaI and Pa metabolites can be considered anti-staphylococcal biofilms agents since their single and mixed biofilm growth was significantly disturbed by both supernatants, regardless their mode of application. However, when Staphylococcal species are entrapped in polymicrobial biofilms with E.coli and P.aeruginosa, supernatants did not exhibit noticeable anti-biofilm activity, mainly when applied against established biofims. In general, all mixed biofilms accumulated more mass and had more metabolic activity when submitted to the supernatants aggression.

It is concluded that P.aeruginosa supernatants as potential as anti-biofilm agents but only against staphylococcal biofilms since they failed in disturb other biofilm consortia that encompassed Gram- bacteria. This trait makes them quite ineffective chemical countermeasures against real biofilms.

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Susceptibility patterns and cross-resistance evaluation of several biofilm-producing _P. aeruginosa_ challenged by antibiotics

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Number: 134

P.aeruginosa (PA) is an opportunistic pathogen responsible for high percentage of nosocomial infections. Its virulence and persistence rises when bacteria switch from planktonic to biofilm state and when facing antimicrobial pressures. This study aimed to evaluate the antimicrobial tolerance and regrowth of several biofilm-producing PA after antibiotic treatment, and the occurrence of cross-resistance to other antimicrobials.

PAO, ATCC, CGCT and an isolated strain were used to form 1-day old biofilms in the presence of ciprofloxacin (CIP). Biofilm regrowth was evaluated after 48h, 72h and 96h after addition of, respectively, fresh medium, medium with antibiotic and antibiotic-free medium again. The final 4-days old biofilms were then attacked with CIP, gentamicin, rifampicin and benzalkonium chloride.

After 24h and 72h of biofilm growth, it was observed a good reduction of the biofilm mass, respiratory activity and CFU/cm^2. However, PA biofilm regrowth with similar or even higher cell numbers, comparatively to control, were noticed always the antibiotic-free medium was added. Data allowed speculating that the adhered bacteria that survived to antibiotic therapy may become more resistant to CIP or to other antimicrobials. Luckily, data did not show evidence of cross-resistance to the antimicrobials tested. Biofilms formed in the presence of CIP appeared to be more susceptible to the anti-biofilm action of antimicrobials.

Although it has been observed marked biofilm regrowth after good levels of biofilm eradication due to antibiotic treatment, the resulting biofilms challenged by CIP seemed to be less tolerant to the action of other antimicrobials.

Acknowledgments: IBB-CEB and FCT (PTDC/SAU-ESA/64609/2006; SFRH/BD/31065/2006; SFRH/BD/47613/2008)

Induction of anti-staphyloccocal activity in a marine environmental biofilm

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Number: 135

Staphylococcus aureus is increasingly virulent and resistant to antibiotics. Previous work has shown that epibionts from marine environmental biofilms have been induced to express anti-*S. aureus* activity. We set out to induce inhibitory activity against *S. aureus* in a complex, multispecies marine environmental biofilm.

Agar plates were prepared with or without the addition of 20% *S. aureus* spent culture media and immersed in coastal seawater (Sydney Harbor, NSW Australia; Boston Harbor, Massachesetts USA) for four days. Nuclepore filters inoculated with *S. aureus* were then applied over the surface of the environmental biofilms, incubated 4-12 hour at 37 deg C. and subsequently stained and analyzed. Total DNA was extracted from the environmental biofilms and nested PCR performed followed by DGGE. Cultured isolates from marine environmental biofilms were also tested individually against *S. aureus*.

S. aureus incubated over biofilms grown on agar containing *S. aureus* spent culture media had significantly larger numbers of dead cells, smaller micro-colonies, and less growth compared to *S.aureus* grown over biofilms grown on plain agar. DGGE of plain and *S. aureus* agar-grown marine environmental biofilms showed different microbial consortia. Cultured isolates from plain and *S. aureus* agar biofilms showed no consistent pattern of *S. aureus* inhibition when tested individually.

Compared to those grown on plain agar, marine environmental biofilms grown on agar containing *S. aureus* spent culture media were significantly more inhibitory of *S. aureus* growth. These results also suggest that mixed bacterial consortia may be more able to defend themselves from competition than individual isolates.

Antimicrobial susceptibility of planktonic and biofilm growth forms of urinary tract infecting bacteria

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Number: 136

Objectives: Catheter associated urinary tract infection (CAUTI) is the most prevalent hospital acquired infection which can lead to serious systemic infection, and increases both patient morbidity and health care costs.

The urease-positive bacterium, *Proteus mirabilis* is particularly problematic as it forms extensive biofilms in urinary catheters and induces catheter encrustation. The aim of this study was to determine the susceptibility of CAUTI causing bacteria including *P. mirabilis* against a range of natural antimicrobials.

Methods: The antimicrobial activity of eugenol, tea tree oil, terpinen-4-ol, cineole and triclosan was measured against planktonic and biofilm growth of bacterial isolates from CAUTIs. Planktonic assays were based on a modified broth microdilution method, whilst biofilm susceptibility involved initial development of biofilms in 96-well microtitre plates followed by exposure to the antimicrobials. Planktonic minimum inhibitory concentrations (MICs) were determined as an 80% reduction in absorbance after 24 h exposure relative to controls. The minimum biofilm inhibitory concentration was similarly measured following re-growth experiments on previously exposed biofilms.

Results: Planktonic MICs were species dependent with *Pseudomonas aeruginosa* generally being least susceptible (>8% v/v for tested essential oils and >512 µg/ml triclosan). For other species, planktonic MICs were 0.25-0.5% (v/v; eugenol), 0.5-2% (v/v tea tree oil), 1-8% (v/v cineole), 0.25-0.5% (v/v terpinen-4-ol), and 1-16 µg/ml (triclosan). Reduced susceptibility of biofilms to these agents was generally observed with inhibitory concentrations enhanced 10-fold on occasions.

Conclusions: Susceptibility of CAUTI causing bacteria against essential oil antimicrobials and triclosan was evident, despite an enhanced biofilm resistance being evident to these agents.

High-throughput flow-based screening for rapid determination of optimum biofilm development and dispersal conditions

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Number: 137

High-throughput assays for studying biofilm development and dispersal have traditionally been limited to microtitre-based static growth conditions. This is due to the inherent difficulties in producing consistent and reproducible flow-cell biofilms for direct comparisons across experiments. This research obstacle is especially troublesome when studying novel biofilm-forming microorganisms recovered from unique environments, where the knowledge of what constitutes the ideal growth nutrients, flow rate or surface attachment

requirements to adequately enable flow-based biofilm studies can be sparse. In order to address this technical hurdle, we utilized the Bioflux 1000 microfluidic flow-cell array system to screen for the optimum shear force, temperature and nutrient conditions for several industrial relevant single culture biofilms of *Burkholderia, Pseudomonas* and *Halomonas* species and mixed species oral biofilms. Once conditions supporting robust and reproducible biofilms were obtained, the Bioflux system enabled rapid screening of the biofilm-disruption via time-lapse microscopy. Among the disruptors tested were several novel compounds, such as nitric oxide donor chemicals as well as marketed oral hygiene products. The bactericidal action of traditional and novel antimicrobial treatments was also evaluated by confocal scanning laser microscopy of live/dead stained biofilms directly in the Bioflux microfluidic flow channels. Comparisons of the effectiveness of oral care products on multi-species plaque biofilm development, dispersal and inhibition were also performed. The results of this research present an exciting new approach to the development of flow-based biofilm techniques, enabling the rapid testing of a broader range of biofilm-forming microorganisms, and reducing the time and resources required for screening of biofilm-inhibiting compounds.

Universal system for cultivation, modulation and sample preparation of bacterial biofilm communities tested in proteomic studies

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Number: 138

The gene expression changes accompanying formation of bacterial biofilm communities are efficiently studied using the proteomic approach. There, the quantitative and reproducible preparation of protein samples is an obvious prerequisite. Similarly, the studies of major attributes of biofilms, such as cell signalling and antibiotic resistance, require availability of flexible and easy to modify laboratory cultivation system. To meet these requirements we adapted cultivation system we had developed for studies of Streptomycetes differentiation (Nguyen et al., 2005). The system uses cheap glass micro-beads (0.3 μm) immersed in a liquid medium and can be used for static cultivation in a Petri dish or assembled into the flow-through reactor. Presence of glass beads facilitates disintegration of biofilm mass during the protein samples preparation. We followed the formation of biofilm on glass beads by observation of whole cultivation dishes in an AQUASEM scanning electron microscope (TESCAN). Proteins were separated by 2D electrophoresis and proteome data were analysed by PDQuest software (Biorad). We compared proteomes from biofilm populations of *Streptomyces granaticolor* and *Mycobacterium smegmatis* with those from planktonic populations in shaken and static cultures. Comparison of two different bead materials, glass and Zirconia/Silica, did not show any difference in support of biofilm formation or in non-specific binding of proteins when used for grinding of the cell material. Presented cultivation system has also the potential for testing of the effect of different materials and antibacterial compounds on biofilm formation when coated beads are used.

Nguyen L.D. et al. *Appl Environ Microb.* **71,** 2848-2852 (2005).

Sulfide oxidation by nitrate-reducing sulfide-oxidizing bacteria in wastewater biofilms. Kinetics and numerical modeling

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Number: 139

Indigenous nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) oxidize sulfide produced by sulfate reducing bacteria, coexisting in biofilms from waste water treatment plants (WWTP). Addition of nitrate stimulates the activity of NR-SOB reducing considerably the net sulfide production. The objective of this study was to determine the dependence of sulfide oxidation on the added nitrate concentration. Kinetics of net sulfide oxidation by NR-SOB was studied in the laboratory, in biofilms grown at the Guadalete-WWTP (Spain). Vertical H_2S and pH profiles were measured with microelectrodes at the water-biofilm interface and within the biofilms, being sulfide oxidation rates calculated by numerical modeling. The decrease in sulfide concentration and the increase in the net rate of sulfide oxidation respect to nitrate concentration followed a Michaelis-Menten kinetics, with a half saturation constant of 21 and 17 μM NO_3^- for total sulfide and net rate of sulfide oxidation respectively. The stimulation of net sulfide oxidation by nitrate was a clearly reversible process. Net sulfide oxidation rate decreased to initial values in 3-6 h after the suppression of nitrate addition. Net sulfide oxidation rate and the recovery rate of sulfide levels after the suppression of nitrate addition were dependent on total sulfide and on nitrate concentrations, suggesting a double-substrate kinetics. Mass balance analysis and the stoichiometry of the process showed that neither sulfide oxidation nor nitrate reduction were completed to sulfate and N_2 respectively, what suggested that intermediary compounds as elemental sulfur and nitrite accumulated in our experimental system.

Adhesion Prevention: A Neglected Strategy in the Control of Dental Biofilms

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Number: 140

Dental caries is a dieto-bacterial disease associated with the oral cavity. The disease and the associated tooth decay (demineralization) are correlated with the utilization of dietary sugars by the oral biofilm which leads to a decrease in pH and an increase in the proportion of acidogenic and aciduric species, especially *Streptococcus mutans*. In the oral cavity, dental biofilm or plaque formation is detectable in minutes, rapidly forming on teeth and dental implants. Micro-organisms derived from the oral mucosa and saliva adhere to the pellicle in a critical first step, necessary for the development of dental biofilm. Antiseptic mouth rinse solutions are used in many situations for controlling plaque, however adhesion prevention is often neglected in the fight against tooth decay. The effect of an enzyme containing mouthwash on bacterial adhesion was examined using the MBEC biofilm model. Mouthwashes containing enzymes prevented bacterial adhesion after a one minute treatment compared with a commercially available mouthwash. These results highlight the relevance of preventing bacterial adhesion when developing oral hygiene strategies.

Theme: Signalling and communication in biofilms

Biofilm formation and development in *Rhodobacter sphaeroides* involves the chemosensory system which is regulated by a two component regulatory system

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Number: 141

Rhodobacter sphaeroides is a purple, non-sulphur α-proteobacteria which is able to form biofilms. It has a complex chemosensory system consisting of three main operons and unlinked loci. It is known that the two major chemotaxis operons, cheOp2 and cheOp3, are essential for chemotaxis and expressed in planktonic cells, however little is known about their role and expression in biofilm bound cells. In this study, we looked at the role of chemotaxis in biofilm development and the expression of cheOp2 and cheOp3, using a plasmid based GFP reporter system, when cells are grown in biofilms. It was shown that both operons are expressed throughout the biofilm lifecycle and that they are expressed in cells which are located in different areas of the biofilm.

We then looked at control of expression and identified a two-component regulatory system, which when deleted, caused biofilms to be altered in their physical appearance and have increased production of exopolysaccharide and increased adhesion to surfaces. Furthermore deletion of the two-component regulatory system alters expression of the chemosensory genes. The data suggests that environmental sensing via this two-component regulatory system is involved in the switch from the free-living to surface-attached growth.

F Plasmid-Mediated Signaling during Escherichia coli Biofilm Formation

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Number: 142

Abstract: The F plasmid of *E. coli* allows horizontal DNA transfer between an F+ donor cell and an F- recipient. Expression of the pilus genes is tightly controlled by a number of factors, including the following plasmid-encoded regulatory proteins: TraJ, and the autoregulators TraM. However, the unusual expression of F pili between two F+ cells (F- phenocopies) has been observed during the development of *E. coli* biofilm. The F+ × F+ mating was resulted from the secondary characteristics of stationary phase-like sessile bacterial population during the formation of microcolonies. Here, we found that traM and traJ genes were up-regulated in microcolony biofilm, and later promoted the development of microcolony to mature biofilm. We then demonstrated that the interaction between traM and traJ involved in the F+ × F+ piliation. The localization of TraMJ expression was found on the substratum inside microcolonies indicated that F pili are the initial cell-to-cell adhesion. We showed that TraMJ signal were quorum sensing-like molecule. TraM and TraJ were secreted and assembled outside bacterial cells. In addition, the interaction between TraMJ was regulated by H-NS from the host cell, and each molecule could be produced from different cells. These indicated the role of F transfer in adaptive physiology in starved or stationary-phase cells during biofilm development.

Monoculture and mixed biofilms of *Listeria monocytogenes* and *Pseudomonas fluorescens* – effect of different culture media and temperatures

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Number: 143
Like most microorganisms, *Listeria monocytogenes* and *Pseudomonas fluorescens* are able to form biofilms and are rarely found as monoculture biofilms in natural environments. Previous works showed that associations between bacteria from different genus commonly found in food-processing environments may affect their growth, attachment and biofilm formation. This work studied *L. monocytogenes* and *P. fluorescens* monoculture and multispecies biofilm formation, and investigated how different culture media and temperatures may influence such bacterial interactions.

L. monocytogenes strains assayed were CECT 4031^{T}, 747 and 994 (food isolates), 1559 (environmental isolate) and 1562 (clinical isolate). *P. fluorescens* strains used were ATCC 27663 and PF7A (food isolate). Each strain was tested for monoculture and mixed culture biofilm formation with each one of the other bacterium's strains. Assays were performed during three days in 96-weel microtitre plates, at 4ºC, 22ºC and 37ºC. Brain Heart Infusion (BHI) and Skim Milk (SM) were the culture media and biofilm formation was assessed by Crystal Violet staining.

Overall results showed that both media and temperature affect biofilm formation, as monoculture and as multispecies biofilms, and confirmed that the influence of different bacterial genus on biofilm formation is dependent on strains. Although a decrease of biomass was observed on multispecies biofilms formed at 22ºC in SM and at 37ºC in BHI, significantly higher OD values were found at 4ºC in both media, and at 22ºC in SM, indicating that the combination of these two bacteria on meat and dairy food processing environments may seriously compromise food safety potentiating higher contamination levels.

Concurrent Quorum Sensing and Quorum Quenching in a Simultaneous Nitrification, Denitrification & Phosphorus Removal Sludge Community
Grant Tan, Staffan Kjelleberg, Wun Jern Ng, Yan Zhou, Scott Rice, Kai Shyang Koh and Chuan Hao Tan, Advanced Environmental Biotechnology Centre (AEBC)

Number: 144
Cell-to-cell communication or quorum sensing (QS) signalling is critical for coordination of social behaviours of bacteria. Despite several reports on the isolation of acyl homoserinelactone (AHL) signal producers from activated sludge, limited studies have documented the occurrence of AHL-mediated communication within the sludge microbial community. We have investigated the potential involvement of AHL-mediated communication between bacterial populations and the turnover of AHL signalling molecules in a Simultaneous Nitrification, Denitrification & Phosphorus Removal (SNDPR) sludge community of a Sequencing Batch Reactor (SBR). Using a culture-based AHL detection assay, AHL signal production was readily detected in the SNDPR sludge community. However, AHL signals were almost undetectable in experiments where a bioassay strain that expresses the GFP reporter gene in the presence of AHLs (i.e., a bacterial strain carrying a broad range plasmid expressing *luxR* and the *luxI* promoter fused to the *gfp* gene) was added to the sludge community. One possibility for this discrepancy, is that signal-degradation or quorum quenching (QQ) activity occurs simultaneously in the mixed community which impacts on the *in situ* detection of AHLs. Indeed, *in situ* assays of SNDPR sludge samples spiked with synthetic AHL signals, demonstrated that the exogenously added signals were consistently degraded in a concentration- and time-dependent manner. Further analyses using crude protein preparations of the SNDPR sludge confirmed that the signal degradation was mediated by enzymatic activities. These findings demonstrate the simultaneous QS and QQ activities in a SNDPR sludge community, providing a rare insight into AHL-mediated communication by microbial communities in complex systems.

Capture and retention of *Cryptosporidium parvum oocysts* by *Pseudomonas fluorescens* biofilms on PVC pipe
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Number: 145
Association of *Cryptosporidium* oocysts with biofilm communities can influence the propagation of this pathogen through bothenvironmental systems and water treatment systems. The purpose of the present work was to determine the adhesion ability of *Pseudomonas fluorescens* ATCC 13525 and evaluated the capacity of *P. fluorescens* biofilm to retain and capture *C. parvum* oocyst in poly (vinyl chloride) (PVC) pipe, commonly used in irrigation system.

The experimental system was made of one PVC pipe reactor, one peristaltic bomb, one glass bottle and, two hoses, previously sterilized. The system worked with circulation flow rates of 20 ml.min^{-1} for 12 h per day, during 5 days. Adhered cells were removed by manual scratching a stainless steel rod with a neoprene disc on the reactor wall and they were quantified through colony forming units (CFU). Oocysts were concentrated and quantified by direct immunofluorescence technique. Hydrophobicity was evaluated through contact angle measurements by the sessile drop technique. The degree of hydrophobicity was expressed as the free energy of interaction.

Results showed that PVC surface is hydrophobic, while *P. fluorescens* cells, *C. parvum* oocysts and the mixed culture of these two microorganisms are hydrophilic. From a physical-chemical point of view, the results suggest that adhesion process of *P. fluorescens* cells, *C. parvum* oocysts, or *P. fluorescens* cells with *C. parvum* oocysts on PVC surface is thermodynamically favorable. Moreover, it was observed that *P. fluorescens* biofilmin PVC pipe could be able to retain and capture *C. parvum* oocyst. These results are important because biofilm communitiescan impact the environmental transmission of *C. parvum*.

yaiC, a GGDEF-domain encoding gene, is a determinant of *Escherichia coli* biofilm-mediated susceptibility to antimicrobials

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Number: 146

Cyclic di-GMP (c-di-GMP) is a second messenger that has been implicated in the developmental processes required for bacterial biofilm formation. Intracellular levels of this messenger are regulated by the enzymatic action of c-di-GMP turnover proteins. *Escherichia coli* K-12 has 29 genes that encode proteins involved in this turnover, each bearing a domain responsible for the synthesis (GGDEF domain) or degradation (EAL domain) of c-di-GMP. These proteins also contain N-terminus domains that may sense outside stimuli. We hypothesized that the N-terminal domains may sense antimicrobial compounds to regulate c-di-GMP turnover, leading to changes in the survivability of GGDEF/EAL mutant biofilms. To test this hypothesis, peg-attached biofilms were grown using the Calgary Biofilm Device, determining that all 29 strains of *E. coli* K-12 bearing single deletions in GGDEF/EAL genes were capable of forming biofilms of equivalent cell densities. Additionally, Confocal Laser Scanning Microscopy demonstrated that mutant biofilms had similar morphological structure and thickness compared to wild type. Of the isogenic mutants tested for antimicrobial susceptibility, *yaiC* and *ycdT* (GGDEF-encoding), and *yoaD* and *rtn* (EAL-encoding) displayed altered phenotypes. In particular, *yaiC* had a discrete role in the susceptibility of *E. coli* biofilms to antimicrobials, specifically aminoglycosides. This response is unique to biofilms and is not evident in either logarithmically growing or stationary phase planktonic cells. Therefore, the tolerance of biofilms to antimicrobial agents may be linked to the regulation of c-di-GMP and certain c-di-GMP proteins may be critical components in the stress response pathways of bacterial biofilms.

Development of fluorescent reporter strains to analyse biofilms

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Number: 147

The significance of biofilms as ubiquitous lifestyles with particular advantages compared to planctonic growth is more and more appreciated. With the growing interest in biofilms there is an increasing need for tools to monitor how biofilms are influenced by their environment. For this purpose fluorescent proteins are commonly used as biological reporters to visualise gene expression.

We developed a suite of fluorescent labelled *Candida albicans* and *Pseudomonas aeruginosa* as fungal and bacterial model systems in order to investigate species- and interspecies-specific communication in biofilms. Reporter genes were placed under control of promotors upregulated in different stages of biofilm formation. The constructs were integrated in the genomes to avoid the urgency of selection markers and to ensure equal amounts of reporter genes in every cell. The reporter strains are analysed using fluorescence microscopy and quantitatively measured at specific wavelengths.

First results show that the reporter strains respond at different time points during the formation of biofilms but are not induced during planctonic growth. This indicates that fluorescence is triggered by biofilm formation. The reporter strains are also used to analyse mixed biofilms of *C. albicans* and *P. aeruginosa*. Furthermore the influence of potential quorum sensing molecules produced by different species on the reporter strains is detected.

This toolbox of fluorescent reporter strains will facilitate analyses of biofilms in more detail and help to analyse pathways involved in the formation of biofilms.

QseBC links the detection of autoinducer-2 to the control of biofilm growth and virulence in *Aggregatibacter actinomycetemcomitans*

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Number: 148

Significance: Previous studies identified two receptors for AI-2 (LsrB and RbsB) but it remained unclear how detection of signal was linked to downstream gene regulation that controlled biofilm formation and virulence.

Results: The expression of *qseBC* in a *luxS* knockout strain was induced 14-fold by exogenous AI-2. In contrast, AI-2 had no effect on *qseBC* expression in a strain that did not express *lsrB* and *rbsB*. Furthermore, inactivation of *qseC* reduced biomass in biofilm cultures of *A. actinomycetemcomitans*. To determine if AI-2 signaling and

QseBC influence virulence, a murine model of periodontitis that measures alveolar bone resorption as the clinical outcome was used. Mice infected with wild type *A. actinomycetemcomitans* showed a significant increase in bone resorption relative to sham infected controls. Inactivation of *lsrB* or *rbsB* reduced alveolar bone loss compared to the wild type and a *lsrB/rbsB* double knockout was avirulent. A *qseC* mutant was also avirulent and did not induce bone resorption over that observed in the sham infected group but complementation with a plasmid borne copy of *qseC* restored virulence to wild type levels. Interestingly, virulence of the *luxS* mutant was similar to the wild type. Given that the *lsrB/rbsB* strain was avirulent, this suggests that the *luxS* mutation may be complemented *in trans* by AI-2 produced by indigenous organisms in the murine oral cavity.

Conclusion: Our results suggest that AI-2 signalling regulates *in vivo* virulence of *A. actinomycetemcomitans* and that QseBC may link the detection of AI-2 to gene expression controlling biofilm growth and virulence.

Bacterial signalling in biofilms in the rhizosphere of barley

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Number: 149

Biofilms are the predominant natural life form for most bacteria. Life in a biofilm affords various advantages, for example an increased resistance to environmental stresses, antimicrobial tolerance, protection from protozoan predation, high population densities and microbial interactions. Biofilm development and the resulting interactions with eukaryotic host organisms require cell-cell communication between colonising bacteria. Thus, biofilms are often the site for quorum sensing. Bacterial communication through quorum sensing using small chemical signal molecules like *N*-acylhomoserine lactones (AHL) enables bacterial populations to regulate their gene expression and to coordinate their behaviour.

In this study, biofilm forming bacteria (*Pseudomonas putida* IsoF and *Serratia liquefaciens* MG1) were localised in biofilms on the root surface of barley using *gfp*-tagging and confocal laser scanning microscopy. The AHL-production in such biofilms was investigated with the help of AHL bioreporter strains. AHLs produced by *Serratia liquefaciens* MG1 could also be found in barley shoots and were quantified using an enzyme-linked immunosorbent assay.

Specific induction of quorum sensing in *Erwinia carotovora* subsp. *atroseptica* by host-plant metabolites: detection and characterization of plant inducers

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Number: 150

Quorum sensing controls formation of a biofilm and multiple bacterial phenotypes. In pathogenic bacteria quorum sensing also controls the production of virulence factors. Quorum sensing describes a set of regulatory systems in which gene expression is coupled to the accumulation of chemical diffusible signalling molecules. In *Erwinia carotovora* ssp. *atroseptica* (*Eca*) one of the quorum sensing systems is mediated by the *N*-acyl homoserine lactones (AHLs), synthesized by LuxI family protein – ExpI. Many environmental factors can affect the function of the quorum sensing system. Plants can respond to attack of bacterial pathogens by producing the AHL mimic compounds – functional analogues of bacterial pheromones found in a variety of plant species.

In the present study we found the plant metabolites that induce the expression of *expI* gene and the synthesis of AHL in *Eca*. These metabolites presumably represent the novel type of the "cross-talk compounds", which act during plant-microbe interaction. Our data suggest that quorum sensing system is integrated with other signal pathways responsible for the recognition of compatible host and can be modulated by the plant-derived compounds on transcriptional and post-transcriptional levels. Taken together, our data demonstrate that the model for quorum sensing regulation and biofilm formation in *Eca* is more complicated than thought previously and includes additional components.The results obtained towards the identification of the chemical structure of plant-derived inducers of *Eca* quorum sensing system and the molecular mechanism of their action will be presented in the talk.

Strength of the lasB promotor

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Number: 151

Certain properties of the *Pseudomonas aeruginosa* are regulated by quorum sensing. Here we present results on the *las* quorum sensing system of *P. aeruginosa,* which is one of the two AHL-mediated quorum sensing circuits that have been identified in *P. aeruginosa*, the other being the *rhl* system. A regulatory connection exists between these two systems, where the *las* system is thought to be placed above the *rhl* system. Using a *las* monitor in *Escherichia coli* we have determined the properties of interaction between OdDHL and the LasR

protein. The monitor strain is constructed as a divergent transcribed P*lac-lasR* and P*lasB-gfp*(ASV) system, producing GFP when the *lasB* promoter is activted [1]. We monitored the independent responses of the simulated *las* system to predetermined OdDHL concentrations (12 concentrations from 2 μM to 1 nM). These measurements showed a fast production of GFP probably due to the *lac* promoter ensuring a high production of the regulator *lasR*. The saturated level of GFP production is 150 % above the spontaneous level. This is very low compared to our previous measured levels of the *ahy* system [2], which makes it a very weak switch mechanism. These results may indicate that the *las* system is less stringent regulated than the *ahy* system.

[1] M. Hentzer *et al*., ***Microbiology*** (2002), **148**, 87–102., [2] C. Garde *et al*., ***J Mol Biol*** (2010), **396**, 849–57.

Quorum sensing in pneumococcal biofilm

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Number: 152

Two models of pneumococcal biofilm formation were exploited to analyze for impact of quorum sensing systems. The models relied either on low inocula allowing for exponential growth (model A) or alternatively on high inocula of stationary cells (model B). Out of the three proposed quorum sending systems including the competence system (ComCDE), the bacteriocin peptide system (BlpRH) and luxS only the competence system had effect on biofilm. Model A showed that biofilm formation occurred at the end of the exponential phase and that the attachment was independent from quorum sensing, while maintenance and stability during stationary phase was strictly dependent on ComCDE functionality. Model B showed also that attachment of single cells to surfaces was quorum sensing independent. Over time pneumococci showed in this model extensive microcolony formation. Both competence receptor mutants and mutants for the competence quorum sensing peptide CSP were impaired in formation of these sessile aggregates; a phenomenon that could be complemented by addition of synthetic CSP to the medium. These data shed new light on the role of quorum sensing competence system in pneumococcal biofilm. The data are reminiscent of the recently demonstration of competence dependent aggregation in mixed planktonic populations leading to fratricide, a phenomenon that frees DNA, which in turn could be identified in the extracellular matrix of pneumococcal biofilms.

Development of yeast colonies: Quorum sensing and differentiation

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Number: 153

Yeast multicellular colonies growing on wet agar surfaces possess many features similar to those found in microbial biofilms. Cells developing within these structures exhibit different unique properties absent in individual yeast cells. These include, among others, cell-cell communication, long-distance signalling and developmental changes linked to cell adaptation and differentiation within the colony (Palková & Váchová, *FEMS Microbiol Rev* 30: 806–824, 2006). One of the molecules important for colonial development is volatile ammonia, which functions as a quorum sensing molecule capable of long-distance transmission of the signal among individual colonies. In aging colonies ammonia triggers induction of adaptive metabolic changes important for long-term survival and specific differentiation of colonial yeast population. Cells carrying proteins of the adaptive metabolism localise to the colony margin, while central chronologically aged cells keep higher activities of stress-defence enzymes and transporters linked to membrane potential maintenance. These divergent properties predestine the fate of colonial cells; central cells undergo apoptotic dying and outer cells provide healthy progeny. In young microcolonies of wild *Saccharomyces cerevisiae* strains forming structured colonies, ammonia induces dimorphic transition and oriented pseudohyphal cell expansion in the direction of ammonia source. This leads to unification of adjacent microcolonies to one more numerous entity. Production of high amount of extracellular glycosylated matrix and presence of Flo11p adhesin are essential for consequent development of wrinkled colonial structure. The work was supported by GACR204/08/0718, LC531, AV0Z50200510, MSM0021620858 and HHMI to Z.P.

Interaction between *luxS* expression and *ica* operon involved in biofilm formation by *Staphylococcus epidermidis*

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Number: 154

Significance and objectives.

Staphylococcus epidermidis emerge as common nosocomial pathogen causing infections in patients with implanted medical devices. The mechanism of gene regulation and signaling during biofilm accumulation is under investigation. Polysaccharide intercellular adhesin (PIA), encoded by *icaADBC* is necessary for biofilm accumulation. It is hypothetically known, that autoinducer-2 molecules (*luxS* gene) block biofilm formation through *icaADBC operon*. There are no data on expression of *luxS* gene in patients with bacteraemia and in healthy people. We hypothesize that *luxS* gene product is stimulating biofilm formation in independent way. To our knowledge this is the first study to show expression of *luxS* gene in clinical samples.

Methods and results
43 clinical specimens of *S.epidermidis* were isolated in case of bacteraemia. A control group of 35 *S.epidermidis* from nose epithelium of healthy people was isolated. RNA/DNA was isolated with magnetic particles. Expression of *luxS* gene was measured in quantitative PCR. The 502bp fragment of *icaA* gene was amplified and tested electrophoretically. Among clinical samples, 44,2% were $icaA^+$ and 55,8% $icaA^-$. However, in healthy people, there were 25,7% $icaA^+$ and 74,3% $icaA^-$ results. In clinical group, the mean expression of *luxS* gene was 21,3% in $icaA^+$ samples and 8,3% in $icaA^+$ samples.

Conclusions.
The was no higher prevalence of $icaA^+$ in clinical group in comparison to control group, showing no role of *icaADBC* operon in case of bacteraemia. Significantly higher expression of *luxS* gene (p=0,0026) in $icaA^+$ clinical cases indicates on eventual *luxS*-dependent downregulation of phenol soluble modulins, which could stimulate biofilm formation.

Theme: Biofilm development: A multidisciplinary approach

Study of very early stages of biofilm formation using several corroborative techniques including in-situ ATR-FTIR spectroscopy
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Number: 155
Attenuated Total Reflectance - Fourier Transform InfraRed (ATR-FTIR) spectroscopy has proven to be an interesting tool for studying and monitoring *in situ*, non-destructively, in real time, the initial stages of biofilm formation and, subsequently, the response of bacteria at the biofilm base to changes in environmental conditions. In these studies, the intensity or area of some characteristic spectral bands of biomass is usually monitored. However this approach only provides one part of relevant information contained in an ATR-FTIR spectrum. A more detailed analysis of the whole spectral profile is needed to gain more insight, at the molecular level, into the physiological and structural changes accompanying bacterial adhesion, biofilm development, detachment processes and environmental condition changes. Nevertheless, one difficulty is to differentiate spectral changes due to metabolic changes from those due to environmental physicochemical condition changes or even to ATR technique features because of, for example, changes in distance between nascent biofilm components and the ATR crystal surface or a non-uniform basal bacteria monolayer.

Does dental biofilm accumulation differ between night and day?
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Number: 156
Objective: The initial microbial colonization of dental surfaces has been studied thoroughly by classical ultrastructural and microbiological studies and further analysed by fluorescent methods. Most of these studies, however, do not differentiate between biofilms formed during night and day. The purpose of the study was to perform a quantitative and qualitative analysis of in situ dental biofilms collected during night and day, respectively. We hypothesised that there is a circadian rhythm in the accumulation of bacteria during initial biofilm formation.

Methods: Biofilms were collected on standardized glass slabs mounted in intra-oral appliances and worn by eight individuals for 12-h during day and night, respectively. Subsequently, fluorescent in situ hybridization was performed using probes against *Streptococcus* spp., *Actinomyces naeslundii,* and all bacteria and analysed by confocal laser scanning microscopy. Quantification of bacteria was done by stereological tools.
Results: The study showed a statistically significant difference between the number of bacteria in the two 12-h groups (Wilcoxon signed rank test, p=0.012) with the highest accumulation of bacteria during day time. The qualitative analysis confirmed this difference within all individuals but with large inter-individual variation in the degree of microbial coverage and bacterial composition.

Conclusions: The study provides firm evidence that initial biofilm formation decreases during night. Low biofilm accumulation during the night may reflect circadian rhythms in the rate of bacterial cell division and/or circadian rhythms in the rate of salivary flow and associated drops in salivary nutrients. This finding is of great importance when studying quantitative aspects of initial biofilm formation.

Hot biofilms - EPS analysis of the archaeon Sulfolobus acidocaldarius
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Number: 157
Biofilms represent the common mode of life for more than 99 % of all bacteria in nature. Bacterial as well as eukaryotic biofilms and their extracellular polymeric substances (EPS) have been intensively studied because of their possible application in biotechnology but also due to their potential role as reservoirs for pathogens. Members of the third domain of life, Archaea, have gained special research interest due to their adaption to extreme environments. However, biofilm formation and synthesis of EPS has been neglected so far. The

current study investigates the biofilm formation and EPS composition of *Sulfolobus acidocaldarius* DSM 639, a thermoacidophilic, aerobic member of the Crenarchaeota, first isolated from acid hot springs at Yellowstone National Park in 1972.

S. acidocaldarius was grown at 80 °C as liquid cultures and as unsaturated biofilm on filters, placed on gellan gum plates. In order to establish a reproducible method for EPS extraction, five extraction methods were compared according to their EPS yield and impact on cells. The applied methods were shaking, shaking in presence of a cation exchange resin (CER) and treatment with NaOH, EDTA or crown ether. For each method carbohydrates, proteins and DNA were quantified with standard photometric or fluorimetric methods and the extracellular proteome was visualized using 2D gel electrophoresis. All methods led to quantifiable amounts of EPS with carbohydrates representing the main component. The obtained results demonstrated that CER treatment was best suited for the EPS isolation, resulting in high EPS yields with no interference of subsequent analyses.

Isolation of extracellular polymeric substances from drinking-water biofilms – a critical assessment of standard isolation procedures

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Number: 158

Extracellular polymeric substances (EPS) are structural and functional components of microbial biofilms. The composition of different kinds of EPS has been extensively studied over the years and various EPS isolation methods have been established. However, EPS isolation still remains a compromise of maximising EPS yields while minimising cell injury. This study presents a critical assessment of methods commonly used for EPS isolation with respect to their applicability for small quantities of biofilms as typically found in drinking-water distribution systems. The applied methods were shaking, shaking in presence of a cation exchange resin (CER), or treatment with EDTA, formaldehyde/NaOH or heat. The methods were assessed by their yield of carbohydrates, proteins and DNA, their destructive effect on biofilm cells and possible interference with quantitative and qualitative analyses.

All methods yielded quantifiable amounts of EPS from drinking-water biofilms. Proteins represented the main components of the EPS, followed by carbohydrates and DNA. Isolation by EDTA, formaldehyde/NaOH and heat resulted in an almost complete loss of culturability of biofilm cells, indicating possible cell damage, and consequently, contamination of EPS with intracellular material. Furthermore, EDTA and formaldehyde/NaOH interfered with quantitative analyses (Lowry, phenol/sulphuric acid, or PicoGreen assay) and qualitative analysis by 2D gel electrophoresis. CER treatment showed increased EPS yields compared to shaking, with no significant loss of culturability or extracellular glucose-6-phosphate dehydrogenase activity, and no interference with quantitative or qualitative analyses.

CER treatment is therefore considered best suited to give high EPS yields and reliable results.

Extracellular DNA enhances bacterial adhesion and surface aggregation by influencing acid –base interactions

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Number: 159

Significance and objectives: Extracellular DNA (eDNA) present in extracellular polymeric substances acts as an adhesive and strengthens biofilm. In this study we investigated the effect of naturally occurring eDNA on adhesion and surface aggregation of several Gram-positive bacteria and there mechanism of interaction.
Methods: Initial bacterial adhesion to hydrophilic and hydrophobic substrata and surface aggregation in presence and absence of eDNA were studied using a parallel plate flow chamber. Atomic force microscopy (AFM) was used to measure adhesion forces between bacteria. Thermodynamic analyses were used to study the mechanism of bacterial adhesion to the substrata and surface aggregation. All experiments were done in phosphate buffer saline.

Results: In the presence of eDNA bacteria adhered faster and in higher numbers after 60 min and a significant increase in the percentage bacteria involved in large aggregates was observed when compared to adhesion in the absence of eDNA. On hydrophilic surfaces presence of eDNA increased the percentage of bacteria in large aggregates compared to on hydrophobic surfaces. AFM measurements showed significant increases in adhesion force between bacteria in presence of eDNA compared to the absence of eDNA. Upon removal of eDNA, the hydrophobicity of bacterial strains decreased significantly according to contact angle measurements. Accordingly, favourable interaction energies in the presence of eDNA became unfavourable, in the absence of eDNA due to changes in acid-base interaction energies.

Conclusions: The presence of eDNA on bacterial surfaces enhances adhesion kinetics, forces of interaction and surface aggregation due to the involvement of acid-base interactions.

Study of instant effects of dehydration on the ATR-FTIR signature of a nascent P. fluorescens biofilm
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Number: 160
Water availability plays a major role in all stages of biofilm development and biofilm properties. Even though that severe cellular dehydration can denature proteins, damage DNA and modify membrane fluidity, biofilm-associated bacteria can survive and even biofilms grow in an environment that is only transiently wet such as in soil systems, in food, or in man-made environments. However, relatively little is still known about how an environment undergone wet/dry cycles can affect biofilm development, in particular during the early stages of formation. To gain more insight into this topic, the Attenuated Total Reflectance - Fourier Transform InfraRed (ATR-FTIR) spectroscopy can be a particularly well-suited tool. With an analysis depth of 1-2 µm, it is able to monitor hydration and to analyze the structure of water embedded in biomolecules through the libration and vibration bands of water, and together, to possibly provide, at the molecular level and in situ, information about biochemical and physiological changes of bacteria through the changes in the ATR-FTIR signatures of main biomolecules. Nevertheless, one difficulty is to differentiate spectral changes due to metabolic changes from those due to changes in biomolecule hydration and swelling- or shrinkage-induced. The present communication addresses this issue by analyzing the instant effects of air or nitrogen drying on the ATR-FTIR spectra of planktonic *P. fluorescens* pellets and of 3 hour-old nascent *P. fluorescens* biofilms formed on a germanium ATR crystal. This analysis is performed with help of water and main cellular component ATR-FTIR signatures acquired separately under the same experimental conditions.

Escherichia coli Biofilms: Gene Expression and Elemental Heterogeneity
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Number: 161
Global analyses examining the differences between bacteria growing in biofilms and their planktonic counterparts have revealed that stress responses are significant in biofilm existence. However, some features of the comparative expression profiles may be unavoidable consequences of growth at high cell densities and not a characteristic feature of the biofilm mode of growth *per se*. Since many biofilm cells are probably anoxic or oxygen-limited, compared to planktonic cells, the gene expression profiles of biofilm and planktonic populations of a facultatively anaerobic bacterium *Escherichia coli* were compared under strictly anaerobic growth conditions. Contrary to some published data, *E. coli* readily forms biofilms under anaerobic conditions and genome-wide transcriptional profiling demonstrated up-regulation in the biofilm of only a small number of genes. A chemostat system was developed with which to expose aerobically-growing biofilms to anaerobic conditions and transcriptomic changes were examined during this transition. As well as predictable changes in transcripts encoding respiratory proteins, biofilms also exhibited changes in the expression of flagella, ribosomal proteins, drug exporters and bacteriophage genes. Biofilms have also been shown to exhibit considerable heterogeneity. To examine elemental heterogeneity in bacterial communities, an emerging analytical technology, laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was used. Distinct variations in the distributions of metal ions were detected in colony and biofilm models. Elementally-distinct sub-populations were isolated and their global transcript levels were compared. When compared to the biofilm interior, cells in the biofilm perimeter exhibited higher levels of transcripts related to motility, aerobic respiration and transport.

Carbohydrate composition of *Pseudomonas aeruginosa* PA14 pellicle
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Number: 162
Pseudomonas aeruginosa is an ubiquitous Gram-negative bacterium. It is a cause of severe nosocomial infections, which are hard to eradicate due to its biofilm lifestyle. Certain clinical strains of *P. aeruginosa*, including PA14, are able to form a thick pellicle, a surface-associated biofilm on the air-liquid interface. In the present work we attempted a systematic study of the carbohydrate components of *P. aeruginosa* PA14 pellicle by chemical methods, NMR spectroscopy and DOC-PAGE.

LPS, the major carbohydrate component of *P. aeruginosa* cell envelope, was an important constituent of the extracellular (EC) matrix preparations. We characterized the chemical structure of the LPS O-antigen of this strain and showed it to be composed of a linear trisaccharide repeating units identical to ones described for *P. aeruginosa* type O:2a,c (Knirel *et al.*, 1982). The lipid-free O-antigen was also present in the EC matrix. A recently characterized family of glycero-phosphorylated cyclic β-(1,3)-glucans was the third carbohydrate component of the pellicle.

Comparison of CNP ratios of biofilm developed under various conditions

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Number: 163

CNP ratios of biofilms naturally developed on riverbed pebbles were compared with those cultivated in natural river water supplemented with nutrients. Natural biofilms were collected from riverbed pebbles taken at sampling locations ranging from the pristine region to the urbanized area. For the cultivation of biofilm, silicon rubber coupons were placed in sampled natural river water under dark at an ambient temperature up to five days. Bacterial counts reached maximum in 24 h, while the maximum ATP was observed at 72 h. The smallest proportion of C was observed at 72 h. The percentage of C was greater in artificial biofilms than in the natural biofilms. In biofilms collected from the urbanized area and in biofilm cultivated with supplement of carbon source, the percentages of C were rather smaller. It was suggested that when there are rise in bacterial activity, the ratio of carbon content in biofilm becomes relatively low.

Biofilms from Flow-cells - a model study with *Pseudomonas aeruginosa*, Confocal Laser Scanning Microscopy (CLSM) and Metabolic fingerprinting

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Number: 164

Relevance: Establishment of biofilm with *Pseudomonas aeruginosa* is known from chronic wounds, exfoliated implants and chronic diseases like Cystic Fibrosis (CF) and Chronic Obstructive Pulmonary Disease (COPD). Environmental persistence and recurrent infections caused by *P. aeruginosa* is demonstrated in divers' ear infections.

Objectives: Development of a tool for evaluation of status in a biofilm by identification of significant extra-cellular metabolites related to various phases in the establishment, proliferation and disintegration of *P. aeruginosa* biofilms.

A main objective is to develop intervention strategies for prevention of infections.

Methods and Results: Flow-cell grown biofilms of *P. aeruginosa* are sampled for analyses of extra-cellular metabolites and visualised in parallel using CLSM. Metabolic analyses are done by fingerprinting mass spectrometry (MS). Mass spectrometric analyses are performed by electrospray full scan MS within the mass range from m/z 20 to 3000 in the positive and negative mode. The MS spectra are analyzed by Principal Component Analysis (PCA), and it has been found that the metabolic patterns change with time during different phases of biofilm establishment. The patterns also reflect differences between different bacterial strains. The present experimental model may be suitable for studies of biofilms in the context of intervention strategies for prevention of infections. The experimental model may open for real-time studies of biofilm establishment and disintegration. High-resolution MS analysis may open for identification of certain metabolites that might be of specific importance and interest as biomarkers for biofilm status. Finally this approach may be an important tool in systems biology studies of biofilms.

Kinetics of biofilm development on nanofiltration membranes during drinking water production, link with membrane fouling

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Number: 165

The aim of the study was to determine the relation between biofilm formation and nanofiltration (NF) membrane performances decrease. Biofilm development on membranes, membrane permeability and longitudinal pressure drop were checked during NF. Membrane foulants were analysed by ATR-FTIR spectroscopy, CLSM after DAPI and lectin staining, rheology and wettability. Biofilm growth was observed all along the experiment. Biofilm thickness varied from 30 mm after 7 days to about 60 mm after 717 days. Biofilm development induced progressive coverage of the membrane surface. The kinetics of evolution of sessile bacteria and matrix polysaccharides were different. Attached bacterial populations increased until 80 days of filtration, remained unchanged from 80 to 475 days and increased again from 475 to 717 days of filtration. Matrix polysaccharides increased all along the experiments with different growth phases: high increase from 7 to 80 days, weaker increase from 80 to 475 days and again high increase from 475 to 717 days. A diversification of the polysaccharides of the matrix and an increase of the biofilm viscosity occurred with time. Biofilm maturation conducted to polysaccharides organization as dense balls inserted in a filamentous network. NF biofilm formation came with progressive decrease of membrane wettability. Membrane permeability was precociously and continuously affected all along the filtration process but longitudinal pressure drop did

not increase as long as biofilm growth raised a threshold. This suggests that biofilm formation may mainly affect longitudinal pressure drop and that other fouling mechanisms may be predominant in early stages of membrane filtration.

Development of a Web-based platform for the systematic and large-scale study of microbial adhesion and biofilms

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Number: 166

High-throughput biofilm studies are rapidly accumulating a large amount of omics-scale data. In other biological areas that deal with large datasets, such as genomics or proteomics, ways for simplifying the visualization and understanding of the obtained results have already been developed. As such, we have started the development of a Web-based platform for analogous management, visualization and exploration of biofilm data. This platform, named Biofomics, is comprised of three main areas: 1) experimental characterization, where users define the conditions under which biofilms have been formed (e.g. microorganism, temperature) and the methods used to analyze them (e.g. crystal violet, XTT); 2) data submission, where users fill in a data form customized according to previous characterization and 3) data visualization, where users can correlate and visualize data from different biofilms and different studies (under development). Its contents are expected to represent the result of curation and automated integration of data from the entire biofilms research community. In particular, it is our belief that our efforts to harmonize data nomenclatures (e.g., methods applied to form and analyse biofilms), and document experiments (e.g. by adding citations and references) will support the work of reviewers of scientific literature. Initial tests, where in-house generated data is being used to feed the platform, are currently underway. The platform can be accessed at http://193.137.90.5/.

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Material age has an impact on biofilm formation potential

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Number: 167

Plastic is often used as material for facility components e.g. pipes. Ageing starts the moment these components are installed and in contact with the environment (water, oxygen) resulting in chem. reactions of additives and/or polymer chains. Sanitisation and disinfection of water installations accelerates ageing due to adding oxidative substances or elevating water temperature. Our investigations focused on the question how this thermo-oxidative "attack" on polymers may influence biofilm formation potential.

Two plastic materials (PE-Xb and Ethylen-Propylen-Dien rubber (EPDM)) were treated with sodium hypochlorite under defined conditions: 4 bar, 40 °C, sodium hypochlorite and chlorine dioxide at 2.5 mg/l or 4 mg/l, 4 weeks. These procedures resulted in changes in chemical and morphological properties (increased hydrophobicity, loss of additives, cracks).

Biofilm formation was investigated with a multi-factor assay simulating different drinking water qualities. Biofilm was quantified with parameter Total Cell Number. Results showed : biofilm formation on new versus "older" material was higher on new nutrients had the biggest impact on biofilm formation biofilm formation depended very much on temperature, AOC and age of PE-Xb and EPDM, but less on water hardness and humic substances influence of temperature is higher when biodegradable substances are available; higher temperature (15°C to 25°C) causes higher biofilm formation

Conclusions:

The age of material has to be taken into account when biofilm experiments are performed, and risk of contamination of water by biofilm may decrease over time

Proteomic study of the marine bacterium *Pseudoalteromonas sp.* strain D41 grown in biofilms

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Number: 168

Bacterial biofilm development represents the first step in the biological colonization of marine surfaces. This development is conditioned by complex processes involving bacterial attachment to surfaces, growth, cell-to-cell communication, mobility and production of exoproducts constituting the biofilm matrix. Our study focuses in the marine biofilm forming bacterium *Pseudoalteromonas* sp. D41. This organism displays strong adhesion onto a wide variety of substrates, promoting subsequent biofilm development. We attempted to unravel the molecular mechanisms responsible for these adhesion properties though a proteomic approach. Proteome profiling of the outer-membrane and the total soluble proteome showed the differential expression

of several proteins when this bacterium was grown under biofilm conditions compared to planktonic cultures. Of particular interest, five outer membrane proteins were strongly induced in biofilms. MS/MS *de novo* sequencing allowed us to identify them as two TonB-dependent receptors, a protein homologous to OmpW, a protein homologous to OmpA, and a type IV pilus biogenesis protein (PilF). We are currently characterizing these candidates, in order to understand their functions in bacterial adhesion and/or biofilm development.

Gravitational effects on biofilm formation by *Pseudomonas aeruginosa*

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Number: 169

There is an urgent need to understand the effects of microgravity on the growth, cellular physiology, and cell-cell interactions in microbial biofilms. This information can then be used to curtail harmful activities of microbial consortia thriving as biofilms on the International Space Station and is essential for the long-term success of human space exploration. Bacterial biofilms were abundant on the MIR space station and were responsible for increasing corrosion and blocking a water purification system. Health and safety hazards linked to the development of biofilms are also of great concern because of the decreased in immune function observed in space travelers. We are using laser scanning confocal microscopy and the measurement of viable cell counts to examine how gravity alters biofilm formation with the goal of developing new strategies to reduce their impact on the operation of spacecrafts and the health of their crew. We are focusing on *Pseudomonas aeruginosa* because it forms biofilms both inside and outside of the human body, can switch between benign and pathogenic interactions with humans, and may be relevant to crew health during extended missions. We are studying how the formation and the three-dimensional structures of biofilms formed by these microbes differ in microgravity compared to normal gravity using both simulated microgravity and spaceflight experiments. We will present our recent results comparing biofilm formation on membranes in normal gravity, simulated microgravity and in spaceflight experiments.

Biofilm-mediated *P. aeruginosa* contamination of a drinking water

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Number: 170

P. aeruginosa is an opportunistic pathogen which can be involved in sporadic or persistent contamination events in drinking water systems. The basis of the present study was the recurring cultural detection of *P. aeruginosa* in water samples from a German drinking water distribution system. In order to track the source of this contamination, both water and biofilms were investigated for the presence of *P. aeruginosa*, using a combination of culture-based and culture-independent molecular methods. Genotyping of 18 *P. aeruginosa* water isolates from throughout the distribution system and the waterworks was performed, using pulsed-field gel electrophoresis. Independent of sampling site and date, a single clone of *P. aeruginosa* was detected, indicating that a systemic contamination was highly probable, which seemed to originate from the waterworks. Additionally 22 biofilm samples were analyzed for *P. aeruginosa*. The bacteria were not detected culturally in any of the biofilms. However, by means of culture-independent fluorescence *in situ* hybridization, *P. aeruginosa* was identified in 17 out of the 22 biofilms. In conclusion, *P. aeruginosa* occurred in biofilms of the distribution network and the waterworks in a viable state which could not be recognized by routine culture analysis. Thus, the biofilms were a reservoir of *P. aeruginosa* and presented a continuous contamination potential for the water phase. A possible explanation for cultural detection of *P. aeruginosa* in drinking water during certain time periods may be the transition from the non-culturable to culturable state under favorable environmental conditions which have yet to be defined.

Differential Fluorescende Induction as a tool to search for genes involved in *Salmonella* biofilm formation

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Number: 171

Salmonella Typhimurium is an important cause of bacterial food-borne diseases. Some of these diseases are related to the characteristic that *Salmonella* is capable of forming biofilms on a variety of surfaces. Global gene regulation and expression profiles are different in biofilms as compared to planktonic cells and are the causes of specific biofilm-associated phenotypes. In order to develop novel anti-*Salmonella* therapeutics, also effective against biofilms, insight in gene expression and regulation under this alternative physiological state is needed.

We applied the Differential Fluorescence Induction (DFI) strategy to identify genes specifically induced under biofilm conditions. DFI is basically an enrichment strategy which uses small random fragments genomic DNA, cloned upstream of a promoterless GFP gene to monitor promoter activity with a fluorescence-activated cell sorter (FACS). A genetic selection was performed by subsequent rounds of positive (biofilm) and negative

(planktonic) selection conditions to eliminate most false positive and negative clones. Sequence determination of the genomic inserts in the enriched pools led to identification of specific biofilm induced DNA-fragments. The major advantage of this approach is the generation of data on single cell level in stead of a mean value for the whole population. As such, the heterogeneity, which is inherent to biofilms, can be taken into account. Another important advantage is the monitoring of gradations in gene expression, and this independent of prior knowledge of gene annotation.

Using this screening technique we found some interesting, new elements involved in *Salmonella* biofilm formation of which the exact role is currently being further investigated.

Verification of disinfection of biofilms by fluorescence analysis

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Number: 172

Microbial pathogens (intentionally) injected into drinking water distribution systems (dwds) will settle in existing biofilms. These pathogens may persist, grow and contaminate the water phase for a long time. As shown by Camper et al. (2000) number of pathogens in biofilms may range from few up to several percent of the whole population distributed in the biofilm. Therefore decontamination (disinfection and/or removal) will be necessary with high levels of disinfectants. In experiments we used Ethylen-Propylen-Dien rubber (EPDM) as substratum. This material is typically applied in dwds for e. g. valves. Bacterial biofilm densely populated EPDM-coupons over several weeks up to 5 x 108 bacteria/cm2. Disinfection was performed with elevated levels of NaOCl (10, 30 and 50 mg/L free chlorine) for 24 h.

Assessment of disinfection efficacy was verified by using fluorescence signals as generated from binding florescence dye (DAPI; Sybr II) to nuclide acids. With fluorescence reader such as TECAN GENios direct quantification of emitted light from biofilm is directly measurable within short time. Results give immediately information on DNA damage and hence killing rate. Our experiments showed disinfection with 10, 30 and 50 mg/l NaOCl resulted in incomplete detachment consequently some pathogens may still be attached. Remaining biofilm aggregates stained with DAPI showed very low fluorescence (background level) compared to biofilm before disinfection. Verifiable killing rate measured with fluorescence reader was not more than 3 log for whole biofilm / pathogens. Our aim is improve proof of killing rate by decreasing detection limit.

Characterisation of diatom biofilm growth, succession and development on natural marine surfaces using multidisciplinary imaging and analysis approaches

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Number: 173

Biofilms are rapidly established on both natural and artificial surfaces immersed in the marine environment and quickly develop large amounts of biomass. The development of anti-fouling methods is precluded by an understanding of the biofilm processes that lead to growth on surfaces. Full characterisation of the constituent cells and spread of biofilms is often hampered by single technique approaches. In this study we present our current work into the characterisation and analysis of diatom biofilm development using a multidisciplinary approach with particular emphasis on the development of diatom biofilms in the initial stages of biofouling.

We have used Scanning Electron Microscopy (SEM), Laser Scanning Confocal Microscopy (LSCM), and epifluorescence with time lapse microscopy to understand how multi-species diatom biofilms spread over surfaces in the natural environment. Image analysis techniques such as the creation of SEM image montages combined with pixel colour values based on diatom cell density and cell type are used to give a much clearer understanding of the distribution and rate of colonisation of diatom cells. We conclude from our studies that there are significant differences in the structure and spread of a diatom film over particular surfaces and this appears to be correlated with surface texture and topography. Cell aggregation and distribution of diatom biofilms on the macro-scale (>1 mm^2) are affected by the underlying substratum. This can be much more readily identified using image analysis techniques normally applied in areas such as geographical information systems (GIS).

Semantic tools for data and model integration in biofilm research

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Number: 174

Ongoing progress in experimental technologies in microbiology enables researchers to identify more and more molecular and cellular building blocks which determine the function and spatio-temporal dynamics of microbial biofilms. Obtaining a systems-level perspective on biofilm development from such experiments requires computational tools for the integration of a multitude of heterogeneous sources of biofilm data. In the emerging field of ecological informatics, tools for semantic integration, clarifying data contents by formal

logical structures, known as ontologies, are increasingly used to match and combine information from different sources.

In the present work, we report on the implementation of the semantic database software S3DB for the integration of the data generated in the former EU-project PHOBIA on phototrophic biofilms (www.photobiofilms.org). We present a formal model of knowledge suitable to describe the PHOBIA data, and compare it with parallel descriptions obtained from more generic ontologies used to describe data and experiments in any kind of ecological system.

We discuss our results by suggesting possible seeds to create controlled vocabularies for biofilm data that may help to integrate diverse sources of knowledge and heterogeneous data types such as, e.g., microscopy images, or taxonomic data. We propose that semantic mediation could furthermore be a promising strategy to link experimental data to the output from numerical simulations, fostering the emergence of a coherent network of biofilm knowledge sources, which might significantly enhance the efficiency of future multidisciplinary biofilm studies.

Raman microscopy and surface-enhanced raman scattering for in situ biofilm characterization
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Number: 175
Raman microscopy (RM) is a promising technique for a non-destructive analysis of biofilms. RM provides whole-organism fingerprints for biological samples with spatial resolutions in the μm-range and allows for correlations between optical and chemical images. Low water background makes RM beneficial for in situ studies of biofilm matrices. We apply RM for chemical characterization of multispecies heterotrophic biofilms, including microbial constituents and extracellular polymeric substances (EPS). However, due to its limited sensitivity, RM is time consuming. Moreover, the Raman spectra of biofilms typically exhibit only a few bands and therefore available chemical information is rather limited. The application of surface-enhanced Raman scattering (SERS) for biofilm analysis allows us to overcome these drawbacks. SERS significantly increases Raman signal intensities when molecules are attached or in the immediate proximity to nanometer-sized metal (e.g. Ag, Au or Cu) structures. SERS results in enhancement factors in the range of 10^3–10^6 due to the electromagnetic ("localized surface plasmon resonance") and chemical ("charge transfer") amplification mechanisms. Under certain conditions (at "hot spots" – closely spaced particles or rough nanostructures) the enhancement factors up to 10^{14}–10^{15} can be achieved. By using colloidal silver nanoparticles for the in situ studies, we achieve good reproducibility of SERS measurements, along with substantial enhancement of Raman signals (>10^4) and highly informative SERS signature. Altogether it enables for rapid label-free SERS imaging of the biofilm matrices. Thus, the detection of different constituents and the determination of their spatial distribution in a biofilm can be performed even at low biomass concentration.

The Search for Small Molecule Probes of Biofilm Development
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Number: 176
The developmental pathway of biofilm formation includes a series of complex but reproducible stages that have been characterized largely by genetic methods. The objective of our research is to search for small molecule probes of biofilm development, potentially uncovering novel mechanisms of biofilm formation and identifying new potential therapeutic targets. **Methods**: A collection of compounds, including small libraries of protein kinase inhibitors, ATP analogues and complex plant extracts, was examined for effects on biofilm development. *Pseudomonas aeruginosa* PAO1 biofilms were grown on 96 peg lids in the presence of test compounds and quantified using crystal violet staining. Compounds of interest were those that did not alter planktonic growth but had effects on the overall level of biofilm formed. Compounds that met these criteria were tested to determine their effects on quorum sensing and levels of the secondary messenger c-di-GMP, key signaling pathways that affect biofilm formation by *P. aeruginosa*. **Results:** We have identified a number of inhibitors of biofilm formation, some of which may inhibit quorum-sensing of *P. aeruginosa*. In addition to biofilm inhibitors, a number of biofilm inducers were identified. Two of these compounds stimulated biofilm formation in cells overexpressing a phosphodiesterase responsible for degradation of c-di-GMP, suggesting that they are targeting a novel pathway capable of inducing biofilm formation in the absence of this important developmental signal. **Conclusions:** Our data show that it is possible to use small molecules to probe biofilm physiology as an alternate to traditional proteomic and genomic studies.

Effects of Small Molecules on the Formation of *Listeria monocytogenes* Biofilms
Uyen Nguyen, Iwona Wenderska, Lori Burrows. Gerard Wright, McMasters University, Canada

Number: 177
Listeria monocytogenes biofilms enable tolerance to sterilization chemicals used in food-processing facilities.

Failure to remove *L. monocytogenes* biofilms can lead to food product contamination. Our research aim is to identify small molecules that alter *Listeria* biofilm development To aid in our understanding of the mechanisms underlying *L. monocytogenes* biofilm formation and potentially identify new techniques for prevention of biofilm formation.

Methods: An optimized static biofilm assay was used for *L. monocytogenes* biofilm growth and screening of a protein kinase inhibitor library for compounds affecting biofilm formation. *L. monocytogenes* 568 biofilms were grown in the presence of test compounds on 96-peg lids for 72h, replenishing the media every 24h. Biofilms formed were quantified using crystal violet staining. Of interest were compounds that inhibited planktonic cell growth or affected biofilm formation (<50% or >200% of wild type) but not planktonic growth. These compounds were tested further using growth, adhesion, and dose-response assays. **Results:** A number of compounds were identified as planktonic growth inhibitors, including sphingosine and BAY11-7082. Palmitoyl-DL-carnitine, which we previously identified as a *Pseudomonas aeruginosa* biofilm inhibitor, was shown to inhibit *L. monocytogenes* biofilms. Other compounds were shown to specifically inhibit biofilm formation but not planktonic growth, or were identified as biofilm stimulators. **Conclusions:** We have optimized a *Listeria* biofilm formation assay for high-throughput screening and used it to identify small molecules affecting biofilm development. Characterization of compound targets may lead to new strategies for removing biofilms or preventing biofilm formation, therefore preventing future food product contamination.

The phenomenon of cell division of *Erwinia carotovora ssp. atroseptica* SCRI1043 in the absence of carbon source

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Number: 178

It is well accepted that the major part of bacteria exist as the structured communities or biofilms. Microorganisms can inhabit various ecological niches, even in the absence sufficient amount on nutrients.

In our work we have shown that even in the absence of carbon and phosphorus bacteria *Erwinia carotovora* ssp. *atroseptica* SCRI1043 (*Eca*) retain sufficient reproduction level. The initial stage of response to starvation was stabilization of density of culturable cells in the range of ~ 10^6 colony forming units per ml (CFU mL^{-1}). When cultures were inoculated with low cell density (~ 10^3-10^5 CFU mL^{-1}) the number of culturable cell increased (up to ~10^6 CFU mL^{-1}).

Bacterial division on the carbon deficient medium was accompanied by the modification of cell ultrastructure. Morphological dissociation of bacterial population and cell division was observed. The energy sources and ways of their utilization during the bacteria propagation in carbon-deficient medium remain to be determined. Our preliminary results suggest that the increase of CFU number occurred likely owing to utilization of storage compounds.

As a result of division the cell density that provides the intercellular communication established. Cell-cell communication provides activation of stress response, virulence and biofilm formation in the number of bacterial species. When *Eca* starving cultures achieved the threshold level of population density (~10^6 CFU mL^{-1}) the induction of acyl-homoserine lacton (AHL)-synthase gene expression and AHL-accumulation occurred. Thus, the termination of cell division in *Eca* starving cultures with low initial CFU values accompanied the induction of system of intracellular communication.

Biological and biophysical properties of biofilm forming drinking water bacteria

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Number: 179

Biofilms are ubiquitous, including on the internal pipe surface of the oligotrophic environment of water distribution systems. Understanding the mechanisms involved in multispecies interactions within water distribution systems is important, as the formation of biofilms affects the water quality by increasing the microbial load within the system. One of the key factors involved in the initiation of biofilm formation is the biophysical cell characteristics, such as surface charge. Surface charge is also a key factor in controlling the stability of bacteria in water and their removal during the treatment process. The objective of this study was to measure and compare the cell surface charge and biofilm formation of bacteria isolated from drinking water. The surface charge of four bacteria (*Sphingomonas*, *Methylobacterium*, *Mesorhizobium* and *Microbacterium*), was determined using phase amplitude light scattering (Brookhaven ZetaPALS) under changing pH, constant ionic strength and different time points. Biofilms produced by either individual species or as mixed cultures were quantified by the crystal violet method. *Sphingomonas* and *Microbacterium* were the most negatively charged of all four isolates across the different conditions. When grown in combination, these two isolates formed the least amount of biofilm. However, when grown as individual cultures, whilst, *Sphingomonas* produced very little biofilm, *Microbacterium* had the greatest biofilm forming capacity of all the isolates. Therefore whilst

preliminary findings suggest that surface charge may influence multispecies biofilm formation, further work is needed to unravel the interconnecting relationships between biophysical characteristics and biological behaviour that drives biofilm formation in drinking water distribution systems.

Influence of increased salinity on the wastewater treatment in submerged fixed bed biofilm reactors (SFBBRs)

Alexander Bahrt, Abdelsalam A Elawwad, Heinz Köser, MLU Halle-Wittenberg, Germany

Number: 180

In some arid or coastal regions, seawater-containing water is used for the toilet flushing. Also industrial wastewater from several processes may contain higher amounts of salt. The aim of this investigation was to evaluate the effect of higher salinity on the carbon and TKN removal in submerged fixed bed biofilm reactors (SFBBRs). SFBBRs model cascade reactors with a total volume of 22 l were used to treat clarified domestic wastewater without and with the addition of 20 g NaCl/l. Four SFBBRs were operated in parallel at 5, 10, 15 and 25°C. PE plates were used as support for the growth of the biofilm. The investigation covered a period of about 50 weeks. After adding the salt there was a period of 5 weeks for the biofilm to adapt. Between 10 and 25 °C, the carbon removal efficiency decreased on average by 19%. The effect of the salt on the TKN removal efficiency strongly depended on the load applied to SFBBR. At a surface specific load of 1 g TKN/m^2*d (German DWA design rules allow up to 1.75 g TKN/m^2*d) the decrease in removal was about 7%, however the decrease at a higher load of 2 g TKN/m^2*d adding 20 g NaCl/l was 40%. Nevertheless the influence of the salt on the treatment of domestic wastewater was found to be lower in comparison to similar investigations which were performed with synthetic wastewater. The effect of the salt on the performance of SFBBRs is smaller than reported for activated sludge processes.

Study the effect of start-up and starvation conditions on the treatment of wastewater in submerged fixed bed biofilm reactors (SFBBRs)

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Number: 181

Efforts on improving the start-up time and starvation tolerance of submerged fixed bed biofilm reactors (SFBBR) are reported in this study. SFBBR are typically used for the treatment of wastewater from smaller communities. Start-up time and activity recovery after starvation are associated with limited treatment efficiency for COD removal and nitrogen oxidation and should be as short as possible. For the start-up experiments, SFBBR reactors seeded with sludge were operated with three different biofilm support materials at different temperature 15, 20 and 25°C each. The results showed that for all materials 90% of the final COD removal is achieved within one week of start-up. The ammonium oxidizing biofilm needed between 3 weeks and 6 weeks to reach its full capacity. Higher temperatures and rough surface characteristics help to shorten the formation of the biofilm. For the starvation experiments, the influence of temperature, oxygen and support material type during starvation on the decay of the biofilm activity was studied. Starvation periods were varied up to 4 weeks. Depending on the support material, the remaining NH_4-activity was only 4 to 28% of the initial activity after four weeks starvation period. Oxygen supply and temperature proved to be important parameter for the decay of the activity of the nitrifying biofilm in SFBBR. Low oxygen and low temperature conditions showed better results. The time required for the reactors to reach its full performance was compared with data experienced during the start-up.

Microbial communities in biofilms inhabiting the drinking water treatment plant of Cluj, Romania

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Number: 182

Biofouling in drinking water distribution systems and its associated undesirable impacts such as water quality deterioration and pipes corrosion may be a consequence of the water network's inner surface colonization by microorganisms.

As a first step of a comprehensive approach of biofilms associated to drinking water, the present study evaluates the naturally formed biofilms in two treatment steps (clarification and rapid sand filtration) of drinking water plant of Cluj, Romania. The tests were performed over a period of six months, searching for the presence and frequency of different metabolic groups of bacteria, indicators of fecal contamination and a number of pathogens. Microorganisms belonging to the following groups were determined by culture-based techniques: UFC at 37ºC; UFC at 22ºC; oligotrophic microorganisms; several physiological groups of bacteria: amonifying, denitrifying, sulfur reducing, sulphate reducing, iron reducing, sulfur oxidizing, iron and manganese oxidizing bacteria, total coliforms; indicators of faecal contamination: fecal coliforms, *Escherichia coli*, fecal enterococci; pathogens: *Clostridium perfringens*, and several other genera: *Pseudomonas*, *Aeromonas*, *Legionella.*

The results revealed high frequency of bacteria with freshwater and soil origin. Bacterial load and frequency increased proportionally with the biofilm's age.

The load of microorganisms well known as involved in microbial induced corrosion (sulphate reducing, iron reducing, iron and manganese oxidizing, acid and slime producing bacteria) will be discussed. The presence of indicators of fecal contamination and of pathogenic bacteria *Clostridium perfringens* was recorded in most of the samples. No species belonging to *Legionella* genera were detected within the biofilm samples tested.

Biofilm formation upon virus infection in *Sulfolobus solfataricus*

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Number: 183

The hyperthermophilic crenarchaeote *Sulfolobus solfataricus* is a facultative autotroph with the ability to grow under aerobic conditions. The genome of several *Sulfolobus* species has been sequenced making *Sulfolobus spp.* ***model*** organisms for studying molecular and physiological processes in ***Archaea.*** Information about biofilm formation in Archaea is still at the stage of infancy. Recently, it has been shown that *S. solfataricus* forms a biofilm mainly made of polysaccharides upon attachment to various surfaces, such as glass, mica etc. The genes possibly involved in the production of the extracellular polysaccharides have been identified (1).

We have investigated the ability of *S. solfataricus* to form biofilm as a stress response to the infection with the Spindle-shaped virus 2 (SSV2) (2). After prolonged growth on solid medium of a lawn of *S. solfataricus* cells spotted with SSV2, production of a white and dense material was observed. Formation of a white matrix was also observed in cells non-infected but grown at high density. The protein fraction of the white matrix was found to be very low and, although not confirmed, it is likely that the white matrix is an exopolysaccharide. We therefore suggest that the formation of the white matrix could be controlled by quorum sensing and cellular stress responses in order to protect cells from external factors. The physiology of the process as well as the chemical composition of the biolfilm-like white matrix, is under way.

1) Zolghadr B *et al*. 2010. J Bacteriol 192:104-10
2) Contursi *et al.* 2006. Extremophiles 10:615-27

Effect of the *kil* gene in plasmid ColE1 on *E. coli* biofilm formation

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Number: 184

Significance and objectives: Biofilm formation by Gram-negative bacteria is a frequent problem in for example long-term use of indwelling urinary catheters. Lipopolysaccharide (LPS) is a major component of the surface of Gram-negative bacteria and its polysaccharide portion is situated at the outermost region. The aim of this study was to examine the possibility of a relationship between alterations in the polysaccharide part of LPS and biofilm formation of *Escherichia coli*, using a series of LPS mutants with different sugar composition. Methods and results: *E. coli* strain BW25113 and its LPS mutant derivatives, including also mobilizable plasmids, pNTR-SD and its derivatives, (from the NIG collection, Japan) were used in this study. We found that both biofilm formation and cell surface hydrophobicity were enhanced by the lack of a specific sugar of LPS inner-core, HepI. Surprisingly, biofilm formation by a HepI-deficient mutant, the *hldE* strain, strongly depended on extracellular DNA and increased drastically when cells carried the cloning vector pNTR-SD, which is a ColE1 plasmid (pColE1) derivative. The plasmid determinant responsible for the hyper-biofilm formation was identified as the *kil* gene, which is present on pColE1 and related plasmids frequently found in clinical isolates of *E. coli* and other Enterobacteriaceae. **Conclusions:** While the *kil* gene is involved in release of colicin protein, our findings demonstrate an alternative role of the *kil* gene in biofilm formation. We suggest that these results should be considered in the context of current development of antibacterial compounds aimed at inhibiting HepI incorporation in LPS because of the potential risk of biofilm-related complications.

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