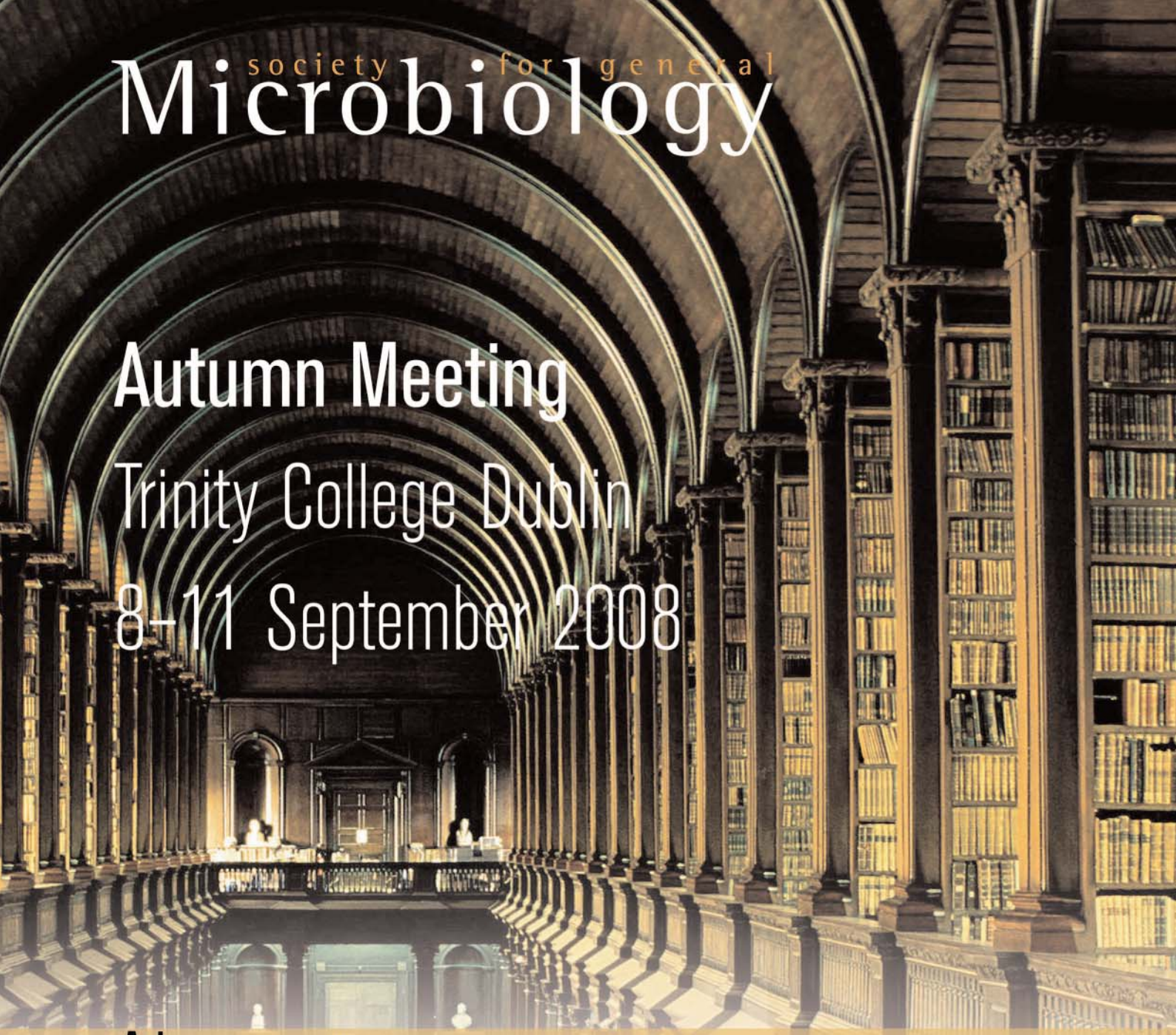

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Microbiology

Autumn Meeting

Trinity College Dublin

8–11 September 2008

Abstracts

Contents

Please note: Abstracts are printed as received from the authors and are not subjected to editing.

Plenary Session

Behaviour of biofilm bacteria: from co-operation and communication to control

Laboratory models for biofilm maintenance and growth-rate control

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The ubiquity, variety and complexity of biofilms has become part of the perceived wisdom amongst specialists. We know that most biofilms are composed of multiple species, genera and often, kingdoms and that there is arguably no unifying biofilm architecture: each biofilm reflects its particular niche. Whilst observing biofilms *in situ* or *ex situ* has revealed the wide distribution and function of sessile bacterial communities, much of our understanding of biofilm physiology and micro-ecology has been gleaned from experiments using *in vitro* biofilm models. The concerted effort to study biofilms has led to the development of a wide range of models in a relatively short time. Broadly speaking, biofilm models can be designed to either replicate environmental conditions in the laboratory or to investigate selected variables using simplified model systems. This presentation will give an overview of some commonly-used biofilm models and their applications. These include the constant depth film fermenter, flow cells, single and multiple Sorbarod systems, perfused membrane biofilm rectors and rotating reactors. Perfused membrane reactors, in particular, enable growth rate to be controlled within thin, relatively homogenous biofilms through modulation of flow rate and medium nutrient composition. A high degree of division synchronicity within eluted cells can be achieved, which is particularly useful for physiological and gene-expression analysis.

Exploring biofilms using molecular methods

Mathew Upton

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The nature of a biofilm can be greatly influenced by the environment in which it forms and, although these communities are classically viewed as being complex, multi-species systems, biofilms causing human infection commonly contain only a single species. The variety of techniques that can be employed in the study of biofilms is probably as wide ranging as the complexity of biofilms themselves, and the most appropriate methods for analysis will vary in relation to the population diversity.

This presentation will introduce some of the molecular methods more widely used for examination of biofilm communities, indicating how the methods are integrated into the model systems being described in this overview session.

The nucleic acid detection and characterization methods introduced will include amplification based approaches for single gene target detection, microarrays, reverse-transcriptase PCR and real-time PCR for examination of gene expression and denaturing gradient gel electrophoresis (DGGE), denaturing high-performance liquid chromatography (DHPLC) and terminal restriction fragment length polymorphism (T-RFLP) analysis for examination of mixed DNA samples.

The material covered will conclude with a look to the future and comment on the potential impact new, high throughput, DNA sequencing methods and metagenomics will have on our understanding of biofilm biology.

The use of model systems and image analysis techniques to study the Saptio-temporal development of biofilms

Alexander H. Rickard

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Biofilms are ubiquitous and typically composed of many bacterial species. How these communities develop and contribute to health, disease or an undesirable activity has been the focus of thousands of research papers. However, only with recent technological and conceptual advances have we been able to switch from single-species biofilm studies to those which study complex multi-species biofilm development and behavior.

It is the aim of this presentation to introduce the audience to various model systems and microscopic techniques to study the spatio-temporal development of single, dual and tri-partite biofilm communities. The importance of such an understanding is clear when one considers that many multi-species biofilms develop through ordered successional integrations of species. Discussion of model systems will include flowcells, Modified Robbins devices, sorbarod systems and 96-well plate assay systems. Microscopic techniques that will be discussed include epifluorescence microscopy and Confocal Laser Scanning Microscopy. The advantages and disadvantages of each system and suitability for studying medically and environmentally relevant biofilms will be compared and discussed. Considering recent advances in computer-based image analysis software, attention will also be drawn to various methods and approached to examine spatio-temporal relationships, such as total and species-specific biofilm biomass and analysis of juxtaposition and co-localization of species within biofilms.

When explaining various model systems and techniques to study biofilms, focus will be drawn to studying dental plaque biofilms as well as freshwater biofilms and relating biofilm development to inter-species communication.

Biofilms: The Hypertextbook

Rockford J. Ross

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Only occasionally does research generate a revolutionary body of knowledge so extensive that it compels fundamental changes in content across many science curricula. Such is the case with the emerging discipline of biofilms. The recognition that micro-organisms on surfaces generally live in heterogeneous colonies (i.e., *biofilms*) with inherent defense mechanisms and other characteriztics not found in those same micro-organisms in aqueous solution is radically changing our view of microbiology and profoundly affecting practice and research in academia, industry, medicine, and dentistry. Important as this topic is, however, there are very few courses and educational resources (e.g., textbooks) that incorporate biofilm topics. *Biofilms: The Hypertextbook* represents a unique teaching and learning resource, constructed around Web 2.0 technologies and authored by many biofilm experts working in collaboration. Subject material in the Hypertextbook is presented in parallel in distinct forms for students at different academic levels. In addition to standard textual presentations of a subject, the Hypertextbook incorporates high-resolution images, slide shows, videos, audio, active learning models of important processes that require active learning on the part of the student, and interactive feedback quizzes—all interwoven into a seamless presentation. A Hypertextbook disseminated on the Web or on DVD can be accessed by virtually anyone, virtually anywhere, by means of ubiquitous Web browsers.

Biofilm dissemination: learning resources

Joanna Verran[1] & Rocky Ross[2]

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Biofilm research is a dynamic and vibrant subject. The diverse but related topics addressed at this plenary, and at other symposia during the meeting (and at other meetings) reflect the importance of the subject to pure and applied microbiology. However, in the undergraduate setting, textbooks do not provide a significant amount of information for students, who therefore rely on searches, recommended reviews, references and websites (of repute, naturally!) to obtain updated and relevant works. Some of these resources will be described during the presentation. *Biofilms – The Hypertextbook*, is an important new resource that will also be demonstrated (Ross).

The Education and Training group of SGM have assembled a number of posters describing resources, courses and learning activities to assist in biofilm education for display at this meeting. Conference presenters are encouraged to consider submitting images and film to curriculum resource collections, and any delegates actively engaged in biofilm research who wish to assist in dissemination of learning resources for biofilm education are most welcome to contact the presenters.

Moving forward, moving back: what is gained can be lost (but then it gets complicated)

Hubertus J.E. Beaumont, Christian Kost, Jenna Gallie, Gayle C. Ferguson & **Paul B. Rainey**

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The reversibility of evolution is a long-standing and fundamental issue in biology. When propagated in spatially structured environments bacterial cells that over produce various adhesive polymers are favoured by natural selection on account of the fact that together the group of cells forms a self-supporting mat (and cells within the mat gain access to oxygen that is otherwise severely limiting). When propagated in a spatially unstructured environment cells that possess the ability to form mats are maladaptive and mutants that reverse their mat-forming abilities are favoured by selection. The genetic and phenotypic pathways to mat formation can be (and have been) unravelled; so too have the routes to mat loss. In this talk I will outline the results of a large-scale experiment in reverse evolution where 12 independent lines have been subjected to repeated evolutionary reversals for gain and loss (and re-gain and re-loss and on ad infinitum) of mat-forming ability. A combination of genetics, genomics (re-sequencing) and phenotypic analyses reveals some striking insights into contingency, epistasis, pleiotropy and the reversibility of evolutionary change (and biofilms).

Forces affecting biofilm structure and organization

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Biofilm cells are typically surrounded by a protective extracellular polymeric slime (EPS) matrix which provides the biofilm with mechanical stability. The main physical forces encountered by a biofilm are often those exerted from the overlying flowing liquid. The ability of the biofilm to remain attached to a surface with overlying flow depends on the adhesive strength between the cells at the base of the surface and the surface, and the cohesive strength which is the strength of the biofilm itself, as a bulk material. Adhesive failure will result in the biofilm 'peeling' from the surface while cohesive failure will cause pieces of biofilm to break away. Biofilm bacteria can respond to changes in fluid shear and modify their mechanical properties to remain attached. Fluid flow can also modulate cell signaling by 'washing away' diffusible signal molecules. Biofilm bacteria can also produce enzymes which dissolve slime matrix components allowing the cells to be released for active dispersal. We have used rheometry and image analysis to measure biofilm viscoelasticity and used expression analysis to identify genes associated with natural detachment or exposure to elevated shear stress. We have also used novel staining and imaging techniques to visualize tertiary structures in biofilm EPS.

Temporal gene expression in biofilms using proteomic approaches

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Biofilm cells differ from their planktonic counterparts in the genes and proteins that they express. Using proteomic approaches, we were able to demonstrate that *in vitro* and *in vivo* biofilm formation by *P. aeruginosa* occurs in a progressive and stage-specific manner with each developmental stage displaying a unique phenotype. To elucidate the mechanisms involved in the stage-specific formation of biofilms, we have begun to identify regulatory proteins and pathways that are critical for normal biofilm development using a proteomic approach. Recent progress in elucidating the regulatory pathways controlling biofilm development by *P. aeruginosa* will be summarized. Specifically, the role of regulatory proteins required for planktonic growth, biofilm formation, microcolony formation and maintenance of the planktonic mode of growth will be discussed. Inactivation of GacAS, BflS1 and BflR2 resulted in impaired biofilm development prior to the stages at which these proteins were produced, in altered protein production patterns and in biofilms that were less resistant to antimicrobial agents. MifR was found to regulate microcolony formation while SclR played a role in dispersion and maintaining the planktonic mode of growth. These findings suggest the existence of an intricate and coordinated regulatory network involved in regulating and coordinating *P. aeruginosa* biofilm development.

Biofilm dispersal: ecology, mechanisms and control

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All organisms need to disperse to and colonize new environments. Studies of several Gram-negative bacteria have revealed that microcolonies in mature biofilms often undergo processes of cell lysis and dispersal. Characteriztics of cells that disperse from biofilms include enhanced biofilm formation and phenotypic variability compared to pre-dispersal biofilm cells. Dispersal in a broad range of Gram-negative organisms is linked to the accumulation of reactive oxygen species (ROS) in the interior of microcolonies. Molecules implicated in biofilm dispersal of this type include nitric oxide and hydrogen peroxide, and add-back of ROS to biofilms can induce a shift from biofilm to planktonic cell physiology. For *Pseudomonas aeruginosa*, this is achieved by addition of nitric-oxide donor compounds to biofilms. Nitric-oxide mediated dispersal in *P. aeruginosa* appears to occur through NO-mediated interference with cyclic-di-GMP signaling pathways which are known to regulate sessile versus planktonic lifestyles in diverse bacteria. Thus new understanding of the mechanisms of biofilm dispersal has revealed novel strategies for the manipulation of biofilms in medical and industrial settings.

The complex life in slime: biofilm organisms and their extracellular polymeric substances

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Biofilms come in a wide variety of manifestations but they have fundamental common characteriztics: all of them consist of microbial aggregates, mostly multispecies, and are kept together by extracellular polymeric substances (EPS). These are biopolymers of microbial origin such as proteins, polysaccharides, nucleic acids etc. which all form hydrogels, immobilizing biofilm organisms which then can establish long-term interactions and synergistic microconsortia. Not only physiological interactions are facilitated but also horizontal gene exchange. The EPS form the immediate environment of the cells and EPS properties directly determine the conditions of life for biofilm

organisms. Biofilm organisms can influence the physico-chemical properties of EPS molecules in composition and molar mass, both resulting in different properties of the matrix regarding sorptive and mechanical properties. By interaction between extracellular enzymes and EPS components, activation of the EPS matrix is achieved. Furthermore, enzymatic activity can lead to modification and degradation of the matrix, thus, influencing its architecture with profound influence on mass transport. As a metaphor, the EPS matrix can be considered a functional 'house of biofilm cells'.

Distributed labour and subpopulation interactions are involved in maintenance of physiological heterogeneity in *Pseudomonas aeruginosa* biofilms

Tim Tolker-Nielsen

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The existence of physiologically distinct subpopulations in biofilms in many cases allows one or more subpopulations to survive environmental insult. It is generally assumed that physiologically distinct subpopulation arise and are maintained in biofilms because of the structured nature of the biofilms which allow different subpopulations to occupy different micro-niches. We show in the present study that maintenance of subpopulation diversity in biofilms may be complex and involve distributed labour and subpopulation interactions. Because biofilms in most medical, industrial and environmental settings are subject to turnover, which can allow subpopulation succession, mechanisms involved in maintenance of physiologically distinct subpopulations may contribute to their innate tolerance to adverse conditions.

Adaptation and evolution of *Pseudomonas aeruginosa* to a biofilm lifestyle in the cystic fibrosis lung

Søren Molin

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Populations of infecting bacteria represent at the same time a challenge to the clinical microbiologists, and an opportunity for the experimental microbiologists to study basic biological phenomena such as mechanisms of adaptation and evolution in connection with shifts of the environmental conditions.

We study chronic infections of the common soil and water borne bacterium *Pseudomonas aeruginosa* in lungs of patients suffering from the genetic disorder cystic fibrosis as a model for long-term microbial infections in humans. Our findings have led to the following hypothetical model: The bacteria infect the patients, either from the outer environment (where *P. aeruginosa* is frequently occurring) or from other infected patients. There is no direct evidence that the infection specifically requires virulence factors – in fact, we have indirect evidence suggesting that at no stage is there a need for these factors. We also find that biofilm development is associated with over-production of the exo-polysaccharide alginate (the mucoid phenotype), resulting in biofilm structures which are different from the well-characterized 'mushrooms' developing *in vitro* for wild type strains. From recent transcriptomic studies we have seen that among the gene expressions changed in the infecting bacteria are efflux pumps and genes encoding amino acid catabolism. We suggest that the infecting bacteria adapt to life in the CF lung in two ways – one, by becoming antibiotic resistant through up-regulation of efflux pumps and by expressing resistance genes such as *bla* (beta-lactamase) and *pmr* (polymyxin resistance), and 2) by overproducing alginate allowing the bacteria to occupy other niches where they can resist the immune system through the production of an alginate capsule. By optimizing their amino acid metabolism they can compete with the attacks from the immune system and the added antibiotics by growing optimally on the available amino acids.

Cell-to-cell signaling in biofilm communities: Implications for understanding and controlling biofilm development

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In the last decade, there has been a tremendous amount of interest in the relationship between intercellular signaling and biofilm development in several bacterial species. Several studies have shown that different types of quorum sensing contribute to biofilm formation in a number of species. In this presentation I will review some of the emergent concepts resulting from these studies. In addition, I will review some key aspects of biofilm communities with the potential to impact the quorum sensing mechanism. In the second half of my talk I will discuss some of the work done on quorum sensing and biofilm formation in *Pseudomonas aeruginosa*. *P. aeruginosa* has a complicated quorum sensing network, with two distinct AHL signals and a non-AHL signal called PQS. Several quorum sensing-controlled functions have been suggested to contribute to different steps biofilm formation. One such function is swarming motility, which contributes to a key step in biofilm development. Additionally, *P. aeruginosa* uses quorum sensing to control expression of antimicrobial functions which could be important in multispecies communities.

Community architecture and signaling in biofilms: species interactions

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A common feature of all human oral bacteria is their ability to interact physically to form networks of genetically distinct cells, a feature called coaggregation. Contact-induced signaling among the participants in these mixed-species communities occurs in suspension as coaggregates and on substrata as biofilms in a flowing environment. The architecture of initial communities that develop on enamel *in situ* repeatedly shows streptococci in juxtaposition with veillonellae and actinomyces as well as other streptococcal species. One streptococcal-veillonellae community has been micromanipulated from enamel; two streptococcal species were cultivated and were coaggregation partners. In a saliva-fed biofilm *in vitro*, veillonellae were unable to grow as a mono-culture, but they coaggregated with the two streptococci and formed three-species communities. Thus, coaggregation appears to foster an advantageous microenvironment for signaling between oral bacteria. This was also shown by forming coaggregates between *Actinomyces naeslundii* and *Streptococcus gordonii* in suspension. In a chemically defined growth medium with low arginine, *S. gordonii* could not grow aerobically in mono-culture or in co-culture (no coaggregates) with *A. naeslundii*. But in coaggregates with *A. naeslundii*, *S. gordonii* up-regulated a regulon of arginine biosynthetic genes and grew to high cell density, indicating a coaggregation-induced signaling and stabilization of arginine biosynthesis.

Metagenomic analysis of microbial communities

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Now in its 10th year metagenomics is becoming an ever more powerful technique for analysing and understanding microbial biofilm communities. Metagenomic techniques allow a truly culture independent analysis of microbial communities, a crucial advantage, given that large proportions of most complex microbial communities are as yet unculturable or indeed, in a viable but non culturable state. With the next generation of technologies able to supply 3–4 Gb of sequence per run, the scale of sequencing project achievable is now bounded only by ones imagination. The metagenomic analyses of formation, development and perturbation of complex multispecies biofilm communities is now therefore an achievable prospect. To compliment large scale sequencing projects functional approaches e.g. phage display technologies can be utilized to, for example, target

adhesions in communities or indeed antibiotics can be added to media to select for clones expressing antibiotic resistance determinants. This approach has been successful in detecting numerous tetracycline resistant determinants from the metagenome of oral microbial communities indeed, a novel tetracycline resistance gene, *tet(37)* has been isolated and characterized. Metagenomics therefore allows us to analyse communities in more detail and faster than previously thought possible; just imagine....

From dental plaque to periodontitis and cardiovascular disease: why worry about the biofilm?

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Cardiovascular disease (CVD) is the leading cause of death in Western societies. Individuals with severe chronic periodontitis have a significantly increased risk of developing cardiovascular disease including atherosclerosis, myocardial infarction and stroke after adjusting for the traditional risk factors. The aim of the present studies was to investigate the role of molecular mimicry as a possible mechanism underlying this association.

hHSP60 was expressed on atherosclerotic endothelial and smooth muscle cells and GroEL-, hHSP60- and P. gingivalis-specific T cell lines were established from peripheral blood and atherosclerotic plaques. These T cell lines demonstrated a predominant Th2 phenotype in the CD4 subset and a Tc0 phenotype in the CD8 subset with a high proportion of CD8 cells also expressing RANTES, MCP-1 and MIP1-alpha. There was an over expression of the T cell receptor Vβ5.2 family in all lines suggesting clonality within the cell lines. Cross-reactivity of GroEL-specific T cell lines to hHSP60 and hHSP60-specific lines to GroEL was further demonstrated.

These results demonstrate the presence of GroEL specific T cells in the peripheral blood as well as in lesions of atherosclerosis and their cross-reactivity with hHSP60 suggesting molecular mimicry between GroEL and hHSP60 may be a fundamental mechanism linking infection and atherosclerosis.

The future of biofilm control using quorum sensing inhibitors

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Antibiotics have saved millions of lives but the backside of the coin is that they by their mode of action strongly promote development of resistant bacterial variants, which rapidly outmatch the original sensitive strains. In order to identify new antimicrobial drug targets, it is imperative to broaden our understanding of the molecular basis of infection, rather than just going in for the kill. The biofilm lifestyle dominates in many of today's chronic bacterial infections. Consequently, they share similar characteriztics; they tolerate the highest deliverable doses of antibiotics and resist the action of the immune system as well as controlling the infectious process by cell-to-cell communication and internal signal transmission. For example, in the biofilm mode a bacteria may employ 'quorum sensing' communication to inform their fellow bacteria that it is time to establish protection against innate immunity while launching a cocktail of other tissue damaging virulence factors. The scaffolds and subsequent development of quorum sensing blockers are therefore interesting and relevant as future antimicrobial measures. In particular their capabilities of assisting the immune system in eliminating infectious bacteria are a highly appealing antimicrobial strategy. Recent scientific developments have elevated small molecules to a position where they pose a truly revolutionizing impact on modern antimicrobial research. One particular advantage is the unique ability of small molecules to rapidly diffuse in and out of cells. Small molecules such as communication blockers offer a rapid, conditional, dose-dependent and often reversible control of bacterial functions, which cannot be achieved with traditional measures.

Gastrointestinal biofilms in health and neoplastic disease

P. Gilbert

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The gastrointestinal tract (GIT) of all mammals is colonized at birth by a complex consortium of micro-organisms, with bacterial cells outnumbering human cells by at least one order of magnitude, predominantly associated with surfaces (mucosal and particulate food residues). Colonization begins in the first hours of life, from the birth canal, the immediate environment and via the diet. Whilst external factors, including diet and lifestyle can influence the development of intestinal biofilms, the relative contributions of host genetics and environment on the composition and metabolic activities of the GIT microbiota remain poorly understood due to of the practicability of separating these two variables. We have used microbial faecal DNA profiling and urinary metabonomics to study various highly inbred mouse strains. Genetically closely related animals exhibited highly conserved faecal microbiotas and urinary metabolite profiles, suggesting the apparent involvement of host genetics. However, implanting a female mouse with embryos of a different mouse strain enabled differentiation between genetic and environmental influences and indicated non-genetic inheritance of the GIT biota with respect to microbial composition and metabolic activity. Additionally, we have studied nude mice, implanted dorsally with various human tumours to show that although GIT microbial richness is unaltered during neoplastic disease, marked tumour-associated differences could be detected using PCR-DGGE and Q-PCR. We conclude that the composition of the intestinal microflora is (i) strongly influenced by environmental factors associated with the mother and (ii) that tumours located distal to the GI tract significantly and reproducibly perturb the GI microbiota.

Cells & Cell Surfaces / Microbial Infection Groups Joint Session

Innate immunity systems

Bacterial elicitation and evasion of plant innate immunity

Gregory Martin, Tracy Rosebrock, Lirong Zeng, Fangming Xiao & Kathy Munkvold

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Many important bacterial pathogens of both plants and animals increase their virulence by using a type III secretion system to deliver effector proteins into host cells. These type III effectors often act by subverting host immunity but their molecular mechanisms are largely unknown. *Pseudomonas syringae* pv. *tomato* DC3000, which causes bacterial speck disease of tomato, delivers ~30 diverse effector proteins into the host cell. We are studying one of these effectors, AvrPtoB, in order to understand the molecular mechanisms it uses to promote bacterial virulence. AvrPtoB is a modular protein with distinct activities encoded by its N- and C-terminal regions. The N-terminal region (NTR) uses two structurally distinct domains to promote bacterial virulence. Interestingly, tomato has evolved two related protein kinases, Pto and Fen, that specifically interact with each of these NTR domains thereby resulting in activation of plant immunity. The C-terminal region of AvrPtoB was found to be a structurally mimic of an E3 ubiquitin ligase of the U box family. The E3 ligase activity subverts recognition of AvrPtoB by the Fen kinase but not by the Pto kinase. I will describe the molecular details of the various virulence activities of AvrPtoB and present a model that places these observations in an evolutionary context. Supported by NIH-R01GM078021 and NSF-DBI-0605059

Recognition of *Salmonella enterica* serovar Typhimurium by pattern recognition receptors

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Inflammatory cytokines produced by the host in response to systemic *S.* Typhimurium infection are critical for controlling the early phase of bacterial growth (the plateau). Initial host recognition of infection depends on pattern recognition receptors (PRRs), specifically Toll-like receptor (TLR) 5 in the gut and TLR4 systemically. PRR recognition of *S.* Typhimurium activates macrophages to produce the oxidative burst and induces cytokine production. In lipopolysaccharide (LPS)-resistant mice (C3H/HeJ; with mutated, non-signalling TLR4) or TLR4$^{-/-}$ mice the early control of bacterial growth does not occur indicating that it is dependent on the inflammatory response induced by TLR4 recognition of LPS. TLR2$^{-/-}$ is dispensable for early control of *S.* Typhimurium infection. TLR4 signals through the recruitment of two adaptor protein pairs Mal/MyD88 and Tram/Trif to stimulate different subsets of cytokine genes. Plateau formation is primarily dependent on signalling through MyD88 independently Mal. Tram/Trif signalling is unimportant for plateau formation. TLR4 is, therefore, critical in generating the host immune response against *S.* Typhimurium.

Fibrinogen binding sites P336 and Y338 of clumping factor A are crucial for *Staphylococcus aureus* virulence

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We have earlier shown that clumping factor A (ClfA), a fibrinogen binding surface protein of *Staphylococcus aureus*, is an important virulence factor in septic arthritis. When two amino acids in the ClfA molecule, P_{336} and Y_{338}, were changed to serine and alanine, respectively, the fibrinogen binding property was lost. $ClfAP_{336}Y_{338}$ mutants have been constructed in two virulent *S. aureus* strains Newman and LS-1. The aim of this study was to analyse if these two amino acids which are vital for the fibrinogen binding of ClfA are of importance for the ability of *S. aureus* to generate disease.

Septic arthritis or sepsis were induced in mice by intravenous inoculation of bacteria. The $clfAP_{336}Y_{338}$ mutant induced significantly less arthritis than the wild type strain, both with respect to severity and frequency. The mutant infected mice developed also a much milder systemic inflammation, measured as lower mortality, weight loss, bacterial growth in kidneys and lower IL-6 levels. The data were verified with a second mutant where $clfAP_{336}$ and Y_{338} were changed to alanine and serine respectively. When sepsis was induced by a larger bacterial inoculum, the $clfAP_{336}Y_{338}$ mutants induced significantly less septic death. Importantly, immunization with the recombinant A domain of $ClfAP_{336}SY_{338}A$ mutant but not with recombinant ClfA, protected against septic death.

Our data strongly suggest that the fibrinogen binding activity of ClfA is crucial for the ability of *S. aureus* to provoke disease manifestations, and that the vaccine potential of recombinant ClfA is improved by removing its ability to bind fibrinogen.

Spatiotemporal dynamics and immune control in bacterial infections

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Understanding the heterogeneous nature of the events that determine the within-host spatiotemporal dynamics of proliferation, spread, and distribution of microbial pathogens is a challenge of clear medical importance and it is necessary for the development of targeted preventive measures and drug regimes.

Salmonella enterica resist the intracellular antimicrobial environment, but also adopt 'hit and run strategies' to spread to new infection foci, thus avoiding the local escalation of the adaptive immune response. This process leads to the concomitant and independent evolution of spatially segregated bacterial subpopulations that expand from the clonal amplification of individual founder bacteria.

Pyroptosis: caspase-1-dependent inflammatory response

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Eukaryotic host cells die upon exposure to toxic chemical or physical insults, but cell death is also an important and regulated host response. The concept of 'programmed cell death' is supported by experimentally observing biochemically distinct mechanisms of cell death dictate equally distinct physiological consequences for the host. Inflammation, one outcome from cell death, plays critical roles in the initial protective responses to infection as well as supporting development of adaptive immunity. Activation of the cysteine protease caspase-1 results in the maturation and secretion of inflammatory cytokines by cells that can also die and release their inflammatory intracellular contents. This process of pyroptosis, from the Greek roots *pyro* relating to fire or fever and *ptosis* (to-sis) to denote a falling or cell death, is highlighted by studies using *Salmonella*, *Yersinia*, and *B. anthracis* as biological probes to query the operation and function(s) of death machinery in eukaryotic cells. Further definition of the distinct mechanism of pyroptosis has revealed it is selected in activated host cells as an alternative to non-inflammatory apoptosis. Pyroptosis

results from the activation of a conserved effector pathway in response to diverse stimuli, with relevance not only to infectious processes, but also cardiovascular disease, stroke, and malignancy.

How *Salmonella* triggers innate B cells to induce protective responses

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Compelling evidence from epidemiological, genetic and animal studies shows that antibody from B cells, in addition to T cells, is important in protecting against *Salmonella* infections. In mice we have shown that *Salmonella* induces a highly atypical B cell response in that is extremely rapid, extensive and does not require T cells to develop. Here I will show our recent data that indicate that *Salmonella* and a limited repertoire of antigens from the organism can rapidly induce a population of innate B1b B cells that produce antibody in the absence of T cell help. Thus these B cells are induced by some outer membrane proteins but they are not induced by LPS or flagellin. Importantly, antibody to these outer membrane proteins is effective at reducing the ability of *Salmonella* to infect and indeed all protection mediated by these proteins was antibody-dependent. This offers a potentially novel line of investigation to help generate new, effective vaccines against *Salmonella*.

Recognition of bacterial PAMPs by plants

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Perception of pathogen-associated molecular patterns (PAMPs) constitutes the first layer of plant innate immunity and is referred to as PAMP-triggered immunity (PTI). Genetic and biochemical studies have recently identified plant pattern-recognition receptors (PRRs) involved in the perception of bacteria, fungi and oomycetes. Interestingly, some of the structural domains present in PRRs are similar in plants and animals, suggesting convergent evolution.

The plant model *Arabidopsis thaliana* provides an excellent system to study PTI, and detects a variety of PAMPs including conserved domains of bacterial flagellin and EF-Tu, or their peptide surrogates, flg22 and elf18, respectively. The related leucine-rich repeat receptor kinases FLS2 and EFR are the PRRs for flagellin and EF-Tu, respectively. They are so far the only known *Arabidopsis* PRRs and the only PRRs recognizing bacterial PAMPs in plants.

Recently, proteins with known roles in development have been shown to control immediate PRR-signalling, revealing unexpected complexity in plant signalling. Although many PAMPs recognized by plants have been described and more are likely to be discovered, the number of PRRs known currently is limited.

Recent results on the identification of new PRRs and downstream signalling regulators will be presented.

Manipulation of innate immunity by oral pathogens to promote their virulence

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Porphyromonas gingivalis is an oral/systemic pathogen implicated in chronic inflammatory conditions such as periodontitis and atherosclerosis, although the mechanism(s) whereby it resists elimination by the host are poorly understood. The virulence of this pathogen depends upon expression of fimbriae, a major colonization factor comprising polymerized fimbrillin (FimA) associated with quantitatively minor proteins (FimCDE). The function(s) of FimCDE have remained elusive and our group has investigated their role using isogenic mutants expressing fimbriae devoid of these minor proteins. Our findings indicate that isogenic mutants lacking FimCDE are dramatically less persistent and virulent in a mouse periodontitis model and express shorter fimbriae than wild-type *P. gingivalis*. Strikingly, these effects could not be readily attributed to differences in colonization but were largely dependent on the ability of native but not mutant fimbriae to mediate immune evasion. Specifically, native fimbriae allow *P. gingivalis* to exploit Toll-like receptor-2 and complement receptor-3 for intracellular entry, inhibition of interleukin-12, and persistence in macrophages. In contrast, this virulence mechanism is abrogated in mutants lacking FimCDE. Therefore, the virulence of *P. gingivalis* is surprisingly dependent upon minor constituents of its fimbriae. These findings support the concept that pathogens evolved to manipulate innate immunity to promote their adaptive fitness.

Innate immune responses to microbial infection in the lung and CNS

Catherine M. Greene

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Toll-like receptors (TLR) are key components of the innate immune system that are activated in response to microbial and host-derived factors. In the airways functional expression of TLRs on both immune and airway epithelial cells orchestrates appropriate responses to infective and inflammatory stimuli. In the milieu of the cystic fibrosis (CF) lung TLR activity is dysfunctional due to chronic stimulation by multiple agonists including factors expressed by *Pseudomonas aeruginosa* and host proteases. TLR-targeted strategies have therapeutic potential for CF. A variety of mechanisms including gene therapeutics, microbial TLR mimetics and antiproteases will be described.

TLRs also have potentially important roles in the host response to infection associated with insertion of a medical device. *Staphylococcus epidermidis* is a frequent cause of neurosurgical device-related meningitis. Expression of polysaccharide intracellular adhesion (PIA) and the ability to produces biofilm are important virulence factors of *S. epidermidis*. Recent studies linking PIA to activation of TLR2 and cytokine expression in human astrocytes will be presented.

The impact of flagellin immunization before, during and after *Salmonella* infection

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Control of primary *Salmonella* infections requires effective interactions between CD4 T cells and innate cells. Flagellin is atypical as it is a potent adjuvant, stimulates the innate system through TLR5 and Ipaf and is an immunodominant *Salmonella* T cell antigen. Consequently, the relationship between the host, flagellin and pathogen is central to understanding how immunity to *Salmonella* Typhimurium develops. Here we show immunization with soluble recombinant flagellin drives a strong Th2-mediated response which is not protective. The adjuvant activity and capacity to drive Th2 responses was not dependent upon a putative TLR5 binding motif. Moreover co-immunization of flagellin and live, attenuated *Salmonella* reduced the protective benefit of the live vaccine partly because fewer IFNγ-secreting flagellin-specific T cells were induced. Lastly, mice primed with live *Salmonella* and then immunized with soluble flagellin experience a rapid hypersensitivity reaction that is related to the presence of residual infection. Thus the innate and adaptive immunomodulatory capacities of soluble flagellin act synergistically to influence responses to *Salmonella* in a neutral or negative manner.

The role of innate immunity in protection, evasion and shaping adaptive immunity to *Bordetella pertussis*

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Respiratory infection with the Gram-negative bacterium *Bordetella pertussis* causes whooping cough, a severe and protracted disease in young children. Acquired immunity against *B. pertussis* develops after natural infection and confers relatively long-lived protection against subsequent infection. However, studies in a murine model have shown that adaptive immunity does not develop until 3–4 weeks after infection and that initial control of the bacteria is mediated by the innate immune system. Macrophages, neutrophils and NK cells are recruited into the lungs early after infection. Infiltrating NK cells act as a source of innate IFN-γ production, which activates anti-bacterial activity of macrophages and together with dendritic cells help to shape the adaptive immune response. IFN-γ-producing Th1 cells and IL-17-producing T cells help to clear the infection, but these cells are suppressed during acute infection. The bacteria produce a range of virulence factors that promote innate IL-10 and TGF-β production and thereby induce IL-10-secreting regulatory T (Treg) cells, which suppress the function of Th1 and Th17 cells. The induction of Treg cells helps the host to limit infection induced immunopathology but has also been exploited by the pathogen as an immune evasion strategy to prolong its survival in the host.

Mechanistic role of CFTR in innate immune resistance to *Pseudomonas aeruginosa* lung infection

Gerald B. Pier

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Background Pseudomonas aeruginosa is the primary pathogen associated with cystic fibrosis lung disease. Prior work has demonstrated that the cystic fibrosis transmembrane conductance regulator (CFTR) is a lung epithelial cell receptor for *P. aeruginosa*. A basic goal has been to use this interaction to define the host responses critical for bacterial elimination in the presence of WT CFTR.

Methods To identify innate immune molecules needed for NF-κB nuclear translocation a variety of transgenic mice lacking factors known to promote translocation were infected intranasally with *P. aeruginosa* strain PAO1 and lung tissues removed 15–360 min post infection and NF-κB nuclear translocation into airway epithelial cell nuclei determined. To find proteins involved in epithelial cell signaling, a proteomic analysis was conducted to identify proteins recruited to lipid rafts within 15 min of *P. aeruginosa* infection of lung epithelial cells. *P. aeruginosa* strain PAO1 was used to infect isogenic epithelial cells expressing WT or mutant CFTR, cells harvested, detergent insoluble membrane microdomains isolated and proteins in these rafts identified by LC-MS.

Results Mice lacking either the MyD88 adaptor protein for NF-κB signaling, or the IL1 receptor failed to translocation NF-κB to the nucleus and were highly susceptible to either acute or chronic *P. aeruginosa* infection. Proteomic analysis identified 150 proteins recruited to lipid rafts within 15 min of *P. aeruginosa* infection of cells with WT CFTR. Prominent proteins identified in rafts included MVP (major vault protein), caveolin, clathrin, CARMA1, migration inhibitory factor (MIF) and PAG (phosphoprotein associated with glycosphingolipid-enriched microdomains). Analysis of cells treated with siRNA for MVP, caveolin, clathrin and MIF showed all but clathrin were needed for CFTR-dependent interactions with *P. aeruginosa*. MVP, caveolin and MIF knock out mice all had phenotypes in either acute or chronic lung infection models.

Conclusions CFTR binding of *P. aeruginosa* is a critical early step in initiating innate immunity to lung infection with this pathogen, activating a plethora of cellular responses that coordinate the clearance of the organism and help maintain airway sterility.

Visualizing an immune response to bacterial infection

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The development of immunity to oral bacterial infection has not been examined in any detail. We have developed TCR transgenic and MHC tetramer systems to visualize the CD4 response to *Salmonella* infection *in vivo*. Our data demonstrate that *Salmonella* infection initiates a highly localized activation of CD4 T cells in the secondary lymphoid tissues of the intestine. More recent data suggest that natural immunity following resolution of primary infection is distinct from acquired immunity after exposure to a live vaccine strain. These observations may explain the incidence of relapse and recurrent infection in areas where typhoid is endemic.

The human cathelicidin LL-37 preferentially promotes apoptosis of *Pseudomonas*-infected airway epithelium via mitochondrial membrane permeabilization

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LL-37, the active fragment of the human cathelicidin hCAP18, is expressed by neutrophils and epithelial cells, and is upregulated in the lung during infection and inflammation. This cationic host defence peptide (CHDP) has both microbicidal and immunomodulatory properties and at high concentrations, can induce apoptosis of airway epithelial cells both *in vitro* and *in vivo*.

Using an *in vitro* model of infected airway epithelium, we demonstrate that physiological concentrations of LL-37 preferentially induce apoptosis (assessed using TUNEL labelling) in cells infected with *Pseudomonas aeruginosa*. LL-37 and *P. aeruginosa* synergistically induce mitochondrial membrane depolarization via Bax-dependent mechanisms, with the release of cytochrome c and AIF. The consequent induction of apoptosis is partially caspase-dependent, with activation of caspases-9 and -3 occurring only in the presence of both LL-37 and bacteria, but not with either stimulus alone. Furthermore, we demonstrate that apoptosis induction requires live bacterial-cell interaction and bacterial invasion of the epithelial cell.

Cationic peptides as selective modulators of innate immunity and novel anti-infectives

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Antibiotics are the underpinning of all modern medicine, but are being undermined by an explosion of (multidrug) resistance, and a dearth of new antibiotics. We are developing novel therapeutics for antibiotic resistant bacteria. Cationic host defence (antimicrobial) peptides are produced by virtually all organisms, ranging from plants and insects to humans, as a major part of their innate defences against infection. Cationic antimicrobial peptides with direct antimicrobial activity are being designed based on peptide array and QSAR approaches. Biochemical and animal model studies demonstrated that these peptides have potential as antibiotics, and phase III clinical trials are underway for prevention of catheter associated infections.

It was recently demonstrated that host defence peptides also modulate innate immunity. Microarrays, sophisticated bioinformatics, and pathway and transcription factor studies have demonstrated that these peptides stimulate innate immunity/inflammation in a unique fashion, boosting protective immunity while suppressing harmful inflammation/sepsis; e.g. it was demonstrated that the human peptide LL-37 triggers multiple signalling pathways while largely suppressing the mainstream TLR to NFκB pathway. Using this principle of selective boosting of innate immunity we have developed novel small peptides with no direct antibacterial activity, that are nevertheless able to protecting against microbial infections in animal models, providing a new concept in anti-infective therapy.

Clinical Microbiology Group Session

Biofilm infection of medical devices

Pathogenic mechanisms of *Staphylococcus epidermidis* biomaterial-related infection

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Staphylococcus epidermidis is the prototype organism involved in medical biofilm disease resulting in infected implants affecting millions of patients worldwide. These infections persist despite antimicrobial treatment regularly requiring device removal. We identified polysaccharide intercellular adhesin (PIA) as the central functional factor in biofilm accumulation. PIA is a homoglycan of β-1,6-linked GlcNAc residues, which are partly de-acetylated. PIA is synthesized by gene products of *icaADBC*. Using isogenic biofilm-negative mutants expression of PIA and biofilm were defined as essential virulence factors of *S. epidermidis* in foreign body infection models. A complex regulatory network controls expression of *icaADBC* and PIA synthesis involving the alternative sigma factor σ^B, the transcriptional repressor IcaR, *sarA*, the new *barAB* locus, and a glucose-induced protein. Molecular epidemiology of *S. epidermidis* strains from port-catheter infections were almost all *icaADBC*-positive and proficient for PIA-synthesis while strains from prosthetic joint infections produced biofilms frequently in an *icaADBC*- and PIA-independent manner. Consequently we discovered a PIA-independent mechanism of biofilm accumulation relying on the highly prevalent accumulation associated protein (Aap). Aap is activated by proteolytic processing by staphylococcal or host proteases generating an intercellular adhesin mediating biofilm accumulation. Apparently, *S. epidermidis* can use factors of innate immunity to generate phagocytosis resistant bacterial aggregates.

Pathogenesis of *Staphylococcus aureus* biomaterial related infection and implications for vaccine development

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Living as a biofilm community on and in about one-third of the human population, as well as in other mammals, *Staphylococcus aureus* is, in fact, mostly a commensal that on occasion causes some usually minor abscess-based skin and soft tissue infections. But on much rarer occasions *S. aureus* causes severe, life threatening infections that are significant medical problems. Many of these infections have a biofilm component to the overall pathogenic picture, and a prominent bacterial factor associated with this life style is production of the poly-N-acetyl glucosamine (PNAG) extracellular polysaccharide that mediates intercellular adherence. PNAG is also a critical factor for the planktonic cells, mediating resistance to host immune effectors in the blood and tissues. Notably, many other bacterial species also make PNAG and, as such, humans have high titers of natural antibody to this molecule. However, these antibodies do not mediate *in vitro* opsonic killing or protection of animals during experimental infection. To understand the basis for the failure to elicit protective antibody we analysed the specificity of antibodies directed to different epitopes on this antigen and found that opsonic, protective antibodies are directed to usually poorly immunogenic epitopes defined by the ability of the antibody to bind to PNAG both before and after extensive deacetylation. Removal of acetates greatly enhances elicitation of antibody to these 'backbone' epitopes and conjugate vaccines containing deacetylated PNAG (dPNAG), as well as fully human monoclonal antibodies that bind to the backbone epitopes, have shown excellent protective efficacy in animal models of *S. aureus* and *E. coli* infections. dPNAG has the potential to serve as vaccine against a range of microbial pathogens, including *S. aureus*, which, if validated by clinical trials, could provide a very effective addition to the armamentarium of anti-infective agents.

Improved detection of biofilm infection

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Total hip replacement with an implanted artificial prosthesis is a highly successful operation with an estimated 50,000 carried out annually in the UK. In a small proportion of cases the primary hip has to be removed and replaced because the patient experiences pain and discomfort. Although it is widely considered that aseptic loosening is the most common cause of failure of total hip replacement, studies indicate that standard microbiological diagnosis using tissue samples underestimates the incidence of infection. When mild ultrasound treatment of prostheses retrieved at the time of revision operation is used to dislodge the adherent biofilm and retrieved prostheses are processed under anaerobic conditions, detection of infection is increased. In addition, *Propionibacterium acnes* is isolated as frequently as coagulase negative staphylococci. Non-culture detection using specific antibodies and immuno-fluorescence microscopy enables the detection of bacteria in culture negative samples. Immuno-fluorescence microscopy reveals characteriztic aggregates of bacteria dislodged from adherent biofilm. This highlights the potential importance of *Propionibacterium acnes* in the failure of total hip replacements and the need to sample from the retrieved prosthesis biofilm to accurately diagnose infection. These techniques are potentially transferrable to the detection of biofilm infection of other implanted devices.

Aggregatibacter actinomycetemcomitans biofilm formation

J.B. Kaplan

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Dental plaque is the prototype for mixed-species bacterial biofilms. Mechanisms that contribute to the development of dental plaque include surface adhesion, autoaggregation, intergeneric coaggregation, and inter- and intraspecies quorum sensing. Work in our laboratory has shown that the human periodontopathogen *Aggregatibacter actinomycetemcomitans* produces poly-N-acetylglucosamine, a sticky surface polysaccharide that mediates intercellular adhesion, biocide resistance, and resistance to killing by human macrophages. Interestingly, *Staphylococcus epidermidis* produces a nearly identical polysaccharide that mediates biofilm formation on medical devices. This talk will highlight studies on the biosynthesis and function of poly-N-acetylglucosamine in *A. actinomycetemcomitans*, and will describe how these findings may lead to improved methods for the treatment and prevention of staphylococcal biofilms on medical devices.

Prevention of chronic *Pseudomonas aeruginosa* infection in patients with cystic fibrosis

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In patients with cystic fibrosis (CF), mucoid *P. aeruginosa* and highly viscous mucous plugs prevent the killing of the pathogen by neutrophils and antibiotics, leading to chronic infection and antibiotic-resistant *P. aeruginosa* strains. Since *P. aeruginosa* isolated from CF patients early after airways colonization are nonmucoid variants which are more readily killed by antibiotics *in vitro* than the mucoid

aggregates, CF patients have been treated as early as possible after onset of *P. aeruginosa* lung colonization. This strategy has led to successful eradication of *P. aeruginosa* from the CF airways and a decrease in the prevalence of chronic *P. aeruginosa* lung infection in this disease. Furthermore, active immunization of CF patients with a bivalent *P. aeruginosa* flagella vaccine revealed the development of high and long-lasting serum anti-flagella IgG titres and a lower risk for infection with *P. aeruginosa* in the treatment group compared to a placebo group. Finally, recent data show that partial inhibition of acid sphingomyelinase with amitriptyline prevents the susceptibility to *P. aeruginosa* infection in CF mice – an approach which may also be successful used in the future in CF patients.

Antimicrobial peptides to prevent initial adhesion and biofilm formation of coagulase negative staphylococci

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Coagulase negative staphylococci especially *Staphylococcus epidermidis* are a major cause of infections associated with the use of indwelling medical devices. The main pathogenic attribute of these strains is the ability to form biofilms. The increasing resistance of biofilm forming strains to current antimicrobial therapies makes them impossible to eradicate. New methods of preventing initial adhesion of bacteria to biomaterials and biofilm formation is therefore of great importance. Antimicrobial peptides represent a potential alternative therapy in the prevention of biofilm infections.

The aim of this study was to assess the activity of semi-pure preparations of anti-microbial peptides 224 (MW 6069 Da) and 229 (MW 2814 Da) both at a concentration of 320 AU/ml against clinical biofilm forming strain *Staphylococcus epidermidis* 156 (abs 0.225 at 450nm). The microtitre plate biofilm assay was used to investigate 4 protocols to determine the peptides effect on strain 156 to produce biofilm.

Results obtained showed both peptides 224 and 229 resulted in complete inhibition of biofilm formation for strain 156 for the 4 protocols tested, resulting in absorbance readings comparable to that of a non-biofilm forming isolate (abs <0.120 at 450nm).

These results clearly indicate the effectiveness and potential use of antimicrobial peptides to prevent initial adhesion and subsequent biofilm formation of clinical biofilm forming isolates of coagulase negative staphylococci.

Novel antimicrobial biomaterials for the prevention of biofilm infection

R. Bayston

University of Nottingham

Abstract not received

Influence of material and microtopography on the development of orthopaedic implant infection

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In vivo studies have shown that polishing the surface of fracture fixation implants reduces tissue adhesion with significant clinical benefit in reducing complications associated with implant removal or soft tissue adhesion. The effect of polishing these implants on bacterial adhesion and infection is evaluated in the present study, prior to clinical implementation.

The common orthopaedic metals titanium and titanium-aluminium-niobium (TAN) with standard rough surfaces were compared to polished equivalents. The topography, chemistry and hydrophobicity of all surfaces were characterized. Adhesion of *Staphylococcus aureus* was performed i*n vitro* and subsequently i*n vivo* infection rates were compared using standard AO locking compression plates (LCPs) in rabbits.

In vitro results showed a significant decrease in the amount of bacteria adhering to polished TAN compared to standard TAN surfaces though we did not detect a difference between polished and standard titanium. No statistical differences in *in vivo* infection rates were observed between the different groups. The *in vivo* infection rates suggest that for the LCP model, which minimizes damage to bone, implant material and surface roughness do not have a large influence on infection rate.

Prevention and treatment of prosthetic joint infection

A.R. Berendt

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Abstract not received

Clinical Microbiology Group Session

Infective endocarditis

The changing face of infective endocarditis

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Even in the modern era of advanced diagnostic imaging, improved antimicrobial chemotherapy and potentially curative surgery, infective endocarditis remains an evolving disease with a persistently high mortality and morbidity. Despite these improvements in health care, the incidence of the disease has remained unchanged over the past two decades and may even be increasing. Almost all aspects of the disease, including its natural history, pre-disposing factors, sequelae and causative organisms are virtually unrecognizable compared with original descriptions from the 19th century. In particular, chronic rheumatic heart disease is now an uncommon antecedent, whereas degenerative valve disease of the elderly, intravenous drug abuse, preceding valve replacement or vascular instrumentation have become increasingly frequent, coinciding with an increase in staphylococcal infections and those due to fastidious organisms. Previously undetected pathogens are now being identified with the disease and multi-drug resistant bacteria challenge conventional therapeutic regimes. Early surgical treatment is potentially life saving, but randomized trials to demonstrate its effectiveness are absent – many indications remain controversial and timing of intervention in individual patients is often difficult. Finally, antibiotic prophylaxis to prevent the disease in at risk individuals is a source of perennial debate, fuelled by fundamental differences in international guidelines. The changing face of infective endocarditis seems set to challenge the endeavours of cardiologists, microbiologists and cardiac surgeons for many decades yet.

Dental treatment and endocarditis: how strong is the association and what is the role of prophylactic antibiotic therapy in 2008?

R.G. Bogle

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Abstract not received

Host–pathogen interactions in the pathogenesis of infective endocarditis

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Staphylococcus aureus is a leading cause of infective endocarditis (IE). *In vitro* studies suggest a critical role for the interaction of bacteria with human platelets in the pathogenesis of IE. In particular, platelet activation leading to aggregation could contribute to the development of vegetations on heart valves. The molecular mechanism of *S. aureus*-platelet binding leading to activation and aggregation has been elucidated and the process involves specific bacterial and platelet surface receptors and plasma proteins. Currently, we are investigating the clinical relevance of these host-pathogen interactions for the development of IE.

Fibronectin binding by *Streptococcus gordonii*

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Oral viridans streptococci, including *Streptococcus gordonii*, are among the most common causative agents of infective endocarditis. Adhesion of streptococci to host glycoproteins such as fibronectin is considered important for endocardial infections. Here, adhesion of *S. gordonii* to fibronectin was shown to be reduced ~50% by pre-treating fibronectin with sialidase. The role of *S. gordonii* sialic acid adhesin Hsa, and of cell surface proteins CshA/CshB and SspA/SspB, in fibronectin adhesion was investigated using isogenic mutants. Knockouts of Hsa or CshA/CshB significantly reduced the levels of *S. gordonii* binding to fibronectin. Disruption of genes encoding SspA/SspB further reduced adhesion of the CshA/CshB mutant to fibronectin and ablated residual fibronectin binding by the Hsa mutant. However, in the presence of functional Hsa and CshA/CshB, deletion of SspA/SspB did not affect fibronectin binding. Therefore, in *S. gordonii* Hsa and CshA/CshB are primary adhesins for fibronectin and secondary adhesion is supported by SspA/SspB. None of the proteins contain consensus motifs for fibronectin binding. These results show that molecular interactions of *S. gordonii* with fibronectin are multi-modal and distinct from those utilized by pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes*.

Endocarditis guidelines

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Healthcare professionals seem to like to have Guidelines, both from a best practise point of view and as a potential insurance for future litigation.

Ideally, Guidelines should be evidence based but in practise this is not easy in Infectious Diseases as there are very few good clinical trials which fulfil the exacting criteria of modern medicine.

This is particularly true in endocarditis, where practise is largely based on animal models and 'traditional' antibiotics, and where an accepted definition of the disease was agreed only in the past 15 years. There are a few clinical trials, but they are very small and are largely pertaining to right sided endocarditis, which is on the benign end of the spectrum of infective endocarditis.

Never the less, recent Guidelines in relation to the prevention of endocarditis have been more radical and controversial and have tried to incorporate the microbiology of the condition and the risk benefit for the individual. It remains to be seen what impact these guidelines have on the incidence of endocarditis, and with the lack of any robust prospective data collection reporting will be anecdotal.

New insights in the pathogenesis of staphylococcal endocarditis

P. Moreillon

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Abstract not received

Enterococcal endocarditis

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Enterococci cause approximately 10% of all infective endocarditis cases, a rate that has stayed constant over recent decades. *Enterococcus faecalis* accounts for the majority of episodes and both native and prosthetic heart valves can be affected. The source of infection is usually thought to be the colonized gastrointestinal or genitourinary tract but colonized intravascular catheters and intravenous drug use are emerging risk factors. Although the clinical course is often indolent and protracted, patients may also present with acute onset of severe sepsis. With appropriate antimicrobial therapy, cure rates can exeed 80% but prolonged intravenous therapy is required, sometimes combined with valve surgery. This presentation will outline the clinical features of enterococcal endocarditis, current approaches and

controversies in treatment particularly in the context of antimicrobial resistance patterns, and an overview of the molecular theories of pathogenesis.

Molecular methods for the diagnosis of endocarditis

B. Cherie Millar

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Infective endocarditis (IE), although a relatively uncommon infective state, is associated with a high degree of morbidity and mortality and continues to remain an important and potentially fatal serious infection of the heart particularly the heart valves. The diagnosis and subsequent antimicrobial treatment remains both a diagnostic and therapeutic challenge, for a successful clinical and microbiological outcome. There has been an increase in the adoption of molecular based techniques to aid with the detection and identification of causal agents of IE, particularly in cases of atypical or culture-negative IE. Several different molecular approaches have been employed, including variations in the examination of cardiological specimen type, nucleic acid extraction, gene target and molecular platform, each presenting their own advantages and disadvantages. This presentation will exam the role molecular approaches play in aiding in the diagnosis if IE particularly in cases due to obligate (*Coxiella burnetii, Tropheryma whipplei*) and facultative (*Bartonella* spp.) intracellular bacteria, fastidious organisms (*Abiotrophia defectiva, Granulicatella* spp.), community-associated MRSA and fungi. The application, adoption and integration of these techniques into routine service algorithms will be discussed, as well the interpretation of molecular results and the implications for their inclusion in the Duke Classification Scheme to aid in the diagnosis of IE.

Streptococcus mitis adheres to sialylated residues on human platelets

Jennifer Mitchell & Paul Sullam

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Platelet binding by *Streptococcus mitis* strain SF100 is mediated in part by the phage encoded surface proteins PblA and PblB. This study involves identification of the platelet ligand for *S. mitis*.

Platelets were treated with enzymes that specifically deglycosylated surface exposed proteins. Desialylation of platelets resulted in a reduction in *S. mitis* adherence. Platelets were subsequently treated with sialydases that removed specific sialic acid linkages. This assay indicated that α(2–8) linked sialic acids are the main sialic acid linkage to which *S. mitis* adheres.

The ability of antibodies to candidate glycoproteins and glycolipids on the surface of platelets to inhibit the adherence of *S. mitis* to platelets was then analysed. Antibodies to GPIb, GPIIbIIIa, CIqR and FcgRIIa had negligible effect on the ability of *S. mitis* to adhere to platelets. However antibodies to GPIV and ganglioside GD3 reduced binding of *S. mitis* to platelets.

This indicates S. mitis adheres to platelets in part via its interaction with α(2–8) linked sialic acids on GPIV and ganglioside GD3.

Streptococcus biofilms: from mouth to heart

Howard F. Jenkinson

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Viridans streptococci are early colonizers of saliva-coated oral cavity surfaces and they pave the way for development of complex biofilm communities on hard or soft tissues. Analysis of the sequenced streptococcal genomes reveals multiple genes encoding secreted or cell wall-anchored proteins potentially relevant to colonization. Three families of cell surface proteins found across the streptococci are involved in biofilm formation: Srr (serine-rich repeat) glycoproteins, Antigen I/II (AgI/II) adhesins, and GTFs (glucosyltransferases). These proteins mediate microbial adherence, community development and biofilm accumulation, respectively. They also provide for versatility in colonization of cardiac sites. Srr proteins interact with platelet receptor GPIb, enabling capture of platelets by bacteria under flow conditions, while AgI/II polypeptides act in concert with Srr proteins to facilitate platelet aggregation. Recently, another high molecular mass surface polypeptide, with extensive amino acid repeat blocks, and involved in biofilm formation, has been identified in *Streptococcus gordonii*. This protein mediates platelet adherence, interacting with receptor GPIIb/IIIa, promotes platelet aggregation, and induces platelet activation at high shear. Viridans streptococci thus express a repertoire of cell surface proteins for oral cavity colonization that can behave as surrogate virulence factors in infective endocarditis.

Environmental Microbiology Group / Irish Branch Joint Session

Industrial bioremediation: from contamination to clean-up

The carbon and sulfur cycles in oil fields: new insights spawn new biotechnologies

Gerrit Voordouw

University of Calgary, Canada

Production of oil from a subsurface field is often achieved by the injection of water to maintain reservoir pressure. Water injection greatly enhances microbial activity because it can relieve nutrient limitation or provide a favorable temperature for growth. Injection of water containing sulfate stimulates growth of sulfate-reducing bacteria (SRB), which couple oxidation of oil organics to reduction of sulfate to sulfide (H2S), a process referred to as souring. Souring has significant negative impact (e.g. increased iron corrosion). Inclusion of nitrate in the injection water can reduce souring by stimulating nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB), catalyzing sulfide + nitrate → sulfur, sulfate + nitrite, nitrogen, as well as heterotrophic nitrate reducing bacteria (hNRB), which oxidize oil organics with nitrate. Nitrate injection has been particularly successful in lowering souring in high temperature (60–80°C) fields, because all SRB activity is near the injection wellbore where the cold injection water comes in. A recent study of the effect of nitrate injection into a low temperature (30–40°C) field for a one-year period has indicated a strong decrease of sulfide concentrations (70% field wide) in the first 5 weeks, followed by recovery. A model explaining these observations is presented.

Hydrocarbons – tough substrates for anaerobic bacteria

Friedrich Widdel

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Hydrocarbons which by definition consist only of carbon and hydrogen are devoid of functional groups and thus exhibit low reactivity and often also marginal solubility in water. Whereas aerobic microbial degradation of hydrocarbons is known since a century, insights into their anaerobic degradation have been gained rather recently. Methane, the simplest hydrocarbon, is anaerobically oxidized by archaea that live in close association with specialized sulfate-reducing bacteria. Utilization of the 'higher' hydrocarbons has been observed in various bacterial branches. Anaerobic degradation of hydrocarbons apparently makes use of diverse mechanisms, depending on the type of hydrocarbon and electron acceptor; so far known, all these mechanisms differ mechanistically and energetically substantially from the established aerobic mechanisms which so far known always involve oxygenases. Saturated hydrocarbons, the main constituents of petroleum and natural gas, belong to the most stable organic compounds. Their utilization as growth substrates by micro-organisms requires activation reactions that overcome particularly high C–H-bond energies. The striking diversity of micro-organisms and pathways involved in the anaerobic oxidation of hydrocarbons may reflect the early appearance of these compounds as natural growth substrates for micro-organisms in Earth's history.

Anaerobic petroleum degradation to methane in the subsurface: microbial communities and processes

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Biodegradation of petroleum has major consequences for oil and gas production. Heavy oils of which there are immense known reserves are more difficult and costly to produce. Evidence is emerging to suggest that in-reservoir petroleum biodegradation was and still is caused by anaerobic hydrocarbon degrading bacteria.

Oil degrading laboratory experiments have been used in conjunction with field data to determine mechanisms of hydrocarbon degradation in petroleum reservoirs. In laboratory microcosms crude-oil hydrocarbon degradation under methanogenic conditions mimicked the sequential removal of compound classes seen in reservoir-degraded petroleum. The degradation of the n-alkanes was accompanied by generation of close to stoichiometric amounts of methane and selection for bacteria and archaea consistent with the syntrophic oxidation of alkanes to methanogenic precursors. These findings suggest a common methanogenic biodegradation mechanism in the anaerobic subsurface. Intriguingly, the dominance of putative CO_2 reducing methanogenic archaea in the microcosms and petroleum reservoirs and, the observed isotopically heavy CO_2 and light CH_4 associated with degraded reservoir oils, suggests that methanogenic alkane degradation is dominated by CO_2 reduction linked to hydrogenotrophic methanogenesis and syntrophic acetate oxidation. Methanogenic biodegradation of oil may offer a route to economic production of difficult-to-recover energy assets from oilfields.

Bugs, buckets and bioavailability: concepts, measurement and application

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In general, the extraction of organic contaminants using organic solvents has little relevance to the measurement of their bioavailability in soil. The measurement of bioavailability is considered important as it can describe the extent to which organic contaminants may be degraded in soil. The determination of bioavailability using chemical methods is desirable as they can be generally more rapid and more reproducible than biological techniques. Previous research has shown that the use of aqueous-based cyclodextrin solutions may extract the microbially degradable fraction of phenanthrene in soil. The data presented here extends this initial research by comparing cyclodextrin extractability of organic contaminants with microbial degradation in laboratory and field contaminated soils, containing single compounds and mixtures. In all cases, there were strong correlations observed between the fraction of the contaminants extracted by the cyclodextrin technique and degradability. Linear correlations also demonstrated direct (~1:1) relationships between the catabolic data and the HPCD extractions. To further examine the linkage between the cyclodextrin extraction and microbial degradation, a study was conducted to investigate the relationship between sequential HPCD extraction and microbial mineralization of phenanthrene (10 mg kg^{-1}) in four soils over time (0, 25, 50 and 100 d). Phenanthrene extractability (desorption) was assessed at 24 h intervals over 10 d and compared to cumulative microbial mineralization using an enriched pseudomonad inoculum. Significant differences were found between mineralization assays performed using single and multiple pseudomonad inoculations; repeated inoculation produced higher levels of ^{14}C-phenanthrene mineralization. The cumulative total of phenanthrene extracted by HPCD exceeded the mineralization asymptote by >20%. However, the overall total extents mineralized, after multiple inoculations, were statistically similar to that extracted after the first 24 h by HPCD; the ratios of extractability to mineralizable phenanthrene consistently approached 1. Furthermore, a good linear correlation between phenanthrene mineralized and extracted was observed (r^2 =0.95; gradient = 1.00; intercept = –0.48), demonstrating that a single HPCD extraction accurately and reproducibly predicted the total fraction of phenanthrene available for microbial mineralization in different soils. From this work, it is proposed that a chemical extraction that rapidly

and reproducibly predicts the 'microbially available' fraction could be extremely useful in the assessment of contaminated land for bioremediation as this technique has the potential to accurately predict the total fraction of contaminant available for biodegradation.

Keywords bioavailability, extraction, biodegradation, soil, contaminants

Recent advances in the bacterial degradation of high molecular weight polycyclic aromatic hydrocarbons: metabolism, proteomic and genomic approaches

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The environmental fate of polycyclic aromatic hydrocarbons (PAHs) is of concern due to their genotoxic, mutagenic, ecotoxic and carcinogenic properties. Extensive efforts have been devoted to develop bioremediation strategies to remove these persistent pollutants from contaminated soils. Recent studies on the biodegradation of high molecular weight (HMW) PAHs have shown that mycobacteria are important members of PAH degrading microbial community in contaminated soils. *Mycobacterium vanbaalenii* PYR-1 was isolated from oil-contaminated estuaune sediment exposed to petrogenic chemicals. *M. vanbaalenii* PYR-1 has exceptional ability to degrade two to five ring PAHs. We have used a combination of metabolism, genomic and proteomic techniques to better understand the physiology, enzymes and genes involved in the catabolism of HMW PAHs. The catabolic pathways by *M. vanbaalenii* PYR-1 have been elucidated for the degradation of naphthalene, phenanthrene, anthracene, fluoranthene, pyrene benzo[*a*]pyrene and benz[*a*] anthracene. The genome of this bacterium has recently been sequenced allowing us to gain insights into the molecular basis for the degradation of HMW PAHs. The 6.5 Mb genome contains 194 chromosomally encoded genes likely associated with the degradation of PAHs. Many identified genes were enriched with multiple paralogs showing a remarkable range of diversity which could contribute to the wide variety of PAHs degraded by *M. vanbaalenii* PYR-1. There are differences in the gene structure and organization as compared to extensively studied PAH degraders including *Pseudomonas, Burholderia* and *Sphinogomonas* species. The results of the metabolism, proteomic and genomic research has provided a deeper understanding of mycobacteria and related organisms ability to degrade PAHs in the environment.

The impact of TNT on soil microbial community structure

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Environmental contamination with recalcitrant toxic chemicals presents a serious and widespread problem to the functional capacity of soil. Explosives are a major cause of such pollution, with 2,4,6-trinitrotoluene (TNT) the most widespread example. TNT has been shown to be highly toxic to a wide variety of organisms. We have examined the microbial community composition within soil from across a long-term TNT-contaminated site, as well as the acute impact of trinitrotoluene (TNT) contamination of soil on the bacterial community composition and function. A polyphasic approach encompassing culture-independent molecular analysis by DGGE, carbon substrate analysis and cell enumeration was taken. A clear change in the genetic diversity and carbon-substrate utilization patterns of the soil occurred when soil was contaminated with TNT. There was a shift from the unculturable α-proteobacteria, prevalent within the control soil, towards more culturable bacteria, in particular Pseudomonads. Phytoremediation potentially offers a means to decrease the toxic effect on micro-organisms. We have found that transgenic tobacco plants overexpressing a bacterial nitroreductase gene detoxify soil contaminated with TNT, with a significantly increased microbial community biomass and metabolic activity in the rhizosphere of transgenic plants compared with wild type plants.

Bioremediation of PAH contaminated ground water; a complex microbiological problem

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Most organic compounds can serve as potential energy sources for a wide diversity of micro-organisms. This concept underpins our approach to employing micro-organisms in complex communities for the bioremediation of polluted sites. Accumulated evidence suggests that the diversity of microbial communities and their catabolic pathways matches that of the complex mixtures of organic compounds available. Whilst the potential for catabolic activity can be well defined under laboratory conditions, quantifying this under remediation conditions in a field process presents a formidable challenge. Dynamic changes in communities, physiology, gene expression and conditions contribute to the overall biodegradative activity. The assessment of microbial gene diversity has become an integral part of monitoring bioremediation processes. Improvements in the methods for the extraction of nucleic acids, the use of techniques such as gene microarrays and quantitative PCR to monitor changes in gene diversity and expression hold out the promise of faster assessment of effective communities. The use and limitations of molecular techniques will be critically reviewed with reference to some data acquired from the remediation of contaminated ground water at two former gasworks sites. These will be compared with the role of conventional microbiological techniques and chemical methods to assess *in situ* catabolic rates.

The acid test: can we clean up our dirty oil?

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The vast heavy oil fields and tar sands in North America represent a 6 trillion barrel resource. These tar sands comprise of complex mixtures of aliphatic or alicyclic carboxylic acids known as 'Naphthenic Acids' (NAs). For more effective oil exploration and production, it is important to understand the processes underlying NA biodegradation, the microbial communities involved and the factors that control it. This project combines analytical and molecular techniques to characterize and quantify aromatic NA-degrading micro-organisms in relation to NA structure and biodegradation rates. GC-MS analysis demonstrated almost complete biodegradation had occurred on enrichments grown on the least-branched aromatic NAs (after 49 days incubation) whilst the more-branched aromatic NAs were more recalcitrant. GC-MS analysis also revealed the presence of an intermediate metabolite which suggests that aromatic NA degradation may involve β-oxidation of the carboxyl side chain. Increases in Eubacterial cell numbers were also observed from 3.7 x 10^2 to 2.48 x 10^4 cells ml^{-1} during incubation on all the aromatic NAs tested. Application of PCR-DGGE analysis of the 16S rRNA genes amplified from DNA extracted from the enrichment cultures revealed shifts in microbial diversity during aromatic NA degradation. This is the first report to characterize aromatic NA-degrading microbial communities.

Low-temperature anaerobic biotreatment of pentachlorophenol-contaminated groundwater

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Low-temperature anaerobic digestion (LTAD) represents a low-cost, low-technology approach for pentachlorophenol (PCP)-contaminated groundwater bioremediation.

Two expanded granular sludge bed-packed bed (EGSB-PB) hybrid bioreactors, R1 and R2, were each seeded with anaerobic granules and employed for the treatment of PCP-contaminated groundwater at 7°C.

Co-metabolic PCP biodegradation was facilitated by providing an alternate carbon source, in the form of either polyhydroxybutyrate (R1) or peat (R2). PCP (1.5 mg l^{-1}) was supplied at an applied loading rate (PCPLR) of 3 g PCP m^3/d (initial hydraulic retention time (HRT), 12 h), which was increased to 9 g PCP m^3/d by step-wise HRT reductions through 9, 6 and 4 h.

Despite transient disimprovements in performance, PCP removal efficiencies of 50–99% were recorded at PCPLRs up to 9 g PCP m^3/d. The detection of lower chlorinated phenols and benzoate in R1 and R2 effluents suggested the primary PCP removal mechanism was reductive dechlorination, and that this was non-regiospecific. 16S rRNA gene fingerprinting indicated that microbial community development was linked to both operating temperature and pollutant loading rates, and suggested the development of stable psychroactive PCP-degrading consortia.

Fungal systems for metal, radionuclide and mineral transformations

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Fungi are ubiquitous components of terrestrial microbial communities and intimately involved in biogeochemical transformations, including organic and inorganic transformations and element cycling, rock/mineral transformations, bioweathering, and mycogenic mineral formation, as well as interacting with such substances as toxic metals and radionuclides from natural and anthropogenic sources. Several of these processes have potential in bioremediation. Solubilization enables removal of metals from wastes and by-products, low-grade ores, and metal-bearing minerals, and is relevant to bioremediation of soil matrices and solid wastes, metal recovery and recycling. Immobilization processes enable metals to be contained and/or transformed into chemically more inert forms. This contribution will outline the main mechanisms by which fungi transform metals, radionuclides and minerals and the resulting consequences for environmental mobility of metals and radionuclides. Free-living fungi as well as mycorrhizal fungi associated with plant roots will be discussed, the latter having significant consequences for the biogeochemical mobility of metals and associated elements in the plant root zone. Specific highlighted examples will include fungal transformations of copper and lead-containing minerals, depleted uranium and uranium oxides, and fungal biodeterioration of concrete which may have implications for containment and storage of radioactive waste.

Putting subsurface micro-organisms to work: applications of metal-reducing bacteria to the bioremediation of contaminated land and water

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The redox cycling of metals by subsurface bacteria has attracted recent interest as these transformations can play crucial roles in controlling the mobility of both inorganic and organic species in a range of environments and, if harnessed, may offer the basis of a wide range of innovative biotechnological processes. These include the bioremediation of metal contaminated land and water, the oxidation of xenobiotics under anaerobic conditions, metal recovery in combination with the formation of novel functional bionanominerals, and even the generation of electricity from anoxic sediments. Rapid advances over the last decade have resulted in a detailed understanding of some of these transformations at a molecular level, with added impetus expected from the imminent availability of complete genome sequences for key subsurface bacteria, in combination with genomic and proteomic tools.

Focusing on 'dissimilatory' processes, I will discuss recent advances in the understanding of the mechanisms of anoxic Fe redox cycling in the subsurface, and the impact of these biotransformations on sediment biogeochemistry and the mobility of trace metals, metalloids and radionculides. The biotechnological application of Fe(III)-reducing bacteria for the generation of commercially useful bionanominerals will also be discussed, alongside their use in a range of innovative ex situ applications.

Geochemical and microbiological transformation of depleted uranium in contaminated soil

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Environmental assessment of depleted uranium (DU) in oxic soil is hampered by limitations on information regarding uranium mobilization and transport. This study aims to determine the relative influence of microbial and geochemical factors and place these within the context of the overall biogeochemical processes, including uranium speciation and mineralogy.

DU contaminated soil was collected from two UK sites, Eskmeals and Kirkcudbright. The Kirkcudbright soil is an organic rich clay, whereas the Eskmeals soil is a quartz-rich sand. Samples up to 50cm from the DU metal were analysed.

Sequential extraction and X-Ray Fluorescence showed the uranium present as U(VI).

DU contamination in Eskmeals lies within 10 cm of the metal, found as a uranium oxide schoepite, $(UO_2)_4O(OH)_6{\cdot}6H_2O$, coating the sand grains. Whereas in Kirkcudbright the uranium was more diffuse and associated with ferric oxyhydroxide minerals.

Bacterial numbers are not affected by DU concentration, but metabolic diversity decreases within the contaminated zone. The pore water in the contaminated zone also contains high concentrations of oxalic acid indicating a significant biological response.

The microbial transformations of phosphorus

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Phosphorus (P) is an essential element for living organisms and is found in the environment in both inorganic form (as orthophosphate) and in a variety of organic compounds. It is a scarce natural resource and micro-organisms have had to develop elaborate methods for its scavenging, storage and recycling. In many terrestrial and aquatic environments P is the growth-limiting nutrient; in others, human activity has led to an excess of P and consequent problems of eutrophication and environmental damage. The Urban Wastewater Treatment Directive requires P to be removed from sewage, with a biological option regarded as preferable by industry. Paradoxically rock-P reserves are declining and possibly sufficient to last only for the remainder of this century.

The cornerstone of any biological-P removal process is the ability of micro-organisms to accumulate polyphosphate. The exact physiological function of polyphosphate remains unknown, yet a wide variety of micro-organisms are able to synthesize this biopolymer, perhaps as a response to stress. A knowledge of what triggers this accumulation (and degradation) could potentially be exploited for biological-P removal and recovery from industrial and municipal effluents. Our work has therefore focused on the links between polyP and the response of microbial cells to nutritional and environmental stimuli.

Application of Confocal Raman Microspectroscopy to the study of non-culturable micro-organisms

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Raman Microspectroscopy is a non-invasive technology to acquire characteriztic chemical information from <1 micron area of a material.

It has been applied to classify different bacterial species and characterize bacteria states, all at a single cell level. Combined with other techniques (e.g. optical tweezers, fluorescent *in situ* hybridization and stable isotope probing), Raman microspectroscopy is now developed to a valuable tool of the study and manipulation of unculturable micro-organisms which account for more than 99% microbial species in natural environment. It opens a new frontier for better understanding biodegradation of contaminants, biogeochemical cycles and discovery new enzymes and medicines.

Bioremediation: lessons to be learned from moving the laboratory findings to the field environment

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While we are very effective at carrying out bioremediation at the laboratory scale, the results at the field scale are less convincing. The reasons for this are two-fold: firstly the ideal remedial method is rarely adopted due to economic aspects, and secondly there is little consideration of experimental design and replication because we need the process to work rather than simply be evaluated. This presentation will report on three key components addressing these issues: the use of a decision support tool (DST) in remedial management, the results of on site *ex situ* remediation methods and the use of emerging technologies. The DST is an innovation that matches the specific needs of a site to current technologies (of which 30 are biologically driven). A set of case study applications will be considered and the merits evaluated. In terms of *ex situ* remediation techniques, the presentation will consider scale-up systems for biopiling and windrow for a range of hydrocarbon forms in different matrices. Finally, the concept of novel devices that remediate soil and water yet couple technology with industrial by-products will be considered together with the role of biological and chemical interfaces.

Detection of DNA adducts on soil DNA during transformation of PAHs

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PAHs e.g., benzo(a)pyrene (BaP), are common soil contaminants that are targets for microbial based bioremediation. This work aimed to develop a new technique based on detection of DNA adducts on soil DNA to provide information of potential use for bioremediation. BaP is known to be transformed to intermediates that form DNA adducts. DNA adducts are used as indicators of genetic damage; thus DNA adduct formation would provide evidence for the production of potentially toxic intermediates during bioremediation. The first stage of the work demonstrated that DNA adducts could be found in microbes exposed to BaP. Subsequent experiments using different soil types containing BaP showed that DNA adducts could be detected on DNA extracted from soil. Amounts of DNA adducts formed both varied over time and with soil type. Results from control experiments suggest that the technique is not able to directly reflect *in situ* bioavailability of BaP (or transformation products). However, this new method provides a potential way to detect mutagenic compounds in soil and to assess the outcomes of soil bioremediation.

Bioinformatic analysis of bacterial glutathione *S*-transferases in environmental soil samples

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Toxic synthetic chemicals introduced into the environment, via industry or agriculture, can penetrate ecosystems and pose a threat to all living species. One family of enzymes that plays an important role in detoxification of a variety of compounds is glutathione *S*-transferases [GSTs: EC 2.5.1.18)], ubiquitous multifunctional proteins found in almost all species from bacteria to humans. Localization of bacterial GST DNA with other genes involved in degradation of toxic pollutants, and substrate specificity analysis of expressed protein, suggest a role for bacterial GSTs in biodegradation. In this project, molecular biological and bioinformatics techniques are used to detect bacterial GST genes (known and novel) in soil samples from selected environmental sites in Ireland. Bacterial GST sequences from contaminated soil DNA samples are compared with known bacterial GST sequences with a view to identifying novel sequences that could be beneficial in detoxification of environmental pollutants. Bacterial GSTs could have potential in bioremediation of contaminated soil in the environment and could be used as bioindicators of environmental toxicity.

Using bacterial whole cell living biosensors to assess pollutant bioavailability from oil

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The availability of organic pollutants in the environment for living beings is often limited by their poor aqueous solubility, strong tendency to sorb to surfaces and, consequently, slow mass transfer rates. Because of the heterogeneity of natural environments at the microscale, bioavailability is very difficult to predict. Here we explore the problem by using living bacterial cells to monitor pollutant mass transfer rates and display their assessment by the production of autofluorescent proteins in the cell. We focused on three types of organic pollutants: short-chain linear alkanes (C6-C12), long-chain alkanes and phenanthrene. Specific bacteria which degrade those compounds were complemented with genetic circuitry to produce intracellular stable egfp or echerry in response to the pollutant. We show that such bacteria can sense different pollutant mass transfer rates, measure pollutant diffusion and differentiate contaminated soils on the basis of their available and accessible pollutant fraction.

Eukaryotic Microbiology Group Session

Nucleic acid packaging and processing

DNA organization by bacterial condensin MukBEF

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Correct folding of the chromosome into its highly ordered structure requires the activity of condensins. Condensins are highly conserved across species and contain at their core the characteriztic V-shaped dimer of SMC (structural maintenance of chromosome) proteins. The mechanism of SMCs remains under debate. Using a combination of biochemistry and DNA nanomanipulations, we investigated DNA organization by the *Escherichia coli* condensin MukBEF. We found that MukBEF acts as an ATP modulated macromolecular assembly that can bring distant DNAs into a stable complex. MukBEF binds DNA in a highly cooperative, ATP-stimulated manner producing oligomeric protein clamps. The resulting clamps are highly resilient to applied force and can form a bridge with another DNA. Both linear and circular DNA can be efficiently bridged by MukBEF. We propose that MukBEF forms a network of loosely linked clamps that divides the chromosome into a set of giant loops.

Organizing the Ter macrodomain of the *Escherichia coli* chromosome

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The organization of the *Escherichia coli* chromosome into insulated macrodomains influences both the cohesion of sister chromatids and the mobility of chromosomal DNA. Here we report that organization of the Ter macrodomain relies on the binding of a newly identified protein designated MatP to a 13 bp motif called *matS* repeated 23 times in the 800 kb-long domain. MatP accumulates in the cell as a discrete focus that colocalizes specifically with DNA of the Ter macrodomain. The effects of MatP inactivation reveal its role as main organizer of the Ter macrodomain: in the absence of MatP, segregation of Ter macrodomain occurs early in the cell cycle, no cohesion step between duplicated Ter macrodomains is observed, DNA is less compacted and the mobility of markers located in the macrodomain is increased. Mutational analysis of *cis*-acting *matS* sites indicates that MatP acts at a distance. Implications for the spatial organization of chromosomes in bacterial cells are discussed.

Nucleoid architecture in bacteria revealed by atomic force microscopy

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The cell-growth-dependent dynamic changes of the *Escherichia coli* nucleoid structure were analysed under atomic force microscopy, after successive treatments with lysozyme and detergent to partially expose the nucleoid fibres. In the log phase nucleoid, '30-nm fibres' were evident, and beaded-fibrous structures with a diameter of ~80 nm that could form higher-order structures of 'loop' were also found. In the later growth phases, the nucleoid was converted into a 'coral reef structure' with the 80-nm beaded units, and, finally, turned into a 'tightly compacted nucleoid' depending upon a nucleoid protein, Dps, and topological flexibility of DNA. RNase A degraded thicker fibres (30 nm and 80 nm wide) into thinner fibres (10 nm wide), while RNase III and RNase H degraded 80-nm fibres into 30-nm (but not 10-nm) fibres. Treatment with the transcription inhibitor, rifampicin, in the absence of RNase A changed most nucleoid fibres to 10-nm fibres. Proteinase-K treatment of nucleoids exposed DNA. Therefore, (i) the smallest structural unit is an RNase A-resistant 10-nm fibre composed of DNA and proteins, (ii) the hierarchical structure of the bacterial chromosome is controlled by transcription itself, and (iii) the formation of 80-nm fibres from 30-nm fibres requires double-stranded RNA and RNA-DNA hetero duplex.

Organization and transcription from the compact nuclear genomes of microsporidia

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The eukaryotic genome is generally large, with extensive gaps separating genes. In certain lineages, however, the genome has undergone both massive gene loss and compaction. This is seen most acutely in microsporidia parasites, which have very small genomes ranging in size from 2.3 Mb to 20 Mb. These show varying levels of genome compaction, which, in some species leaves spaces between genes as small as 4 base pairs. The effects of this extreme compaction on the process of transcription will be discussed.

Expressed sequence tag (EST) surveys and RACE analysis have been used to study the nature of mRNAs in microsporidia, and this has shown that many transcripts encode fragments of, or complete copies of several genes. These multi-gene transcripts however are not transcribed from operons. Instead they are likely the consequence of transcription promoter and termination signals being displaced from tiny intergenic regions into adjacent genes.

This overlapping transcription pattern appears to be a common consequence of severe genome compaction: Sequenced ESTs from even more highly compacted nucleomorph genomes of endosymbiotic green and red algae also possess multi-gene mRNA transcripts but at even higher frequencies.

So whilst there appears to be strong selection for compaction in these organisms with small genomes, it may have important effects on the way in which the genome functions and is transcribed.

Analysis of the dynamic genome of lager yeasts

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The genomes of lager yeast arose from the fusion of two yeast species most closely resembling *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. The resultant strains have subsequently undergone genome duplications and rearrangements leading to the generation of a complex aneuploid genome. Recombination between the two parent genomes has led to the presence of mosaic chromosomes.

The complex nature of the genomes of lager yeasts poses many interesting questions relating to genome stability and gene transcription. Using competitive genomic hybridization (CGH) analysis, we have determined the gene copy number of *S. cerevisiae*-like genes in three lager yeasts. Our analysis confirmed the aneuploid nature of the lager yeast chromosomes and identifies at a single gene resolution the regions where recombination between homeologous chromosomes

has occurred. Sequence analysis in these regions of recombination identifies the presence of novel genes in the lager yeasts and points to a mechanism for adaptive evolution in these species.

The knowledge of the copy number of *S. cerevisiae* and *S. bayanus* genes in the lager yeasts allows us to develop methods to determine the gene expression patterns from the two constituent genomes and to address questions regarding gene dosage in these complex yeasts. To examine the effects of gene dosage on the expression patterns of lager yeasts, a yeast artificial chromosome containing *S. bayanus* chromosome XVI from lager yeast was introduced into isogenic polyploid strains of *S. cerevisiae*. Analysis of the expression of the *S. bayanus* genes revealed that increasing the copy number of *S. cerevisiae* genes, caused a decrease in the expression of the *S. bayanus* homeologous gene, indicating the gene dosage effects may prevent over expression of genes in polyploid strains of yeasts.

Organization and expression of the unusual plastid and mitochondrial genomes of dinoflagellates

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Dinoflagellates are a diverse group of eukaryotic algae, most of which are photosynthetic and contain chlorophylls *a* and *c*. They are widespread and of major ecological and economic importance. For example, they grow as symbionts with corals and other organisms, but are expelled from their hosts in coral bleaching. They are also responsible for a number of toxins that can accumulate in shellfish.

Dinoflagellates have a reputation for anomalous cell biological features, such as the possession of permanently condensed chromosomes, and work over the last few years from our lab and elsewhere has shown that their organelle genomes are equally unusual. Most of the genes that are present in the chloroplast in other organisms have been lost to the nucleus in dinoflagellates, and the remaining ones are located on small plasmids, each containing at most a few genes. This organization contrasts markedly with other chloroplast genomes, where the remaining genes are on a single molecule. The dinoflagellate mitochondrial genome is also very unusual, with fragmented rRNA genes, a bewildering array of partial pseudogenes and an apparent absence of tRNA genes. We will review recent work on the organization and expression of these extraordinary genomes.

The genomic and topological organization of eukaryotic chromatin

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In vivo nucleosomes often occupy well-defined preferred positions on genomic DNA. An important question is to what extent, if any, these preferred positions are directly encoded by the DNA sequence itself. We have derived from accurately mapped *in vivo* positions a different translational positioning signal can be derived that identifies the midpoint of octamer-bound DNA. This signal corresponds to the averaged sequence organization of cloned '*in vivo*' octamer binding sequences and also correlates to ±10bp with ~75% of sampled accurately mapped positions in yeast. We conclude that in yeast the genomic code for nucleosome positioning comprises a particular pattern of DNA bending anisotropy and that nucleosome occupancy may be modulated by the overall DNA flexibility. I will also argue that a topological coupling between the wrapping of DNA around the histone octamer and the degree of coiling is fundamental to the folding and unfolding of an array of nucleosomes. This coupling determines the extent of compaction of the fibre. By varying the extent of DNA wrapping both core histone acetylation and variant core histones can directly influence the equilibrium between fibre folding and unfolding.

A newly observed S-phase checkpoint that links histone mRNA abundance with DNA replication

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The correct packaging of genomic DNA into chromosomes, allowing for appropriate gene expression and successful cell division, is essential for cell proliferation and animal development, and is dependent on the finely balanced coordination of histone protein synthesis with DNA replication. Insult to genomic DNA results in the activation of cell cycle checkpoints to allow for DNA repair prior to progression to the next cell cycle phase. In S-phase, checkpoint activation causes an inhibition of DNA replication, and this is paralleled by the down regulation of histone gene expression, to maintain the balance between DNA replication and histone synthesis. we have exposed S-phase arrested cells to the kinase inhibitors, caffeine and LY294002. This uncouples DNA replication from histone mRNA abundance, altering the efficiency of replication stress-induced histone mRNA down-regulation. RNAi-mediated interference with caffeine-sensitive checkpoint kinases ATR/ATM does not affect histone mRNA down-regulation, indicating that ATR/ATM alone cannot account for such coupling. The small molecule inhibitor LY294002 potentiates caffeine's ability to uncouple histone mRNA stabilization from replication, but only in cells containing functional DNA-activated protein kinase (DNA-PK), indicating DNA-PK is the target of LY294002. DNA-PK is activated during replication stress, and DNA-PK signaling is enhanced when ATR/ATM signaling is abrogated. Histone mRNA decay does not require Chk1/Chk2. Replication stress induces phosphorylation of UPF1, but not HBP/SLBP, at S/TQ sites, preferred substrate recognition motifs of PI3K-like kinases, indicating histone mRNA stability may be directly controlled by ATR/ATM- and DNA-PK-mediated phosphorylation of UPF1.

Factors that control the folding, stability and assembly of higher order nucleic acid structures

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Our laboratory has demonstrated that local alternations in DNA structure, such as sequence-directed curvature and single stranded breaks, can significantly alter the size and shape of particles formed when DNA is condensed from solution by multivalent cations (including peptides and non-histone proteins). These studies have informed our investigations of natural DNA packing mechanisms in viruses, sperm cells and bacterial cells. In the case of mammalian sperm cells, we have investigated the particles formed upon the mixing of DNA with isolated protamines, the small proteins that replace histones during spermatogenesis. Our results demonstrate the creation of a reconstituted sperm cell chromatin. A model for this protein–DNA complex suggests the formation of an extensive network of intermolecular protamine disulfide bonds that explain the high salt stability of mammalian sperm cell chromatin. With regards to bacterial DNA packaging, we have discovered that proteins associated with DNA in the nucleoid (HU and IHF), which do not condense DNA by themselves, can work synergistically with polyamines and crowding agents to control the morphology of condensed DNA. These results suggest a role for DNA bending proteins in controlling local DNA organization in the nucleoid of bacteria.

Centromeric heterochromatin in fission yeast

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Proper assembly and maintenance of heterochromatin is important for many aspects of genome stability, including normal chromosome segregation. A major obstacle that has prevented elucidation of the precise steps leading to establishment of heterochromatin has been the inability to dissociate the establishment of heterochromatin from its maintenance. Studies in many organisms have suggested that heterochromatin assembly can be driven by siRNA-mediated sequence specific targeting of chromatin-modifying complexes to specific loci. The best characterized of these is the RITS complex in fission yeast, which was thought to be recruited to centromeres via siRNA homology. Recently we developed a novel mutant in the RITS complex which allows us to separate requirements for heterochromatin establishment from maintenance. Using this mutant, we have shown that the histone H3 K9 methyltransferase, Clr4, is required to establish centromeric heterochromatin, whereas dicer, the enzyme responsible for siRNA generation, functions after the establishment step to maintain heterochromatin. Accordingly, we find that the Chp1 component of RITS binds K9 methylated H3 with high affinity. Our crystal structure of the Chp1 chromodomain bound to K9 methylated H3 peptide has pinpointed residues which when mutated decrease Chp1's binding affinity. Interestingly, these mutants block efficient establishment, but not maintenance, of centromeric heterochromatin.

The fission yeast HIRA histone chaperone and the maintenance of genomic integrity

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It is becoming increasingly apparent that the eukaryotic RNA polymerase II transcription machinery is promiscuous and that the integrity of chromatin is required not only to ensure the proper regulation of bona fide promoters, but also to limit transcription initiation from cryptic sites. We are currently employing the fission yeast *Schizosaccharomyces pombe* as a model system to study the role that the HIRA histone chaperone complex plays in this global regulation of gene expression. Comparison of transcript profiles in wild type and mutants cells has revealed that the HIRA complex is required to repress the transcription of numerous genes throughout the genome. In particular, the HIRA complex plays an important role in repressing genes located in subtelomeric regions. Furthermore, using strand-specific RT-PCR, we have found that levels of spurious antisense transcripts are substantially increased by mutations in genes encoding HIRA proteins. Consistent with this, loss of the HIRA complex is synthetically lethal in combination with mutation of the nuclear exosome that targets and degrades such illegitimate transcripts. Moreover, mutants defective in HIRA show increased sensitivity to agents that cause DNA double strand breaks and preliminary results suggest that this is because their genomes are more accessible to these DNA damaging agents. Our findings suggest that the HIRA histone chaperone plays an important role in maintaining the integrity of chromatin in fission yeast.

Centromeres in trypanosomes

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Centromeres are the chromosomal loci that govern segregation and are the site of kinetochore assembly. In *Trypanosoma cruzi* chromosomes, we have shown that transcriptional 'strand-switch' domains, composed predominantly of degenerate retrotransposons, are central features of regions required for mitotic stability. Etoposide-mediated topoisomerase-II cleavage, a biochemical marker for active centromeres, is concentrated at these domains. Topoisomerase-II plays a major role in chromosomal biology, and is required to resolve the centromeric DNA catenations that represent the last physical linkage between sister chromatids. In *Trypanosoma brucei*, topoisomerase-IIα activity is also focussed at single chromosomal loci which encompass regions between directional gene clusters containing transposable elements and additionally, domains composed of AT-rich repeats. RNAi-mediated knockdown of topoisomerase-IIα in bloodstream-form *T. brucei* results in the abolition of centromere-specific activity and is lethal within 48 hours. Both phenotypes can be rescued by co-expression of the *T. cruzi* enzyme. This suggests that the elements governing centromere-specific topoisomerase-II activity have been conserved within trypanosomes. The variable C-terminal domain of topoisomerase-II is thought to modulate function. We generated *T. brucei* lines expressing *T. cruzi* topoisomerase-II enzymes containing C-terminal truncations, and examined centromere-specific enzyme activity after the RNAi-mediated knockdown of endogenous topoisomerase-II. We have delineated the region necessary for nuclear localization to 6 amino acids.

Functional analysis of proteins involved in mitochondrial RNA editing in trypanosomes

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RNA editing in kinetoplastid protozoa transforms mitochondrial pre-mRNAs into translatable mRNAs by the precise insertion and deletion of uridine residues as specified by small guide (g) RNAs. It is achieved by a coordinated series of enzymatic steps that cut the pre-mRNA, add or remove uridines, and ligate the mRNA. The process is performed by the editosome, a macromolecular complex of at least 20 proteins, and additional proteins that are not stable part of the editosome. We and others have recently characterized the structure and function of the MRP1/2 complex in *Trypanosoma brucei*, which is involved in matchmaking specific guide RNA and pre-edited mRNA. Moreover, by analysing the function of TbRGG1, another protein involved in RNA editing, we have purified a novel mitochondrial protein complex, called MRB1 complex. It is composed of up to 14 subunits and its function remains unknown. Using RNA interference, we are currently investigating the role of this complex in RNA editing and guide RNA expression.

The role of F-Box proteins in the regulation of histone mRNA levels in *Saccharomyces cerevisiae*

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In lower eukaryotes, such as *Saccharomyces cerevisiae*, histone mRNA biogenesis is cell cycle regulated. The replication dependent histone mRNAs accumulate during S-phase and are rapidly degraded as the cell cycle progresses to G2-phase. Both transcriptional and post-transcriptional events contribute to the accumulation of histone mRNAs in S-phase. We are interested in understanding the underlying mechanism that control the rapid turnover of histone mRNAs as cells enter G2.

The nuclear exosome, a macromolecular complex of 3' to 5' exonucleases, is required for the post-transcriptional processing of a variety of RNAs. This complex has also been shown to play a role in the degradation of aberrantly processed mRNAs, through the action of its 3' to 5' exonucleases. We explored the possible role of the nuclear exosome in the cell cycle regulation of histone mRNAs. Using a mutant strain lacking the RRP6 gene, a component of the exosome, we have shown that histone mRNAs continue to accumulate in the S-phase of the cell cycle in the absence of Rrp6p. This appears to result from a delay in exit from S-phase in *rrp6* cells. The accumulation of HTB1 mRNA is influenced by the interaction of the nuclear exosome with the mRNA 3'-end processing machinery.

Using a three-hybrid screen, we identified a number of proteins that interact with the 3' end of the histone mRNAs. One such protein identified was Skp1 a component of the SCF complex, which plays a role in cell cycle progression through the action of ubiquitin ligases. Proteins targeted for degradation by SCF are recognized by specific F-box proteins, which are responsible for substrate recruitment. Since Skp1 appears to interact with histone mRNAs, we hypothesized that F-box proteins may play a role in regulating histone mRNA levels though its interaction with Skp1p. To examine this, 19 genes encoding putative F-box proteins have been identified; using deletion mutants of these genes we have examined both the steady state levels and the efficiency of transcription termination of the histone mRNA HTB1. Our results show that none of the mutants alter transcription termination; however the steady state levels of HTB1 mRNA are altered in several of the mutants.

Alternative RNA editing produces a novel protein involved in mitochondrial DNA maintenance in trypanosomes

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The mitochondrial genome of trypanosomes is composed of thousands of topologically interlocked circular DNA molecules that form the kinetoplast DNA (kDNA). Most genes encoded by the kDNA require a posttranscriptional modification process called RNA editing to form functional mRNAs. Here we show that alternative editing of the mitochondrial cytochrome c oxidase III (COXIII) mRNA in *Trypanosoma brucei* produces a novel DNA binding protein, alternatively edited protein-1 (AEP-1). AEP-1 localizes to the region of the cell between the kDNA and the flagellum and purifies with the tripartide attachment complex (TAC), a structure believed to physically link the kDNA and flagellar basal bodies. Expression of the DNA binding domain of AEP-1, results in aberrant kDNA structure and reduced cell growth indicating that AEP-1 is involved in the maintenance of the kDNA. Perhaps most important, our studies show a gain of function through an alternatively edited mRNA and for the first time, provide a link between the unusual structure of the kDNA and RNA editing in trypanosome mitochondria.

Organization and replication of trypanosome kinetoplast DNA as studied using various electron microscopy approaches

Eva Gluenz

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Kinetoplastid mitochondrial DNA (kDNA) is organized into a network of concatenated mini and maxicircles, forming a complex structure termed kinetoplast, positioned at the base of the flagellum to which it is physically attached. To understand fully the mechanisms of kDNA replication and segregation, there is a need to develop descriptions of kinetoplast nucleic acid and protein component locations at the electron microscopic level of resolution and determine their 3D spatial positioning during the kDNA replication cycle. We have used a number of different electron microscopy approaches to define structural and functional domains involved in replication and segregation of the *Trypanosoma brucei* kinetoplast. Using electron microscope cytochemistry we discovered a hitherto unrecognized structure containing DNA and basic proteins that links the kDNA discs during their segregation and is maintained between them for an extended period of the cell cycle. Serial section reconstruction was used to determine the 3D spatial positioning of the kDNA and associated filaments during successive stages of kinetoplast segregation. These studies provide new insights into the final stages of kinetoplast replication and organelle positioning. Analysis of mutant phenotypes generated by RNAi knockdown of kDNA replication proteins are used to test these models.

Histone modifications recruit Rad9 to uncapped telomeres

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The telomere is a specialized DNA protein complex that regulates the elongation and protection of DNA at the end of linear chromosomes. Proteins such as Cdc13 bind to the end of chromosomes forming a cap. Temperature sensitive *cdc13-1 Saccharomyces cerevisiae* arrest at the G2/M phase checkpoint when grown at temperatures of 28°C and above. The growth of *cdc13-1* mutants can be rescued by inactivating genes involved in signalling DNA damage such as *RAD9*. Transient phosphorylation of histone H2A at serine 129 is one of the earliest events occuring after DNA damage whilst methylation of histone H3 at lysine 79 by Dot1 is constitutive. Our experiments show that mutation of serine 129 to a non-phosphorylatable alanine residue and inactivation of the histone methyltransferase *DOT1* both partially rescue the temperature sensitivity of the *cdc13-1* mutant by affecting the integrity of the G2/M phase checkpoint. We are testing the hypothesis that H2A S129 phosphorylation and Dot1 dependant histone methylation independently recruit the checkpoint protein Rad9 to uncapped telomeres.

Chromatin and the response to DNA damage

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In eukaryotes, genomic DNA is packaged into the nucleus primarily by association with histone proteins to form chromatin. This structure, while necessary for compaction and chromosome segregation, is inhibitory to processes that require access to DNA, such as transcription, replication and repair. For this reason, cells have two powerful mechanisms for manipulating the structure of chromatin; covalent modification of histones and ATP-dependent chromatin remodelling activities. We and others have identified multiple chromatin modifying activities involved in DNA double-strand break repair. We have recently focused on the role of the RSC chromatin remodelling complex in DNA damage responses. RSC accumulates at a DNA break very quickly and remodels the nucleosomes adjacent to the lesion. This activity helps to facilitate a second chromatin modification in response to DNA damage; the phosphorylation of histone H2A by the DNA damage activated kinases Mec1 and Tel1. Interestingly, we find there is specificity to the activity of the complex depending on the type of DNA damage that is sustained. The response to DNA damage is critical to the maintenance of genomic integrity and the prevention of tumourigenesis. Because these activities are highly conserved, these studies may have implications for understanding the development and progression of cancer.

Epigenetic regulation of gene expression and genomic integrity

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Chromatin assembly and disassembly has long been acknowledged to accompany the process of DNA replication. Accordingly, we previously discovered a key histone chaperone involved in the assembly of chromatin during DNA replication, termed Asf1 (Tyler *et al.*, *Nature* 1999). It appeared likely, but unproven, that chromatin would also need to be disassembled to enable DNA repair and transcription to occur, and then reassembled after these processes are complete. By continuing our analysis of the defects of yeast that lack specific histone chaperones, we have found that disassembly of chromatin from promoter regions is a broadly-utilized mechanism for the regulation of Eukaryotic activation, while chromatin reassembly onto promoters is required for transcriptional repression (Adkins *et al.*, *Molecular Cell*, 2004; Adkins & Tyler, *Molecular Cell*, 2006). We will present our most recent mechanistic insights into these chromatin dynamics. We

will also present data demonstrating that chromatin is also disassembled and re-assembled during double-strand DNA repair, and the influence of these chromatin dynamics on cell survival and ageing.

Neocentromere formation in *Candida albicans*

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C. albicans has small regional centromeres (*CENs)*. We found that DNA that normally provides *CEN* function was not necessary for centromere function: the Δ*CEN5::URA3* strains exhibited ~wt levels of stability, implying that a neocentromere (functional centromere associated with new DNA) formed elsewhere on the chromosome. Selection on 5-FOA, which kills cells expressing *URA3*, yielded Δ*CEN5::URA3* strains that formed colonies on both 5-FOA and SD-Uri and the FOA^R strains could subsequently grow on SD-Uri and vice versa. Southern blot analysis and RT-PCR data indicate that *URA3* is present, but silenced, on Chr5 in FOA^R cells. Chromatin-immunoprecipitation with antibody to CENPA in Δ*CEN5::URA3* strains revealed that the *neoCEN* position was dependent upon the status of *URA3:* CENPA was associated with (and presumably silenced) the *URA3* DNA in cells growing on 5-FOA; in Ura+ cells, CENPA was associated with DNA adjacent to, but not on, *URA3*. This implies that the neocentromere can transiently associate with, and therebyt silence, different DNA sequences, depending upon the selective growth conditions. Thus, *C. albicans* provides a very simple model for studying the function of regional centromeres and the positioning of neocentromeres.

Acetylation of histone H3 lysine 56 preserves genome stability through the regulation of DNA replication coupled nucleosome assembly

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Acetylation of histone H3 lysine 56 (H3K56Ac) marks newly synthesized H3 molecules and is critical to maintain genome integrity during DNA replication. Cells lacking H3K56Ac due to mutations at lysine 56 or the histone acetyltransferase Rtt109, which we have identified, are highly sensitive to DNA damage agents and exhibit spontaneous chromosome breaks. However, the molecular mechanisms by which this modification contributes to genome stability are not known. In the present studies, I will present experimental evidence supporting a model that H3K56Ac regulates DNA replication coupled nucleosome assembly and thereby maintain genome integrity.

Fermentation & Bioprocessing Group sponsored by Bioscience for Business Knowledge Transfer Network

Fuels and chemicals from renewable feedstocks

Renewable chemicals: a fundamental shift and a new opportunity

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Concerns about future availability of current fossil carbon based feedstocks, and the environmental impact of many common materials and processes, are pushing us towards the development of an industry based on renewable feedstocks. And that means chemicals derived from plants. Bio-based feedstocks coupled with physical, chemical and biological processing.

This is a fundamental shift. Plant derived feedstocks are completely different to petrochemical feedstocks in physical form and chemical structure. They will require new chemical and biochemical transformations, new processes, and will produce new and unfamiliar chemicals. We will need to learn how to build these into products and how to produce new products to meet our application needs. Many areas of industrial chemistry will change dramatically.

The reshaping of the chemical and chemical using industries is also an opportunity. We can learn a lot from those existing industry sectors that already successfully use plant based materials about the specific challenges and the commercially successful strategies. Products from plant feedstocks are already competing effectively with petrochemicals, and the range of materials and applications will only increase with time.

Development of a second generation bioethanol process using *TM242* – a thermophilic bacillus

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A major obstacle to developing a commercially viable, second generation, bioethanol process utilizing cellulosic feedstocks is to find an ethanologenic micro-organism able to efficiently convert the sugars from biomass to ethanol. TMO Renewables Ltd has developed such a strain TM242, a thermophilic bacillus capable of efficiently fermenting monomeric & oligomeric, C6 & C5 sugars, to ethanol at high yields. Additionally the strain's ability to grow at high temperatures offers a range of process advantages including rapid feedstock conversion, low enzyme requirements for biomass hydrolysis and low microbial contamination, and hence lower capital and operating requirements.

Early commercial openings for this technology will be with existing corn to ethanol producers, with the addition of a side-door plants adding value by fermenting the residual sugars present in distiller's grain or fibre fraction side products to ethanol, so increasing plant ethanol yields by 10 to 15%. The complete TMO process is outlined from pre-treatment & hydrolysis through to fermentation & the ability of TM242 to realise a commercially viable cellulosic ethanol process is illustrated on distiller's grains.

Wastes into energy: an integrated system for the hydrogen economy

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Tomorrow's clean energy will depend heavily on hydrogen as an energy vector because the only product of its combustion is water. H_2 can be burnt, or used in a fuel cell to generate electricity. Fuel cells contain precious metal (PM) catalysts. Like oil, the price of PMs is rising sharply and this will impact on the future hydrogen economy.

Much of the global supply of PMs is being used in automotive catalysts for control of atmospheric pollution and metal recycle lags behind consumption. At the same time increasingly stringent controls on disposal of waste to landfill are prompting the search for ways to use food wastes as resources. Bacteria can make hydrogen from organic wastes by various fermentative methods. No one organism can achieve total conversion of sugar to H_2. By coupling a dark fermentation and a photofermentation it is possible to achieve this goal. The 'biohydrogen' is clean enough to use directly in a fuel cell. The waste biomass can be used to recover PMs from metallic wastes and scrap. The metallized biomass is active in the generation of electricity in a fuel cell run using the biohydrogen. Hence, electricity is made using wastes from the food processing and automotive industries. The only waste is CO_2 but the process is, overall, carbon neutral. CO_2 can be trapped and turned into high value product to achieve a positive carbon balance.

Acetone-butanol fermentation revisited

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The genus *Clostridium* is one of the largest prokaryotic genera, and as a group exhibit extreme biocatalytic diversity. Over the last century, this biodiversity has been much fated by the industrial biotechnologist. Nowhere is this more apparent than in the case of the solventogenic clostridia. Indeed, the production of acetone-butanol (AB) by *C. acetobutylicum* was one of the first large-scale industrial processes to be developed, and in the first part of the century ranked second in importance only to ethanol production (Jones DT & Woods DR, 1986, *Microbiol Rev* 50: 484–524). Since its development, however, its fortunes have shown considerable fluctuations. From the peak of activity between the 1st and 2nd world wars, there was a steep decline as new technologies made it more economic to produce these chemicals from fossil fuels. Interest was briefly revived in the wake of the oil crisis of 1973–74, and then waned. In recent years, with current concerns over global warming and severe rises in the costs of crude oil, there has been resurgence in interest. Still, the greatest obstacle to the improvement of biobutanol production through metabolic engineering is the limited genetic amenability of the solvent-producing clostridia. Developments made at Nottingham, and in particular ClosTron technology, suggest that this hurdle to progress has now been removed.

The authors wish to acknowledge the financial support of SysMO (project number 2; www.sysmo.net), the BBSRC (BB/F003390/1, BB/D522289/1, BB/D001498/1 and BB/E021271/1), Morvus Technology Ltd and TMO Renewables Ltd.

Development of Biobutanol

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DuPont Biofuels, Delaware, USA

Abstract not received

Synthetic biology for advanced biofuels

J.D. Keasling

University of California, Berkeley, USA

Abstract not received

Corn, kernels, catalysis(bio) and chemicals (phenolic biorenewables)

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Corn kernels contain nearly 70% of starch, an abundant source of glucose that has been widely used for commodity level production of lactic acid, high-fructose syrups, ethanol, and other products obtained by fermentation or large-scale biocatalysis. Less attention has been afforded to other chemical components found in corn such as lignins and lipids. This presentation will review past and present opportunities for biocatalysis to produce value added chemicals from corn. Major emphasis will be on biotransformations of ferulic acid found at 2–4% w/w in corn kernel hulls in almost pure form. This phenolic cinnamic acid derivative and the closely related *p*-coumaric acid occur together in varying proportions in other parts of the corn plant. The nature of ferulate in corn, major ferulic acid biotransformation pathways, micro-organisms and enzymes involved in biocatalysis will be discussed. The source of numerous phenolic products found in the Distillers Dried Grains with Solubles (DDGS) relates to biotransformations occurring during dry milling ethanol fermentation. Bioorganic and mechanistic features of enzymatic decarboxylation, β-oxidation, styrene hydration, and carboxylic acid reduction will be described as will properties of the novel biocatalyst, carboxylic acid reductase and its applications to reductions of ferulic acid, vanillic acid, and fatty acids.

Artificial metalloenzymes based on the biotin-avidin technology

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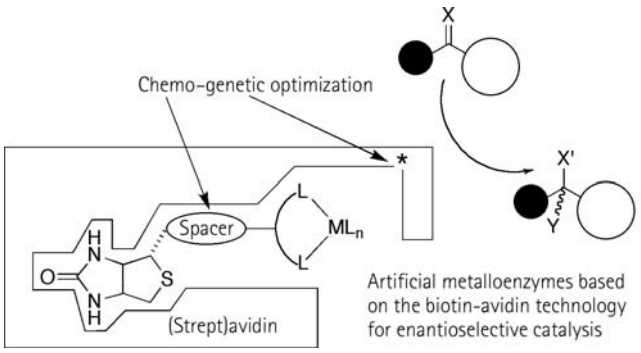
Artificial metalloenzymes can be created by introducing a catalytically active achiral organometallic moiety within a host protein. For this purpose, we rely on biotin-avidin technology to anchor non-covalently a biotinylated moiety within (strept)avidin.

As a starting point, this talk will present the structural characterization of an artificial transfer hydrogenase based on the biotin-avidin technology. Having identified close contacts between the streptavidin host protein and the biotinylated ruthenium piano stool moiety, we present a designed evolution protocol for the chemogenetic optimization of the performance of these hybrid catalysts.

This chemogenetic optimization strategy can be extended to other artificial metallenzymes for the hydrogenation of dehydroaminoacids, oxidation- and C-C bond forming reactions. These reactions are used to present relevant kinetic-, thermodynamic- and statistical modelling aspects.

It thus appears that artificial metalloenzymes based on the biotin-avidin technology combine attractive features of both homogeneous- and enzymatic catalysis.



Food & Beverages Group Session

Microbial adhesion to food matrices

Bacterial persistence in food processing environments with particular reference to *Listeria monocytogenes*

Brigitte Carpentier

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Cleaning and disinfection of open surfaces in food industry premises leave micro-organisms behind; these build up a persistent microbiota on the surfaces. Among non-pathogenic micro-organisms *Pseudomonas* and coagulase negative *Staphylococcus* species are some of the most frequently found. Some pathogens are also known to be able to persist in processing sites, *Listeria monocytogenes* being one of the most famous one. Experimental persistence of bacteria, in conditions as close as possible as those encountered in a food processing site, needs a minimum initial bacterial load which is quite different depending on the species considered. The second condition allowing persistence is that growth must be greater than reduction obtained by cleaning and disinfection. The analysis of the literature on the factors impacting on *L. monocytogenes* attachment to surfaces and on the conditions where *L. monocytogenes* is most prone to be found in food processing environment strongly suggests that acting towards decreasing attachment would be far less efficient than acting towards reducing its growth potential.

Attachment of bacteria in the meat processing environment

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Listeria monocytogenes is a significant contamination problem in processed food products and can persist in the production environment acting as a source of contamination. This persistence may be related to biofilm production as well as desiccation resistance. We have examined the influence of food components on *Listeria* attachment to food processing surfaces. *Pseudomonas fluorescens* is a common spoilage organism of raw and cooked meats; in dual culture with *Listeria* the production of *Listeria* biofilm on stainless steel surfaces was impaired. However *Listeria* showed improved attachment to pre-formed *Pseudomonas* biofilms. To examine the influence of the food itself, cooked meat juices from beef, pork, lamb, chicken and duck were prepared and attachment of three *Listeria* isolates, one *L. innocua* and two *L. monocytogenes*, to stainless steel coupons in their presence was investigated at 5 and 25°C. Significant differences were seen between attachment in the presence of different juices and differing temperatures with duck at 25°C allowing high levels of attachment. *L. monocytogenes* grown in minimal medium with duck juice supplement showed a higher cell hydrophobicity. The differences in attachment seen when cultured in different meat juices could give an indication that different cleaning and sanitizing procedures are needed according to the meat product being processed.

Investigation of key factors influencing the surface properties of lactic acid bacteria

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Cell surface molecules play an important role in the interactions between probiotic micro-organisms and the host. In this study, we investigated the surface properties of *Lactobacillus rhamnosus GG* cells taken at different points in the growth curve. The ability of the cells to adhere to Caco-2 cells was also studied in an effort to establish the correlation between surface properties and adhesion. A combination of physicochemical analyses on the cell surface was conducted to measure the cell hydrophobicity and charge, and analyse the surface proteins. It was observed that the hydrophobicity increased considerably as the cell growth progressed; it reached a maximum at the early stationary phase, after which it decreased. In addition, the cells from early stationary phase exhibited lower levels of proteins compared to the cells from the exponential phase. Overall, the results indicated that during the exponential phase small size hydrophilic proteins and negatively charged polysaccharides were most likely present in the surface. In contrast, in the early stationary phase cells negatively charged hydrophobic non-proteinaceous substances, most likely lipo–teichoic acid, dominated the surface. Treatment with various proteases and LiCl verified the little contribution of surface proteins to the overall surface properties. The adhesion of *Lactobacillus GG* to Caco-2 cells varied, depending on the status of the bacterial cells; the highest was for cells from early stationary phase. The results indicated that the level of adhesion to Caco-2 cells is linked to the hydrophobic nature of the cell surface and its chemical composition.

Effect of organic food soil on surface hygiene and cleanability

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The presence of food soil and residues of cleaning product alongside micro-organisms on surfaces may well affect subsequent cleanability and soiling, and thus hygienic status. We have demonstrated during repeated soiling and cleaning of hygienic food contact surfaces, using a micro-organism-food soil mix, that micro-organisms are removed during cleaning more easily that the food material: indeed, whilst no accumulation of micro-organisms is apparent, organic material seems to build up on the surface. This food soil may protect the micro-organisms, provide some element of nutrient, and interfere with the activity of cleaning and disinfection treatments – thus a knowledge of its behaviour on the surface is important in food hygiene.

A range of analytical techniques have been used in order to differentiate soil from cells. The most simple is differential staining, using fluorescent stains. Other more sophisticated techniques have been used to compare the behaviour of different soil components on surfaces, and to evaluate the sensitivity of the detection techniques.

One aim of this work is to enable the development of methods which will provide a more realistic, yet rigorous and reproducible *in vitro* simulation of fouling and cleaning *in situ*.

Knowledge of the behaviour of food soil on surfaces will potentially inform on the subsequent behaviour of micro-organisms associated with/attached to this soil, and on selection of the most appropriate cleaning and disinfection regime.

The effect of functional materials on microbial attachment in breweries

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Material science has lately introduced coating materials that could be useful for the hygiene management in the brewing industry. We studied photocatalytic TiO_2 coated steel on brewery filler surfaces for up to 18 months on 3 bottling lines, and found out that they reduced the total microbial load on the filler surfaces on average 90% for 6 months. An obstacle in the use of photocatalytic materials is the requirement of UV light to activate the surfaces. Combining antimicrobial metals with photocatalytic surfaces improves their action. By adding a low concentration of silver ions to TiO_2 surfaces we were

able to reduce the total microbial load 99–99.9% in production environment, where the illumination is based on day light and luminescent lamps. The antimicrobial effect of surfaces, however, did not last for 18 months. Material characterizations revealed that surfaces were covered by Si-O containing precipitates, which probably decreased photocatalytic activity. It was also seen that silver ions had dissolved from the surfaces. These problems may be overcome by eliminating the formation of process based inorganic films and further surface development. Functional surfaces at critical sites of the process could reduce the attachment of micro-organisms and facilitate their removal in cleaning operations.

The attachment of brewing yeast to glass surfaces

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In most circumstances production environments such as a brewery the formation of microbial biofilms is undesirable and associated with severe risks to product quality and integrity. However, in the case of bottle-conditioned beers the formation of a stable biofilm of brewing yeast is essential both to the maturation of the product and to its successful dispense. Bottle-conditioned ales are those in which at packaging the beer contains fermentable sugar, a limited supply of oxygen and a low concentration of suspended yeast cells. In the subsequent maturation phase the yeast multiplies at the expense of the oxygen and sugar. The products are carbon dioxide, various flavour-active metabolites and an increase in biomass. The beer is ready for consumption when this secondary fermentation is completed and the yeast forms a compact sediment in the bottle which is not disrupted during dispense. The formation of the sediment involves interactions between individual yeast cells and between yeast cells and the glass surface of the bottle. It is known that some brewing yeast strains are better able to form stable sediments than others. Here is described the mechanisms which underlie the formation of stable yeast sediments and an explanation is provided which explains strain-specific differences.

The effect of surface topography on the retention of food soil and micro-organisms

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In the food industry surfaces must be easy to clean. Wear of food contact surfaces through abrasion, cleaning and impact damage increases the surface roughness, thus can introduce topographical features which may increase the retention of both organic soil and micro-organisms. The presence of organic soil on the surface may affect the hygienic status of the surface by providing protection for the micro-organisms and interfering with disinfection protocols. One approach to reduce bacterial colonization of surfaces is to modify the surface topography or chemistry.

The aim of this study is to specify the linear topographical features which affect microbial retention, in order to minimize fouling and enhance cleanability. Surfaces with defined linear features of the magnitude of scratches and finish lines (diameter 1 micron and below) encountered *in situ* were fabricated. These surfaces were coated in titanium using PVD magnetron sputtering, which provided a uniform surface chemistry, enabling comparisons between surface topographies to be carried out. Retention assays were performed using *Staphylococcus sciuri* and *Escherichia coli*.

Using atomic force microscopy (AFM), it was shown that interactions between cell shape and surface feature size affected the force required to remove cells. In order to model realistic situations the AFM was used to assess the removal force in the presence of an organic food soil. *E. coli* was retained poorly on titanium coated surfaces without the organic food soil. The results showed differing levels of retention dependant on the topography of the surface and the presence of an organic food soil. The results of this work could be applied to the design of hygienic surfaces in order to minimize fouling and enhance cleanability.

Adhesion of human enteric pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* to salad leaves

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Contaminated salad leaves have emerged as important vehicles for the transmission of enteric pathogens to humans. For example, in the USA recent outbreaks of *E. coli* O157:H7 (EHEC) have been traced to consumption of contaminated pre-washed spinach, while in the UK, infections with *Salmonella enterica* serovar Senftenberg resulted from the consumption of contaminated basil. These outbreaks prompted us to investigate the mechanisms employed by these bacteria to adhere to the epidermis of salad leaves. We found that EHEC strains attach to leaves via a filamentous type III secretion system that is also involved in attachment to mammalian enterocytes. In contrast, *Salmonella enterica* serovars Senftenberg and Typhimurium adhere to fresh basil and salad leaves mainly via flagella. Mutant strains defective in FliC adhered at reduced levels. These results show that enteric pathogens use similar 'virulence factors' to colonize the gut mucosa and the leaf epidermis.

Plant genetic factors affecting microbial colonization of lettuce leaves

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Recent outbreaks of food poisoning have been associated with contamination of cut-salad produce, with the *Enterobacteriaceae* and *Listeriaceae* being implicated in many cases.

These human pathogens, however, will be part of the large and diverse naturally occurring microbial communities which the phyllosphere supports. Although environmental factors are known to influence the structure of these phyllosphere communities, much less is known about the effect of plant phenotypic factors and the underlying genotypes on community composition and dynamics.

16S rRNA Terminal Restriction Fragment Length Polymorphism profiles of the microbial populations occurring naturally on the phyllosphere of a genetically diverse range of field grown lettuce lines were compared at 4-weeks post emergence and at a mature head stage. Clone library data was used to investigate the structure of the populations occurring on three lines (the parents of two lettuce genetic mapping populations) in greater detail. Evidence was found for subtle plant genotype effects on natural microbial populations, particularly in respect of the structure and diversity of the family *Enterobacteriaceae*.

Tentative route of plant infection by human pathogenic *Salmonella* serovars

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The availability of knowledge of the route of infection and critical plant and microbe factors influencing the colonization efficiency of plants by human pathogenic bacteria is essential for the design of preventive strategies to maintain safe food. In this study the physiological and molecular interaction between *S. enterica* serovars and lettuce was evaluated. Investigation of the localization of *S. enterica* on/in lettuce plants revealed the presence of significant populations on the surface and inside the plants. In addition, differential interaction of *S. enterica* serovars with lettuce cultivars was found in terms of prevalence and degree of endophytic colonization of lettuce. Further studies provided clear differential gene expression profiles

between non-colonized and colonized lettuce plants based on transcriptome analysis by cDNA-AFLP. Functional grouping of the expressed genes indicated a correlation between colonization of the plants and an increase in expressed pathogenicity-related genes. Moreover, chemotaxis experiments revealed that *S. enterica* serovars actively move towards root exudates of lettuce. Subsequent micro-array analysis identified genes of *S. enterica* that were activated by the root exudates of lettuce. A sugar-like carbon source was correlated with chemotaxis, while also pathogenicity-related genes were induced in presence of the root exudates. The latter revealed that *S. enterica* is conditioned for host cell attachment during chemotaxis by these root exudates. In summary a tentative route of infection is presented that includes plant-microbe factors, herewith enabling further design of preventive strategies.

Binding of *Escherichia coli* and commensal bacteria to insoluble plantain non-starch polysaccharide

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Inflammatory Bowel Diseases (IBD) are becoming increasingly prevalent in westernized countries and dysbiosis of commensal gut bacteria has been partly implicated in disease formation. Soluble polysaccharide from plantain may potentially confer health benefits to IBD sufferers by manipulating the gut microbiota.

The binding capacity of bacterial strains was assessed following incubation with insoluble plantain fibres for 6 hours. Fibres were then extensively washed, fixed for fluorescent hybridization and the fibre-bacterial complex viewed using fluorescence microscopy. All strains tested were able to bind to insoluble plantain, with extremely high numbers of some *E. coli* strains binding.

Commensal *Bacteroides* strains, *E. coli* strains present in the ECOR collection and some *E. coli* isolates from IBD patients were able to grow on the soluble fraction of plantain. However, the ability to bind to the insoluble fraction was independent of the ability to use the soluble fraction for growth.

Other commensal bacterial strains that had been selected by their ability to grow on soluble plantain were not able to bind to the fibres as efficiently as *E. coli*. This binding to, and growth on, plantain may selectively alter the composition of the gut microbiota.

Physiology, Biochemistry & Molecular Genetics Group / Irish Branch Joint Session

Sealed membranes; the structural basis of transport and energetic processes

Ktn domains: flexible regulators of transport and metabolism

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Ktn domains are ubiquitous among potassium transporters and channels and exert regulation over the activity of the membrane complex. A number of crystal structures have been obtained for these domains, but the precise identity of the co-regulators is poorly understood. We will review the complex literature to provide delegates with an overview of the flexibility of this domain in providing regulatory switching in a range of important biological systems. We will describe in detail the most detailed of these systems KefC in *E. coli* an electrophile-regulated potassium efflux system. The system is maintained closed by the binding of glutathione and is activated by the formation of glutathione adducts. The system functions to regulate K^+ flow in response to potential cell damage by electrophiles leading to a lowering of cytoplasmic pH. We will present the latest insights into the regulation of the system based on genetic and structural studies.

Structure of a 6 TM potassium channel

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The 6 TM tetrameric cation channels form the largest ion channel family, some members of which are voltage-gated and others are not. There are no reported channel structures to match the wealth of functional data on the non voltage-gated members. We have determined the structure of the trans-membrane regions of the bacterial cyclic nucleotide regulated channel MlotiK1, a non-voltage gated 6 TM channel. The structure shows how the S1-S4 domain and its associated linker can serve as a clamp to constrain the pore's gate and possibly function in concert with ligand binding domains to regulate the opening the pore. In particular we hypothesize a new gating mechanism in 6 TM channels by which reversible conversion of the 4th TM from an α-helical to a 3(10)-helical conformation results in opening and closing of the channel gate.

Function and mechanism of the novel TRAP family of membrane transport proteins

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Tripartite ATP-independent periplasmic (TRAP) transporters are novel solute transporters that are widespread in prokaryotes. They consist of an extracytoplasmic solute receptor (ESR) which is located in the periplasm of Gram-negative bacteria or tethered with a lipid anchor in Gram-positive bacteria, and two unequally sized integral membrane components, a large and small membrane component, with 12 and 4 transmembrane (TM) domains, respectively. A rapidly expanding body of work on the ESR components is being produced from a number of TRAP transporters from different organisms, but the integral membrane components and the mechanism of transport is a relatively unstudied area.

The integral membrane component and ESR of a TRAP transporter from *Haemophilus influenzae* have been overexpressed and purified to homogeneity. The entire system has been reconstituted into liposomes and Na^+-dependent uptake of substrate has been observed.

Ammonia channels from bacteria to man

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The movement of ammonium across biological membranes is a fundamental process for nearly all living organisms and is mediated by a conserved family of membrane channel proteins known as the Amt (ammonium transport) family. Amt proteins are ubiquitous in archaea, eubacteria, fungi and plants, whilst in animals, including humans, they are represented by the closely related Rhesus proteins.

We have developed the AmtB protein from *Escherichia coli* as a model system for the analysis of ammonium channel proteins. A combination of genetic, biochemical and X-ray structural studies is now allowing us to begin to understand the mode of action of Amt proteins. Similar approaches have also elucidated the mechanism whereby the flux of ammonia through bacterial Amt proteins is regulated by complex formation with the cytoplasmic signal transduction protein GlnK. The interaction of these two proteins allows integration of extracellular ammonium availability with intracellular nitrogen metabolism in bacteria. Recent genome sequencing projects have also revealed that bacteria occasionally encode members of the Rhesus protein family and our solution of the structure of one such protein has shed new light on these clinically important proteins.

Structure and mechanism of ABC transporters

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ABC transporters are ubiquitous membrane proteins that couple hydrolysis of ATP to the translocation of diverse substrates across cellular membranes. Whereas ABC importers (only present in prokaryotes) catalyze the uptake of essential nutrients from the environment, ABC exporters facilitate the extrusion of various compounds, including drugs and antibiotics, from the cytoplasm. ABC transporters share a conserved architecture: two nucleotide-binding domains (NBDs) hydrolyze ATP, while two transmembrane domains (TMDs) provide a pathway for the substrate.

Recent crystal structures of full ABC transporters suggest that a key step of the molecular mechanism is conserved in importers and exporters. Binding of ATP appears to promote an outward-facing conformation, whereas the release of the hydrolysis products ADP and phosphate promotes an inward-facing conformation. This basic two-state scheme can in principle explain ATP-driven drug export and binding protein-dependent nutrient uptake.

Communication mechanisms between nascent polypeptides and the ribosome

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The targeting of membrane proteins is a challenging cellular process in which highly hydrophobic transmembrane domains must remain in an insertion competent conformation while they are transferred from the

ribosome to the lipid bilayer through the aqueous milieu of the cell. This process is universally controlled by the signal recognition particle (SRP), which docks at the ribosome exit tunnel sequestering signal sequences as they emerge and targeting them to the membrane insertion machinery. In mammalian cells this process is stringently controlled and both membrane and secretory proteins are targeted co-translationally by SRP to the Sec61 complex for insertion or translocation respectively. However in bacteria SRP recognizes only membrane proteins, some of which are thought to be targeted co-translationally and some post-translationally. In addition bacteria have developed numerous ways of inserting membrane proteins utilizing both the SecY and YidC proteins in the membrane. Therefore despite considerable conservation in the structures of SRP and the ribosome between eukaryotes and prokaryotes it appears that the early stages of targeting dictate quite different mechanisms for membrane protein integration. This presentation will discuss the comparative communication mechanisms used between the ribosome and the nascent peptide to affect membrane protein targeting in eukaryotes and bacteria.

Biosynthesis of complex bacterial respiratory enzymes

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Many prokaryotes have a remarkable respiratory flexibility being able to use a vast array of different organic and inorganic compounds to obtain energy for growth. The facultative anaerobe *Escherichia coli* produces enzymes that allow the use of, amongst other things, nitrate, nitrite, trimethylamine *N*-oxide (TMAO) and dimethyl sulfoxide (DMSO) as alternatives to oxygen as electron acceptors and molecular hydrogen as an electron donor. The enzymes behind this respiratory flexibility are amongst the most complex of proteins, being frequently comprised of multiple subunits, containing multiple prosthetic groups, and are often membrane-bound. Indeed, respiratory electron transport chains usually have enzyme components localized on both sides of the cytoplasmic membrane and the bacterial cell therefore faces a basic problem in how to target complex, cofactor-containing proteins to the outside of the cell. The broad aim of this research is to understand at the molecular level the cellular processes that govern cofactor insertion, protein folding, and oligomerization of complex enzymes, and the processes that co-ordinate those assembly events with protein targeting pathways. In many cases the assembly of complex enzymes is governed by dedicated accessory proteins, or 'chaperones', that recognize and bind to only one particular enzyme. The structure and function of two such chaperones, TorD and NapD, will be discussed.

Where does this bit go? Piecing together the structure of bacterial tripartite efflux pumps

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Drug efflux in Gram-negative bacteria is carried out by tripartite pumps. These consist of a trimeric inner membrane H+ antiporter (such as AcrB in *Escherichia coli*) coupled to the periplasmic domain of an outer membrane exit duct (represented by TolC in *E. coli*). The coupling together of these inner and outer membrane components is due to an elongated flexible adaptor protein (AcrA in the *E. coli* system).

The adaptor is a multi-domain protein with a central helical hairpin that has a structural similarity to the lower region of the TolC helical periplasmic domain. Site specific crosslinking *in vivo* has confirmed that the helical domains of TolC and the adaptor interact (Lobedanz *et al.* 2007, *PNAS* 100 4612–4617). This suggests that the other domains of the adaptor bind to the surface of the inner membrane antiporter.

Only two of the other three adaptor domains have been ordered sufficiently in crystals for structural solution up to now. But we have recently determined a low resolution structure for the missing domain. This completes the adaptor structure and, using experimentally determined crosslinks from AcrA to AcrB, we can assemble together these inner membrane associated structures.

Combining this with our previous results on the helical interface with TolC, produces the first complete, experimentally verified, model for the arrangement of the subunits of the tripartite pump. The adaptor hairpin domain locates the TolC in register with the top of the AcrB antiporter. The lower domains of the adaptor are positioned adjacent to the regions of AcrB that are believed to participate in its peristaltic pumping mechanism – suggesting that they may also be involved in this function.

Unravelling the structure of the water splitting site of photosynthesis and implication for mechanism of catalysis

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Photosystem II (PSII) is a multi-subunit membrane protein complex which catalyses the splitting of water to molecular oxygen and reducing equivalents. The reaction occurs at a catalytic centre composed of 4 Mn ions and a Ca ion, is thermodynamically demanding and generates highly oxidized species. It is this fundamental reaction that has given our planet its aerobic atmosphere while at the same time providing the reducing potential to convert carbon dioxide into the organic molecules of life. Thus understanding the molecular mechanisms of this reaction is of considerable importance. To this end structural biology has contributed enormously. Initially the positioning of various protein subunits and their transmembrane helices were determined by electron microscopy (Barber 2003). This was followed by the elucidation of a refined structure of the cyanobacterial PSII by X-ray crystallography (Ferreira et al 2004) giving details of specific environments of the redox active cofactors. The implications of these and more recent structural studies, will be discussed in relation to the unique facets of PSII function, particularly the water splitting reaction.

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What X-ray structures of components of transhydrogenase tell us about the mechanism of proton pumping

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Transhydrogenase is found in the inner membrane of animal mitochondria and the cytoplasmic membrane of many bacteria. It couples the transfer of hydride-ion equivalents between NADH and $NADP^+$ to the translocation of protons across the membrane.

$$NADH + NADP^+ + H^+_{out} \leftrightarrow NAD^+ + NADPH + H^+_{in}$$

The enzyme has three components: dI, which binds NADH, and dIII, which binds $NADP^+$, protrude from the membrane, and dII spans the membrane. There is a number of X-ray structures of isolated dI and isolated dIII from several species, and of the dI_2dIII_1 complex of *Rhodospirillum rubrum* transhydrogenase. We have solved the structure of this complex loaded with $NADP^+$ and a redox-inactive NADH analogue (H_2NADH). At the dI/dIII interface the nicotinamide rings of the two nucleotides lie in apposition; their organization resembles that of a low-energy transition state for hydride transfer which would lead to the experimentally observed stereochemistry. However, in the dI component which does not have a dIII partner the nicotinamide ring of the H_2NADH has a different conformation. We propose that movement of the nicotinamide ring driven by conformational changes in a local polypeptide loop is responsible for gating the hydride-transfer step and is coupled to proton translocation through dII.

Electron transport to the microbe-mineral interface: inner-, inter- and outer-membrane electron transfer

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Bacteria of the genus *Shewanella* are notable for their ability to respire in anoxic environments utilizing insoluble minerals or Fe(III) and Mn(IV) oxides/oxyhydroxides as extracellular electron acceptors. The process is dependent on decaheme electron transport proteins that lie at the extracellular face of the outer membrane where they can contact the insoluble mineral substrates. Mechanistically, in a cellular context, these extracellular proteins must be 'charged' with electrons provided by an inter-membrane electron transfer pathway that links the extracellular face of the outer membrane with energy-conserving inner cytoplasmic membrane and thereby intracellular electron sources. In this talk a study on the modular organization of a novel trans-outer membrane icosa-heme complex from *Shewanella oneidensis* that can provide this electron transfer link will be presented.

Structure-based analysis of ion and protein transport systems specific to Gram-negative bacterial pathogens

Trevor Moraes, Calvin Yip, Raz Zarivach, Thomas Spreter & **Natalie C.J. Strynadka**

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Clinically important Gram-negative pathogens such as *Pseudomonas aeruginosa* and enteropathogenic *E. coli* (EPEC) have evolved alarming levels of antibiotic resistance, due in part to the presence of two membrane barriers in these bacteria. Using an integrated structural biology approach including X-ray crystallography, electron microscopy, mass spectroscopy and NMR coupled with *in vitro* and *in vivo* biochemical and cellular analysis our group is piecing together structure/function relationships in large macromolecular assemblies in Gram-negative bacteria that allow for the specific transport of ions and proteins essential to subsequent pathogenesis. Recent work on the OprP phosphate specific transporter from *Pseudomonas aeruginosa* and the Type III protein secretion system common to many Gram-negative pathogens will be discussed.

A carbohydrate selective transporter from *Pseudomonas aeruginosa* – production, crystallization and preliminary X-ray diffraction

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Pseudomonas aeruginosa is an important causative agent in nocosomal infections. The organism has two inducible pathways for carbohydrate uptake. The low affinity pathway is responsible for the transport of gluconate and 2-ketogluconate. The high affinity route moves glucose directly into the cytoplasm. To understand the molecular mechanism of carbohydrate uptake with a view to rational drug design we are working to solve the crystallographic structure of key components in this transport system. Accordingly, bioinformatic analyses were used to identify an outer membrane protein in *P. aeruginosa* involved in high affinity carbohydrate uptake. The target has been expressed in *Escherichia coli* as inclusion bodies. The protein was refolded by rapid dilution of solubilized inclusion bodies into detergent solution and was purified by sequential nickel affinity, anion exchange and size exclusion chromatography. Circular dichroism showed that the protein is predominantly β-sheet and is likely to be a β-barrel. Optimized *in meso*-grown crystals diffract to 2.8 Å.

Colicin retrotranslocation: discovering strategies to cross bacterial cell membranes

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The nuclease-type colicins are SOS-induced protein antibiotics that penetrate and kill cells in competing *Escherichia coli* populations. The entry of colicins into susceptible cells begins with binding to a cell surface receptor followed by translocation across the outer membrane. For Tol dependent colicins, this occurs through interaction of the N-terminal natively disordered region of the toxin with the cell envelope spanning Tol-Pal complex. Once in the periplasm the nuclease type colicins, or at least their cytotoxic domains, must pass into the cytoplasm where they act enzymatically on DNA, tRNA or rRNA depending on their specific nuclease domain. The absence of any sequence or structural similarity between these different nuclease domains or indeed between nucleases that import via the Tol or Ton pathways, emphasizes that whatever route(s) exist for their entry to the cytosol they are insensitive to the structure of the nuclease. We present data that are consistent with a direct, electrostatically mediated interaction between colicin nucleases and the bacterial inner membrane. Subsequent translocation across the cytoplasmic membrane involves neither the Sec nor Tat systems but is dependent on the presence of functional FtsH, an inner membrane ATPase and protease that dislocates misfolded membrane proteins to the cytoplasm.

Structural insights into channels and pores

J.H. Naismith

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Abstract not received

Young Microbiologist of the Year Competition

'Vomocytosis' of live pathogenic yeast by macrophages

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Phagocytosis is the first line of defence against invading organisms and many pathogens have therefore evolved strategies to survive within a phagocyte following uptake. Nevertheless, most of these pathogens must eventually kill the host cell in order to escape and infect other tissues. However, by using long-term timelapse microscopy, we have now identified a novel 'escape' mechanism used by the human fungal pathogen Cryptococcus. This expulsive process, which we have termed 'vomocytosis' can occur either into the extracellular milieu or, remarkably, into neighbouring host cells, thus resulting in direct cell-to-cell transmission. Vomocytosis is extremely rapid and can occur many hours after phagocytosis of the pathogen in both cultured cell lines and primary macrophages. After vomocytosis, both the host macrophages and the expelled cryptococci appear morphologically normal and continue to proliferate, suggesting that this process may represent an important mechanism by which pathogens are able to escape from phagocytic cells without triggering host cell death and thus inflammation. Moreover, direct cell-to-cell spread of cryptococci would allow the pathogen to remain concealed from the immune system and protected from antifungal agents, thus achieving long-term latency.

Characterization of novel type III effectors in EHEC O157

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Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 is an attaching and effacing (A/E) pathogen of the human gastrointestinal tract. EHEC possesses an arsenal of proteins termed 'effectors' that can be delivered directly into the host enterocyte cytoplasm through a needle-like type III secretion system (T3SS). The components of this system along with several effectors are encoded by genes within the locus of enterocyte effacement (LEE). These effectors have been shown to subvert normal host cell processes and functions such as the maintenance of tight junctions, ion transport and cytoskeletal rearrangement. Recently 39 effectors encoded outside the LEE were discovered – we aimed to discover the function of these novel effectors. Non-LEE encoded effector proteins were endogenously expressed in yeast and mammalian cells and their effects on cytoskeletal function and cell growth were studied. Six effectors including two members of the novel NleG family affected cell division and morphology of yeast.

Role of platelet receptor polymorphisms and growth in whole blood on *Staphylococcus aureus*-induced platelet aggregation

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Bacteria-induced platelet activation and aggregation may play an important role in the pathogenesis of infective endocarditis (IE). Although *Staphylococcus aureus* surface proteins (MSCRAMMs) and their interaction with platelet receptors have been well characterized *in vitro*, their relevance to pathogenesis is unclear.

We measured platelet aggregation by *S. aureus* isolates from IE, bacteraemia and the nasopharynx of healthy individuals following growth in nutrient broth or whole blood. There were no significant differences in platelet activation between the clinical groups of isolates. Although several strains grown in nutrient broth did not cause platelet aggregation, all strains grown in blood did so, suggesting that growth in blood may stimulate the expression of surface factors which induce platelet activation.

We determined the frequency of polymorphisms in platelet receptors involved in *S. aureus*-induced activation among 21 IE patients and 164 healthy volunteers. There was no significant difference in the prevalence of polymorphisms in patients with IE versus healthy volunteers. However, platelets with the FcγRIIa R/R genotype had an increased lag time to *S. aureus*-induced platelet aggregation ($P<0.05$) suggesting an influence on bacteria-platelet interactions. These data highlight host factors which could influence the outcome of *S. aureus* bacteraemic infection.

A novel pathway of dimethylsulfide metabolism in *Methylophaga thiooxidans* sp. nov.

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Dimethylsulfide (DMS) is a volatile organosulfur compound which has been implicated as playing key roles in both climate control and in the biogeochemical cycling of sulfur. Bacterial metabolism is known to be a key sink of DMS in the marine environment, however, the physiological pathways and genes encoding the enzymes of which they are composed are, as yet, uncharacterized. Investigation of the physiological pathway of DMS metabolism in the marine methylotroph *Methylophaga thiooxidans* sp. nov., from the γ-subclass of the *Proteobacteria*, has revealed a novel pathway, resulting in a surprising new sink for DMS-sulfur in the marine environment – tetrathionate ($S_4O_6^{2-}$). This is the first known production of a polythionate from an organosulfur compound and, as such, represents a new step in the biogeochemical sulfur cycle. The pathway of DMS metabolism in *M. thiooxidans* has been characterized in full and ATP formation directly coupled to the oxidation of thiosulfate to tetrathionate has been demonstrated.

Broad-scale screening for poxvirus modulators of type I interferon

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Multiple modulators of type I interferon are encoded by mammalian poxviruses. Fowlpox virus (FWPV) is resistant to chicken type I interferon (chIFN) but genome divergence and the scarcity of avian interferon reagents has made it difficult to identify the genes responsible.

We therefore used two broad-scale genetic approaches to identify interferon modulators: (i) production of a library of recombinant Modified Vaccinia Ankara (MVA) viruses, each chimaeric for about 8 kbp of FWPV genome, screened for increased resistance to low dose chIFN, and (ii) production of an FWPV gene knock-out library, screened for loss of the ability to block induction of the chicken IFN-beta promoter in infected avian cells.

Two knock-out mutants (out of fifty non-essential mutants) have demonstrated significantly decreased ability to block induction of the chIFN-beta promoter. Two chimaeras (of sixty-five spanning the 266 kbp FWPV genome), each containing a small cluster of FWPV genes, have demonstrated significantly increased resistance to chIFN. These clusters have been termed FWPV interferon resistance (FIR) loci. Further recombinant MVA viruses, containing individual candidates from one of the FIR loci, identified a gene conferring increased chIFN resistance.

Fold and structural properties of the essential Omp85-family POTRA domains

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The outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts contain integral β-barrel Outer Membrane Proteins (OMPs). Folding and insertion of OMPs into membranes utilizes a multiprotein complex employing Omp85 family proteins, represented by YaeT in *Escherichia coli*. Members of the Omp85 family contain a characteriztic set of N-terminal repeats, known as POTRA domains. The precise function of the POTRA domains is unclear but is important in recruiting nascent OMPs from the periplasm and directing them towards the Omp85 complex. The NMR structure of tandem POTRA domains of YaeT has been determined, revealing the POTRA domain fold. Each POTRA domain comprises a three-stranded β-sheet overlaid with a pair of antiparrallel helices in a β-α-α-β-β order.

PigZ modulates secondary metabolism in *Serratia* sp. ATCC 39006 via transcription regulation of a putative four-component RND efflux pump

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Multi-drug resistance in bacteria is often due to the actions of multi-drug efflux pumps. The resistance-nodulation-cell-division (RND) family of multi-drug transporters are particularly notorious due to their high substrate promiscuity. The *E. coli* AcrAB-TolC pump serves as a prototype for the RND family. This tripartite pump consists of a membrane fusion protein (MFP; AcrA), an inner membrane transporter (AcrB) and an outer membrane channel (TolC). This presentation describes a putative four-component RND pump (ZrpADBC) in the Gram-negative enterobacterium *Serratia* sp. ATCC 39006 (39006).

39006 synthesizes several secondary metabolites, including prodigiosin (Pig) and a carbapenem antibiotic (Car). A complex hierarchial network of regulators control Pig and Car production. A mutation in *pigZ* resulted in differential regulation of Pig and Car. These effects are due to the overexpression of a divergently transcribed putative RND pump, encoded by *zrpADBC*, in the absence of the PigZ repressor. Unusually, this pump contains two predicted MFPs, ZrpA and ZrpD. Genomic deletions indicate that ZrpD, but not ZrpA, is essential for pump activity. Bioinformatic analysis revealed that putative four-component RND pumps are not uncommon, particularly amongst plant-associated, Gram-negative bacteria. Based on phylogenetic analysis, we propose that these pairs of MFPs consist of two distinct sub-types.

Start-up of expanded granular sludge bed bioreactors for the low-temperature anaerobic treatment of trichloroethylene contaminated wastewater

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Low Temperature Anaerobic Digestion (<20°C; LTAD) of several wastewater categories has been recently proven feasible, although the quality of seed biomass used to inoculate these systems is vital to sucessful outcomes. This is of particular importance when dealing with problematic wastestreams, contaminated with potentially carcinogenic compounds, such as Trichloroethylene (TCE). To this end, the microbial composition and metabolic capabilities of three candidate inocula (A, B and C) were assessed. The resultant profiles provided a basis for the selection of one suitable inoculum for four laboratory-scale expanded granular sludge bed (EGSB) bioreactors (R1-R4). R1 and R3 were test reactors treating TCE at 37°C and 15°C, respectively, while R2 and R4 acted as TCE-free controls, again at 37°C and 15°C respectively. Reactor performance was monitored throughout the trial by the analysis of COD depletion, methane production and TCE degradation. Molecular analysis of the microbial community structure and dynamics was carried out, at key time points, using terminal restriction fragment length polymorphism (TRFLP), 16S rRNA clone libraries and fluorescent *in situ* hybridization (FISH).

Escherichia coli as a cellular factory for Fab antibody production: effects of batch and fed-batch process modes on product yield and microbial physiology

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The use of bacteria as 'factories' which can be utilized to manufacture industrially, and medically, desirable moieties is well established and is a major part of the multi-billion dollar biopharmaceutical industry. Greater understanding of the effects of these kinds of processes on the physiology of the cell is important for increasing the yield, and improving the cost-effectiveness of purification strategies. Using flow cytometry along with traditional analysis techniques on both batch and fed-batch modes of operation, it has been observed that the ability of *E. coli* to manufacture optimally is dependent on induction and feeding being provided at suitable times. Significant detrimental effects have been elicited from this organism in both batch and fed-batch processes, these have been monitored at-line using multi-parameter flow cytometry and have enabled observation of impaired cellular function before they were made apparent by the more traditional methods in use.

Population genomic analysis indicates avian host adaptation by *Staphylococcus aureus*

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Staphylococcus aureus is an important human and animal pathogen. Population genetic studies have shown that most strains of *S. aureus* are restricted to a single host species. To investigate the diversity of strains infecting poultry we carried out multi-locus sequence typing of 50 poultry isolates from 6 countries in 4 continents. The majority of isolates belonged to a single major human lineage but had phenotypic differences to human strains suggesting host-adaptation. Our data suggest that a host jump from humans to poultry has recently occurred followed by wide geographical dissemination, possibly due to the globalization of the poultry farming industry since the 1950s. To investigate the genetic basis for avian host adaptation by *S. aureus,* we determined the 2,824,409 bp genome sequence of a representative poultry-specific strain. This facilitated comparative analysis with published genomes of closely-related strains of human origin. Mobile genetic elements (MGE) unique to the avian strain were the major source of variation, including a novel *S. aureus* pathogenicity island (SaPIav), prophage, putative transposon, and plasmids. Several MGEs encoded novel putative virulence determinants which may play a role in host-specific pathogenesis. These data represent new insights into *S. aureus* host adaptation and the role of human activities in the evolution of pathogens, in general.

Quorum-sensing and related protein expression in the cyanobacterium *Gloeothece*

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Cyanobacteria are a group of photosynthetic organisms that have both beneficial and harmful effects in the environment. Dense growth of these organisms has been found to be associated with blooms and in

biofilms. It is of particular interest to examine mechanisms such organisms have, in order to overcome problems of stress and survival, associated with growth at a high cell density. Recently, quorum-sensing, via homoserine lactones, has been demonstrated in a group of proteobacteria, in which it functions to communicate at high cell density. However, to date there is no evidence of such processes operating in the cyanobacteria. Our research provides the first evidence that a C8-homoserine lactone (C8-HSL) accumulates in cultures of a cyanobacterium. A number of proteins displaying altered expression levels in response to treatment with C8-HSL in *Gloeothece*, suggests that the cyanobacterium also responds to the homoserine lactone through changes in gene expression.

Peter Wildy Prize Lecture

Stripping down science: The Naked Scientists

C. Smith

University of Cambridge

Chris Smith is a clinical lecturer in virology and fellow of Queens' College at the University of Cambridge. He's also the founder of The Naked Scientists, a weekly science radio talk show, podcast and website.

He joined the combined MB/PhD programme at Cambridge University in the mid-1990s working on the development of viruses as gene therapy vectors. Mid-way through his PhD, and during his time as a junior doctor, he found working 100 hours a week was leaving him feeling under-employed, so he set up The Naked Scientists radio show and website.

This was initially a hobby, but it has since taken over his life, won several awards, and grown to become one of the world's most downloaded science podcasts; since 2005, over 5 million copies of the show have been distributed internationally. Chris also appears live every week on national radio in Australia and South Africa to talk about the latest scientific breakthroughs.

When he's not behind a microphone, Chris teaches and examines medical and science undergraduates at Cambridge, and works as a specialist registrar in a diagnostic laboratory at Addenbrooke's Hospital, where he has a special interest in human and avian influenza and emerging viral pathogens.

Now busy completing his third and fourth popular science books, Chris also has a young daughter who ensures that his immune system remains on high alert by infecting him with everything circulating in Cambridge.

The Peter Wildy Prize is awarded annually for an outstanding contribution to microbiology education.

Posters

Cells & Cell Surfaces Group

CCS 01 Multiplicity of cell surface proteins function in *Streptococcus pyogenes* interactions with gp-340 and fibronectin

Linda Franklin[1], Aras Kadioglu[2] & Howard F. Jenkinson[1]

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Streptococcus pyogenes is associated with a range of infections including pharyngitis, toxic-shock syndrome, and puerperal sepsis. Adherence factors are important in early stages of infection and also contribute to invasion and modulation of host responses. Genes encoding for the antigen I/II (AgI/II) family of cell wall anchored oral streptococcal adhesins have been found in invasive *S. pyogenes* M types. The aim of this study was to determine AgI/II function in various M28 *S. pyogenes* strains. Genetic profiles of fibronectin-binding proteins SfbI, SfbX, and Sof, pilus adhesin Cpa, and AgI/II protein were determined for five M28 and M2 strains by PCR, and abilities to bind host proteins tested by plate assays. Overall adherence levels to innate immunity glycoprotein gp-340 and fibronectin were generally not correlated with differential genetic profiles for relevant adhesins. Isogenic AgI/II mutants of M28 strains 71-298 and MGAS6180 were constructed and found to be approximately 90% and 50% reduced, respectively, in binding to immobilized gp-340. However, mutants were not affected in binding to fibronectin. These results suggest a role for AgI/II in recognition of gp-340 by *S. pyogenes* that is strain dependent. Moreover, within a single M type, multiple interacting proteins govern *S. pyogenes* recognition of gp-340 and fibronectin.

CCS 02 Staphylococcal interactions with Continuous Ambulatory Peritoneal Dialysis (CAPD) fluid

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Renal failure is increasingly being treated by Continuous Ambulatory Peritoneal Dialysis (CAPD). However, infection leading to peritonitis and possible CAPD failure is a frequent complication of this form of kidney dialysis. Infections are often caused by commensal skin bacteria such as the staphylococci, and *Staphylococcous epidermidis* and *S. aureus* are responsible for over half of all CAPD infections. The host risk factors that influence CAPD patient susceptibility to infection currently revolve around issues such as patient hygiene, and little thought has been given to the role that the host factors released into the CAPD dialysis fluid may play. We therefore analysed the protein profiles of CAPD dialysates from a large number of new and established CAPD patients, as well as examining how the different CAPD dialysates affected the growth and virulence of bacteria such as the staphylococci. We found there were significant differences between patient dialysates in terms of protein profile and how the bacteria grew and expressed virulence factors. Considered collectively, our data suggests that the host factors released into the dialysis fluid of CAPD patients could be an additional factor that influences their chances of developing a potentially life threatening infection.

CCS 03 Functional & computer-aided analysis of the KfiB (*Escherichia coli*) and its homologous HyaE (*Pasteurella multocida*)

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Computer-aided analysis of protein sequences is a powerful tool in molecular biology for the estimation of structures, functions and the evolutionary relationship of proteins. To support the structure-function studies of KfiB, computer-aided analysis of this protein was undertaken.

Also the hyaE gene was amplified and the PCR product was cloned into pBlue script KSII. In-f frame insertion of the hyaE gene was confirmed. The resulted plasmid was unable to complement the KfiB mutation and restore K5 capsule biosynthesis.

By comparing the MTK and MTDIK outputs using COILS program, high probabilities for coiled coil formation (>90%) were seen with both matrices for KfiB from residues 360–445 and for HyaE from residues 310–390 and 410–507. This confirms the notion that KfiB may well interact via coiled-coil interactions either with itself or with other proteins involved in K5 polysaccharide biosynthesis.

The similarity of HyaE to KfiB suggested that both *hyaE* and *kfiB* must code for function related to the common sugar, possibly in the synthesis of the N-acetylglucosamine component.

CCS 04 Exploring probiotic efficacy *in vitro*: towards a mechanistic understanding

James Collins[1,2], Nicholas Coldham[1], Martin J. Woodward[1], Glenn Gibson[2], Bob Rastall[2] & Roberto M. La Ragione[1]

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Probiotics are used widely to reduce the contamination of food producing animals with *Salmonella,* but their exact mode of action remains unknown. To determine potential molecular mechanisms underlying probiotic efficacy, a proteomic analysis was adopted. The effect of the cell free supernatant (CFS) from *Lactobacillus plantarum* was determined on the proteome of *S.* Typhimurium at pH 7.2 and pH 4.5, along with appropriate lactic acid and pH controls.

A urea extract from *S.* Typhimurium was analysed using Multi-Dimensional Protein Identification Technology (MuDPIT) and the relative expression of proteins was determined using the spectrum count method.

Changes in metabolic enzymes associated with central metabolism in *S.* Typhimurium were observed by the addition of lactic acid and CFS to culture medium. Significant differences were evident between *S.* Typhimurium exposed to CFS or lactic acid. However, the presence of lactic acid or CFS had pH independent antimicrobial activity and global regulatory effects on proteins involved in the phosphotransferase system (PTS), *de novo* amino acid synthesis, chemotaxis, efflux and central metabolism. Subsequent phenotypic analysis consisted of *in vitro* organ culture, electron microscopy and invasion assays. Interestingly, these studies suggest that CFS may modulate *Salmonella* pathogenesis *in vitro*.

CCS 05 Protein glycosylation in Streptomyces coelicolor A3(2)

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Protein glycosylation is found in all kingdoms of life and is the most common post-translational modification in eukaryotes. In the past few years, glycosylation has also been found in the S-layer and flagella of many prokaryotes and archaea. In *Streptomyces coelicolor* A3(2), mutants isolated as resistant to phage ϕC31 infection were found to be deficient in genes homologous to protein *O*-glycosylation genes found in *Saccharomyces cerevisiae*. All three mutants have reduced glycosylation of membrane proteins. Further study into the phenotypes

of strains deficient in polyprenol phosphate-mannose synthase 1 (*ppm1*) revealed reduced ability to transfer GDP-[^{14}C]-mannose onto native membrane lipids. Mass spectrometry identified the mannosylated lipid as C_{45} polyprenol phosphate mannose (C_{45}-Ppm) in wild type membranes. Polyprenol phosphate mannose synthase is believed to function in association with a putative membrane protein, Ppm2, which is predicted to have a C-N hydrolase activity. Ppm2 was targeted for mutagenesis and the mutants (eg AV301) are phage resistant and fail to glycosylated proteins. An antibody has been raised to Ppm2 and preliminary results indicate that Ppm2 is indeed membrane located. Further studies are currently underway to determine the role of Ppm2 in the protein glycosylation pathway in *Streptomyces*.

CCS 06 Fibronectin-binding protein B variation in *Staphylococcus aureus*

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Fibronectin-binding protein B of *Staphylococcus aureus* mediates adhesion to fibrinogen, elastin and fibronectin. The gene encoding FnBPB (*fnb*B) occurs in seven out of the nine fully sequenced *S. aureus* genomes. Alignment of FnBPB amino acid sequences indicated substantial variation in the N-terminal fibrinogen and elastin-binding A domain while the C-terminal fibronectin-binding motifs were highly conserved. Four FnBPB variants (isotypes I–IV) were identified based on divergence in the minimal ligand-binding N23 sub-domains, which are 66–76% identical to one another.

Ligand binding by recombinant FnBPB N23 isotypes I–IV was compared by ELISA-based solid phase assays. Each bound to immobilized fibrinogen, elastin and fibronectin dose-dependently and saturably with similar affinities. Binding to fibronectin was surprising because the A domains do not contain any known motifs that mediate binding to fibronectin. This raises the possibility that the A domain of FnBPB binds fibronectin by a novel mechanism.

Four isotype-specific DNA probes recognizing DNA encoding the highly divergent N3 sub-domains were used to identify *fnb*B variants by DNA hybridization in a *S. aureus* strain collection. Some strains that appear to cluster by phylogenetic analysis contain different *fnb*B variants, whereas other strains that are more divergent, contain the same *fnb*B variant. This suggests that *fnb*B genes have been horizontally transferred between strains.

*fnb*B DNA from *S.aureus* strains, which did not hybridize to the type I–IV specific probes, was cloned and sequenced. The deduced amino acid sequences revealed three novel FnBPB A domains called isotypes V, VI and VII.

Posters

Clinical Microbiology Group

CM 01 Novel tea-tree oil based treatment of *Candida* biofilms

Steven Milligan, Shauna Culshaw, Jeremy Bagg, Petrina Sweeney & Gordon Ramage

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Candida albicans is the predominant yeast associated with oropharyngeal candidosis (OPC), which presents clinically as thrush or denture stomatitis. These infections are characterized by multispecies biofilms forming on the oral epithelium or artificial denture surfaces. Current antifungal treatments have limited success due to resistance, with recurring infections common. Therefore, alternative methods for suppressing or eradicating biofilms are desirable. The aim of our study was to evaluate the efficacy of tea tree oil (TTO) and key derivatives against biofilms formed by 100 oral *Candida albicans* isolates. These were formed in microtitre plates, treated with individual components and the fungicidal activity evaluated using a metabolic dye to evaluate killing. It was shown that although generic TTO was effective against planktonic cells, it was poorly active against sessile cells (MFC_{90} >2%). However, terpinene-4-ol and terpineol exhibited anti-biofilm activity (MFC_{90} = 1%). In addition, lower concentrations (0.25%) of these two agents were able to inhibit filamentous growth. Overall, TTO-based mouthwashes may offer a suitable alternative to conventional azole treatment.

CM 02 Lysostaphin and ranalexin act synergistically to kill multidrug-resistant *Staphylococcus aureus* (MRSA) cells in established biofilms

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Multidrug-resistant *Staphylococcus aureus* (MRSA) is a serious global pathogen that infects hundreds of thousands of patients each year and can result in permanent debilitation or even death. Patients with indwelling medical devices such as catheters or intravenous drips are at greater risk of MRSA infection as this bacterium can form persistent biofilms on the surfaces of such medical devices. Using various biofilm viability measurements, including a microtitre plate assay, we show that a novel drug combination of lysostaphin (an anti-staphylococcal endopeptidase) and ranalexin (a natural antimicrobial peptide) acts synergistically to kill established MRSA biofilms. The synergy is highly potent and the ranalexin and lysostaphin concentrations can be reduced by ~10–50 times to give the same antibacterial effect when these compounds are tested individually. This new drug combination may find application in the eradication of established MRSA biofilms or could be incorporated into medical devices at manufacture to prevent subsequent biofilm formation.

CM 03 The influence of tigecycline on gene expression in meticillin-resistant *Staphylococcus aureus* biofilms

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Staphylococcus aureus is a leading cause of hospital-acquired infections in the UK and approximately 45% of *S. aureus* isolated are resistant to meticillin (MRSA). These organisms can cause a range of infections including those of surgical sites, endocarditis and pneumonia. *S. aureus* can also readily colonize artificial surfaces and grow as biofilms on implanted medical devices. Bacterial cells within biofilm structures are inherently resistant to antimicrobial challenge and difficult to eradicate from the infected host. Tigecycline is a novel glycylcycline antibiotic which has shown efficacy against *S. aureus* grown in free-floating planktonic culture. This study examined the influence of tigecycline on gene expression within biofilm-associated cells of a clinical MRSA isolate, using BμG@S *S. aureus* microarrays. The results of the microarray study were validated using real-time RT-PCR. In the presence of tigecycline, genes encoding several virulence factors were down-regulated including toxins, adhesion proteins and biofilm-associated proteins. This study suggests that tigecycline may be a useful antibiotic for the treatment of biofilm-mediated MRSA infections and may reduce the expression of life-threatening virulence factors, ultimately improving morbidity and mortality rates in infected patients.

CM 04 Neuraminidase activity in viridans group *Streptococcus*

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Viridans group streptococci (VGS) are the second leading cause of infective endocarditis. Although the virulence factors associated with the primary steps of attachment and colonization of a damaged heart valve are recognized, little is known about the degradative enzymes and toxins that are potentially produced by these organisms.

A panel of 38 isolates, comprised of 21 reference strains and 17 clinical isolates, were screened for neuraminidase production using a colorimetric substrate (2-*O*-(*p*-nitrophenyl)-α-D-*N*-acetylneuraminic acid). The influence of exposure at sub-minimum inhibitory concentrations of various antibiotics, including penicillin and gentamicin (1/2x, 1/4x and 1/8x MIC) on the expression of this enzyme was then analysed for the positive strains.

Although the majority of the isolates tested were uniformly negative for neuraminidase production, members of the mitis group, namely *S. oralis* and *S. oligofermentans*, revealed a high activity for this enzyme.

Even though the role of neuraminidase is not clearly understood, it has been suggested to contribute to the *in vivo* persistence of VGS growing in an endocardial vegetation by providing essential nutrients for growth. Alternatively, it may assist bacterial adhesion through the exposure of endothelial binding sites. Evaluation of the antimicrobial effect on neuraminidase production may allow an insight into the pathogenesis of streptococcal endocarditis and potentially offer new therapeutic approaches.

CM 05 In a novel, *in vitro* model of osteomyelitis, *Staphylococcus aureus* biofilms are resistant to gentamicin and daptomycin at clinically relevant concentrations

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Staphylococcus aureus is a common causative agent of bacterial bone infection (osteomyelitis). Despite prolonged antibiotic therapy, often in combination with surgery, osteomyelitis is rarely resolved and infection recurrence common. Bacterial resistance to antimicrobial therapy may be partly due to poor antibiotic penetration into bone and bacteria growing as biofilms. Here we show in a novel, *in vitro* model of *S. aureus* bone infection, using uniform bovine bone blocks, that exposure to gentamicin and daptomycin at clinically achievable concentrations results in a negligible decline in *S. aureus* biofilm burden. Drug concentrations at 10 x achievable concentrations did not demonstrate bactericidal (3 $\log_{10}$ decline) activity over 24 h, and resulted in only 2.17 and 1.85 $\log_{10}$ reductions in CFU/mL for gentamicin and daptomycin respectively.

These data closely reflect the observed clinical situation in osteomyelitis, where the activity of antimicrobial agents is generally poor. This supports the further development of our model.

CM 06 Cost of processing contaminated blood culture samples

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My study project (Oct'06–April'07) was related to calculating cost and implications of processing contaminated Blood culture samples. Approximately 40,000 blood cultures samples are processed each year in Royal Hallamshire and Northern General Hospital. Positive blood cultures are expensive to process and costs are little different for both true positives and contaminants. Evaluation by audits indicated substantial contaminant rates in blood cultures (8.8% overall). Contaminated blood cultures have potential to trigger chain reaction of wastage of health resources, clinical expertise and precious time of medical & technical staff and above all adversely affecting patient's care, a problem that can be solved by effective teaching and training.

In my project I calculated time of laboratory staff and microbiologists, wasted in processing these samples and overall cost including cost of consumables. Results indicated Approximately fifty five working days/year of microbiologists and laboratory staff, wasted on this activity, and adding together overall expenditure was approximately 30,000 £/year. Overall it was an underestimate of true cost as it did not include cost of patients' stay in hospital and antibiotics patients received only due to samples being contaminated!

CM 07 Detection of bacterial 16S rRNA genes from a variety of environmental sites in an intensive care unit

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Hospital acquired infections (HAI's) are a major cause for concern with approximately 0.5–1 million patients acquiring a hospital infection in 2002–2003 alone. Although all hospitalized patients are at risk of developing an HAI those in the intensive care unit (ICU) are often significantly immunocompromised and highly susceptible. With many of these organisms being antibiotic resistant (often multidrug resistant) researchers have been studying patient isolates since the introduction of antibiotics into clinical medicine. However, there is limited research that has investigated the hospital environment itself. This study uses molecular techniques to identify the presence of bacteria in the intensive care unit (ICU) environment. Through PCR particular antibiotic resistance determinants can be detected. The presence of a range of genes has been investigated: including *mec*A (meticillin resistance – MRSA), $bla_{SHV,\ TEM,\ CTX\text{-}M}$ (extended spectrum beta-lactamases), *tet*(M,O,W) (tetracycline resistance). Before commissioning of the ICU, samples taken from floor, chair, intercom, blind and light switch were all negative for 16S rRNA genes. Equivalent samples taken after patient admission were positive for such genes, which suggests an increase in bacterial colonization upon commissioning of the ICU. To date resistance determinants have been detected with only a very low frequency.

CM 08 Gentamicin – medical staff knowledge, antibiotic guidelines and patient safety

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Antibiotic guidelines are commonplace in clinical medicine. Rises in hospital acquired infections and their publication in the general media ensure that antibiotic usage is changing. Continually varying protocols and the introduction of newer antibiotics make it difficult for practitioners to stay up to date.

Objective This study investigates the knowledge of medical staff with regards to the practical use of the aminoglycoside Gentamicin.

Results A prospective study questioning 42 Doctors and 49 Nurses. 58% of Doctors and 22% of Nurses recalled the correct dose of Gentamicin. 26% of Doctors and 31% of nurses knew when to take the first Gentamicin levels and 37% of doctors and 57% of nurses knew when to take subsequent levels.

Nephrotoxicity (86% of Doctors and 33% of nurses) and ototoxicity (53% of doctors and 10% of nurses) were reported as side effects.

Conclusion Medical staff responsible for prescribing and administering Gentamicin lack knowledge concerning its dosing, monitoring and potential complications. From this, It can be concluded that, patient safety may be compromised due to infrequent monitoring, incorrect dosing and under recognition of side effects.

CM 09 Unravelling *Streptococcus gordonii*–platelet interactions using bacterial proteomics

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At sites of periodontal damage, oral bacteria such as *Streptococcus gordonii* can gain entry to the circulation. Platelet adhesion and subsequent aggregation plays a critical role in the pathogenesis and dissemination of the infective process. Here we identify an interaction between fructose bisphosphate aldolase (FBA) and human platelets. *S. gordonii* strain DL1 induced platelet aggregation (57±1%) with a lag time of 12±1mins while strain Blackburn failed to induce platelet aggregation after 20mins (1± 0.3%, n=20). Using a proteomic approach mass spectrometry identified peptides from 87 proteins that were present in DL1 and absent in Blackburn. One protein of particular interest was FBA, as it has recently been shown to bind epithelial cells through flamingo cadherin. The presence of FBA in the cell wall fraction of DL1 was confirmed by immunoblot and the expression of flamingo cadherin on platelets was shown by immunoblot. Flow cytometry identified 1146±174 copies on the platelet. A blocking flamingo cadherin antibody failed to affect the percent platelet aggregation induced by *S. gordonii*, however extended the lag time from10±1mins to14±2mins, $p<0.05$, n=5. Our results suggest FBA may play a role in *S. gordonii* induced platelet aggregation.

CM 10 A novel *Streptococcus* surface protein associated with platelet interactions

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Streptococcus gordonii, a viridans *Streptococcus*, is a recognized agent in infective endocarditis (IE). On gaining entry to the vascular system, these bacteria adhere to platelets and this is thought to be crucial to their pathogenic potential in causing IE. A range of platelet-interactive proteins are expressed on the surface of *S. gordonii*, including Antigen I/II proteins SspA and SspB, and Hsa/GspB. Here we identify a novel cell wall anchored protein (mol. mass 397 kDa) designated platelet adherence protein A (padA,) which is expressed from a locus containing three genes transcribed co-directionally. Mutants in *padA* were generated by allelic exchange of *aad9* (encoding spectinomycin resistance) with an internal segment of the *padA* gene. Mutants were also generated by allelic exchange with sequences within an upstream gene encoding for a thioredoxin signature protein, and a downstream gene encoding a putative cell wall anchored protein of unknown function. Only the *padA* mutant showed deficiency in production of PadA protein, and was also deficient in platelet binding, platelet aggregation, and in binding platelet receptor GPIIb/IIIa. None of the mutants were affected in Hsa or Antigen I/II production. These results identify a novel platelet-interactive protein that may be a virulence factor in IE.

CM 11 Characterization of avian beta-defensin 10 antimicrobial peptide expression in three distinct lines of chickens reared in low and high hygiene environments

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Antimicrobial peptides (AMPs) are a group of host defence peptides 1–10 kDa in size which contain spatially separated hydrophobic and cationic amino acids. To date a number of chicken (*Gallus gallus domesticus*) AMPs have been reported; these include the avian β-defensins (AvBD) or 'Gallinacins'. This study investigated the effects of age and either low (LH) or high hygiene (HH) environments on Avian beta defensin 10 (AvBD10) gene expression in three lines of male birds aged 0, 7 and 35 days respectively. RT-PCR analyses indicated AvBD10 gene expression in most tissues analysed but marked differences were noted between birds maintained in the LH and HH environments. The AvBD10 peptide was hyper-expressed *in vitro* and shown to have antimicrobial activities against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* spp. These data suggest important functional roles for this peptide in the avian innate defences and in particular in young birds. Moreover environmental parameters can potentially affect gene AMP expression levels.

CM 12 Isolation of the highly pathogenic *Stenotrophomonas maltophilia* ('Steno') from the poultry red mite *Dermanyssus gallinae*

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Stenotrophomonas maltophilia also known as 'Steno' has recently made headlines as one of the most dangerous Gram-negative bacteria responsible for a rapid increase of nosocomial cases in patients. Some strains are known to be resistant to a broad range of antibiotics making it as difficult to control in hospitals if not more problematic than MRSA or *Clostridium difficile*.

It was therefore unexpected when *Stenotrophomonas maltophilia* was found growing from sheep blood agar inoculated from external washes with PBS and even bleach from the surface of the poultry red mite *Dermanyssus gallinae* collected in a farm in UK.

After PCR using 16S rRNA primers, the 700 bp amplified fragment was found to be 99% similar to 5 *Stenotrophomonas maltophilia* sequences from Genbank.

Following previous observations of this highly pathogenic bacteria from soils and marine samples it is now more worrying to have isolated this bacteria from a poultry farm showing how endemic the situation could be.

CM 13 '*Synergistetes*' in periodontal health and disease

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Introduction Members of the phylum '*Synergistetes*' have been frequently detected in the human mouth at sites of dental disease, but rarely in health. Only two oral '*Synergistetes*' taxa are cultivable.

Objectives To investigate the diversity of '*Synergistetes*' in the mouth; to establish whether '*Synergistetes*' taxa are more strongly associated with periodontitis than health; and to visualize unculturable '*Synergistetes*'.

Methods Sixty samples (saliva, dental plaque and mucosal swabs) were collected from five periodontitis cases and five periodontally-healthy controls. Using phylum-specific 16S rDNA primers, '*Synergistetes*' were identified by PCR, cloning and sequencing of 45 clones/sample. Subgingival plaque samples were labelled with probes targeting rRNA of unculturable oral '*Synergistetes*' using fluorescent *in situ* hybridization (FISH).

Results Analysis of 1664 clones revealed twelve '*Synergistetes*' taxa (five novel). '*Synergistetes*' 4Div2, was found in more cases than controls (p=0.048) and was more abundant in subgingival plaque at diseased sites (cases) than healthy sites in cases (p=0.019) or controls (p=0.019). FISH analysis revealed unculturable oral '*Synergistetes*' cells to be curved rods.

Conclusion The human mouth harbours a diverse population of '*Synergistetes*'. '*Synergistetes*' 4Div2 is strongly associated with periodontitis.

CM 14 Characterization of *Bacillus subtilis* isolated from the human gastrointestinal tract (GIT)

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Bacillus spores present in the soil enter the gastrointestinal tract (GIT) associated with ingested organic matter and this explains the abundance of spore formers in soil-dwelling animals. It has been shown recently that spores of a laboratory strain of *Bacillus subtilis* can germinate in the small intestine of mice dosed orally. Surprisingly, germinated spores could outgrow and then, as they progressed into the upper colon, re-sporulate. This phenomenon was also observed with other, natural isolates of *B. subtilis* that had been recovered from human faeces suggesting that *B. subtilis* could use the GIT for both growth and sporulation. This raises the intriguing question of what is the real habitat for spore-formers?

If *Bacillus* species are intestinal residents or commensals then not only should it be possible to isolate them readily from the GIT but also, to identify strains that have carry attributes that enable their survival within the gut. We examined *B. subtilis* isolates recovered from the faeces as well as the small intestine of human volunteers. This work shows that a considerable diversity of *B. subtilis* isolates can be found in the human GIT and in some cases they show traits that could be beneficial to an intestinal micro-organism.

CM 15 Time-resolved metabolic footprinting for cystic fibrosis pathogen profiling

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Bacteria of the *Burkholderia cepacia* complex (Bcc) and *Pseudomonas aeruginosa* are important pathogens in cystic fibrosis. Because the metabolism of any cell is influenced by its genetic background and its surroundings, we monitored time-dependent changes in the culture supernatants of these bacteria by NMR-based metabolic profiling (footprinting). We tested whether sampling at more than one point of growth provides additional information and if stable contributors to strain and/or species separation can be found. To this end, the intensity changes of individual resonances over time were fitted to non-linear equations. The growth phase in which sampling took place was the major factor determining the metabolic phenotype. Further, the approach provided evidence for tightly regulated patterns of compound uptake in rich media that were fundamentally different for the investigated bacteria. Applying hierarchical pattern analysis on the uptake parameters generated a purely phenotype-based separation of Bcc species and revealed secondary metabolic consequences of the *mucA* mutation in *P. aeruginosa*. We conclude that time-resolved footprinting could serve as a tool in physiology, functional genomics and taxonomy.

CM 16 Partial purification on inhibitory substances, produced by *Staphylococcus* spp. in different optimized media and showing activity against MRSA

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Lantibiotics are a group of peptide antibiotics that are produced by some bacteria and show activity against closely related species. The most prominent representative, nisin, has already a long history of use in the protection of foodstuffs. Lantibiotics have also been considered for application in humans. Fundamental process issues have to be addressed in order to provide such lantibiotics that are maximally produced in desired growth media.

Production on inhibitors by *Staphylococcus spp* was optimized in different modified media. The initial detection of cell-associated and liberated inhibitors showed that supply of monosaccharide sugar was a significant requirement. This suggests that supply readily available basic requirements, including carbon source in the form of monosaccharide enables the producer organism to focus its stored energy in the production of the inhibitor(s) rather than breaking down polysaccharide sugars. A further observation suggested that adsorption of the inhibitor(s) to red blood cells could be a reason for the low levels of inhibitor(s) titre(s) when an increased concentration of blood was used in purification media.

CM 17 Investigation into the potential of sub-lethal Photodynamic Antimicrobial Chemotherapy (PACT) to increase resistance to antibiotics

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In PACT, a combination of a sensitizing drug and visible light causes the selective destruction of microbial cells *via* singlet oxygen production. As singlet oxygen is a non-specific oxidizing agent and is only present during illumination, development of resistance to this treatment has been thought to be unlikely. This study determined whether treatment of bacterial strains with sub-lethal doses of methylene blue (MB) and meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP)-PACT resulted in reduced susceptibility to both antibiotics and further PACT. Treatment of both *P. aeruginosa* and *S. aureus* with sub-lethal concentrations of photosensitizer resulted in significant decreases in susceptibility to previously lethal photosensitization. Exposure of *P. aeruginosa* to sub-lethal photosensitizer concentrations resulted in decreased susceptibility to meropenem with no effect apparent with tobramycin and piperacillin. Similarly, antibiotic and photosensitizer specific changes in susceptibility were apparent following exposure of *S. aureus* to sub-lethal concentrations. The results show that although sub-lethal PACT increased bacterial resistance to previously lethal PACT the percentage kill of each organism remained high.

CM 18 Case report: an epidemic hypervariable *Staphylococcus epidermidis* strain causing persistent bacteremia with myositis and meningitis after haematological stem cell transplantation

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We review the case of a patient with acute lymphoblastic leukemia who received a hematopoietic stem cell transplant and thereafter, during a period of prolonged aplasia, experienced a persistent, generalized, and eventually fatal *Staphylococcus epidermidis* infection. Over a six-week period, a genetically and phenotypically unstable *S. epidermidis* strain was detected in blood cultures, muscle, and cerebrospinal fluid with a striking selection for biofilm-forming and antibiotic resistant variants in the late stages of the infection. The proven affiliation of the strain to a recently identified epidemic clone that is known to cause health-care associated infections in Europe, America and Japan points to the importance of distinct *S. epidermidis* clonal lineages as serious pathogens for immunocompromised patients. Identification of such isolates requires specific attention because they can easily be mistaken for contaminants due to their pronounced variability that often mimics mixed cultures.

CM 19 *Abstract withdrawn*

CM 20 Prevalence of enteroaggregative *Escherichia coli* in cases of diarrhoea in North of Iran

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Diarrhoea is an important case of morbidity and mortality among children in developing countries. Enteroaggregative *Escherichia coli* (EAggEC) is an emerging diarrhoeal pathogen.

The aim of this study was determination of the prevalence of enteroaggregative strain in cases of diarrhoea in Gorgan by PCR method.

During the year 2006–7, 455 diarrheal samples were studied. At first, all the samples were cultivated on the MacConkey and EMB agar media. All colonies suspicious to *E. coli* were subjected to DNA extraction followed by PCR amplification using the related primers and gel electrophoresis.

4.4% of samples were positive for EAggEC, that 12 cases were female and 8 cases were male, 17 cases (85%) were under 5 years old (45.8% under 1 year old). The distribution of this gene in different season were as follow; summer, autumn, winter, spring were 5.3% , 4.2% , 4.1% and 1.8% respectively.

EAggEC were found in 4.4% of diarrheal samples in North of Iran, which was more in girls under 5 and more prevalent in summer.

CM 21 *Abstract withdrawn*

CM 22 *Abstract withdrawn*

CM 23 *Abstract withdrawn*

CM 24 *In vitro* studies on adhesive properties of indigenous and exogenous potential probiotic lactobacilli

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Lactobacilli belong to normal flora of human and animal oral cavity, the vagina and the gastrointestinal tract. Because of their proposed health promoting properties, Lactobacilli species are widely used as probiotic. An important property proposed for a probiotic bacterium is the ability to adhere and colonize host tissue, which enhances multiplication and survival of bacteria in host and prevents colonization by pathogenic bacteria. In the present study adhesive properties of indigenous and exogenous potential probiotic Lactobacilli was observed. For this, total of 20 isolates of *Lactobacillus* sp. were recovered from food and fecal samples.

All the isolates were screened for cell surface hydrophobicity and production of lectin like substances. Cell surface hydrophobicity was determined by bacterial adherence to hydrocarbons assay in LAPTg broth and hydrophobicity was calculated as percentage decrease in Optical Density at 600 nm. The production of lectin like substances in Lactobacilli was determined with an agglutination assay of microbial cells through a suspension of glutaraldehyde –treated yeast cells at 25°C. The general range of hydrophobicity in Lactobacilli was found between 5–40%. Exceptionally, the isolates of human fecal origin, i.e. *L. plantarum* and *L. fermentum* showed 70% hydrophobicity in Xylene and 56% in Toluene respectively. High value of hydrophobicity could point toward a greater ability of Lactobacilli species to adhere to epithelium cells. Another property i.e. production of lectin like substances with *Saccharomyces crevices* NCDC49 , *Lactobacillus* sp. isolated from food samples showed minimum (almost nil) and isolates from human fecal origin showed maximum rate of agglutination within 6.0 h of inoculation . The presence of lectin like compounds in the outer layer of human fecal lactobacilli would favor colonization by its ability to bind specific glycoprotein in the membranes of intestinal epithelial cells and prevents the colonization by harmful enteric pathogen.

In view of all the results and interpretation we can conclude that *Lactobacillus* sp. have good adhesive properties which help them to adhere to surface epithelium of host cell. The isolates of human fecal origin i.e. *L. plantarum* and *L. fermentum* showed appreciable results and could be designated as potential probiotic.

Keywords lactobacilli, probiotic, hydrophobicity, optical density, agglutination

Posters

Environmental Microbiology Group

EM 01 Impact of an oil spill on the uncultivated microbial community in mud flat sediments

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Crude oil contains a mixture of hydrocarbons, most of which are toxic to organisms but many serve as a source of carbon and energy for suitably adapted micro-organisms. The aim of this work is to investigate the changes in the autochthonous microbial community within the mud flat sediment of an estuarine habitat (Colne Estuary, Essex, UK), using tidal mesocosms.

The determination of the structure of microbial community was achieved by DGGE analysis of the V3 region of 16S rRNA and subsequent sequence of selected bands. DGGE and sequence analysis revealed a large variety of micro-organisms and little obvious difference in the profiles of oiled and non-oiled sediment communities. At day 12 of the experiment, community changes became more evident and oil communities were dominated by the presence of Gammaproteobacteria and Cyanobacteria.

The stressful effects of oil in sediments are likely to be diminished because certain components adsorb to sediment. In order to detect micro-organisms particularly related to oil degradation, it is necessary to concentrate on functional genes.

EM 02 Characterization of 'aromatic naphthenic acid'-degrading bacteria

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Naphthenic acids (NAs) are complex mixtures of predominantly cycloaliphatic and alkyl substituted acyclic carboxylic acids found in mainly weathered crude oil. Aromatic compounds make up a small, but unexplored fraction of this mix. NAs cause severe environmental problems as they are recalcitrant, corrosive, and toxic. This project aims to characterize aromatic NA-degrading micro-organisms in relation to NA biodegradation, metabolite production and NA structure. The NAs tested differed in the branching of the alkyl group and were: 4-(4'-*n*-butylphenyl)butanoic acid; 4-(4'-*iso*-butylphenyl)butanoic acid (*iso*-BPBA); 4-(4'-*sec*-butylphenyl)butanoic acid (*sec*-BPBA) and 4-(4'-*tert*-butylphenyl)butanoic acid (*tert*-BPBA). GC-MS analysis of enrichment cultures inoculated into media containing individual NAs as the sole carbon source demonstrated complete degradation of the least-branched, *n*-BPBA after 14 days, whilst the other NAs were more recalcitrant. During degradation, a metabolite was identified which corresponded to the loss of two CH_2 groups from the carboxyl side chain and suggests that NA degradation follows the β oxidation pathway. DGGE analysis was applied to characterize the shift in the community structure during degradation. Pure cultures of NA degrading microbes were isolated from these consortia and identified by 16S rRNA gene sequencing. Further experiments using *Pseudomonas putida* KT2440 demonstrated that increasing concentrations of *n*-BPBA up to 4mg/L were toxic. This is the first report to characterize aromatic NA degradation and the micro-organisms involved.

EM 03 Changes in bacterial community structure and geochemistry during technetium redox cycling

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Biogeochemical and geomicrobiological techniques were combined to understand the behaviour of the redox-active radionuclide technetium (Tc) during reduction and reoxidation in anoxic sediments, which is essential in innovating new *in situ* remediation technologies.

Aquifer sediments representative of the UK Sellafield nuclear site and synthetic groundwaters at (i) pH 7 (no added nitrate), and (ii) pH 5 with 10 mmol l^{-1} added nitrate (HNO_3 is a common co-contaminant at nuclear sites), were spiked with Tc(VII)O_4^- and incubated under anaerobic conditions for ~ 250 days. Tc was removed from solution in the 'bioreduced' sediments, presumably through reduction to insoluble Tc(IV). Bioreduced sediments were then exposed to both air and high concentrations of nitrate (25 mmol l^{-1}) to investigate Tc behaviour during environmentally relevant reoxidation. PCR-based 16S rRNA and *narG* gene analyses were used to investigate changes in the bacterial community during both reduction and reoxidation.

During anaerobic incubation over ~ 250 days, the microcosms poised at pH 7 (without added nitrate) and pH 5 (with added nitrate) progressed through a sequence of terminal electron accepting processes (TEAPs) including nitrate, Mn(IV) and Fe(III) reduction, with Tc(VII) removed from solution concomitant with microbial Fe(III) reduction, presumably via reduction of Tc(VII) to Tc(IV) mediated by biogenic Fe(II). 16S rRNA gene analysis demonstrated the presence of *Janthinobacterium* spp., *Pseudomonas* spp., *Burkholderia* spp. and *Desulfosporosinus* spp. during Fe(III) and Tc(VII) reduction in the pH 7 microcosms. *Herbaspirillum* spp. and *Geobacter* spp. were predominant in the pH 5 microcosm (with added nitrate) during Fe(III) and Tc(VII) reduction.

Upon exposure of the pH 7 microcosms to oxygen, reoxidation of Fe(II) was accompanied by ~ 60% remobilization of Tc to solution, presumably as soluble Tc(VII). During reoxidation 16S rRNA gene analysis demonstrated the presence of *Pseudomonas fluorescens*, *Rhodoferax ferrireducens* and *Sphingomas* spp. The addition of nitrate to the prereduced Tc(IV)-containing sediments resulted in microbially-mediated nitrate reduction coupled to the reoxidation of Fe(II), additionally a small amount of Tc was remobilized (< 5%). 16S rRNA gene analysis revealed that most sequences were affiliated with *Betaproteobacteria* from the genus *Janthinobacterium* (79%). Most sequences retrieved using the *narG* gene specific PCR primers were similar to the nitrate reductase gene sequence of *Herminiimonas arsenicoxidans*.

This study demonstrates that Tc(VII) can be reduced and immobilized in sediments under anoxic conditions, with Fe(III)-reducing bacteria implicated in this process. Pre-reduced Tc(IV) can be mobilized by treatments with air, whereas microbial communities dominated by *Janthinobacterium* species were stimulated by high nitrate conditions and reoxidized Fe(II), but did not remobilize Tc. These results have implications for the long-term stewardship of land contaminated with radioactive waste, and treated via biostimulation of metal-reducing bacteria.

EM 04 Photocatalytic inactivation of *Escherichia coli* using fluorescent light irradiated paints

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One current area of interest deals with studying the photocatalytic activity of TiO_2 in order to minimize microbial pollutants in indoor air pollution. Hospitals, clean rooms and other public facilities are places

where a heightened awareness and strict control of bacterial levels are needed. Photocatalytic antimicrobial paints can play an important role in such facilities.

This study examines the effect on inactivation of bacteria, *Escherichia coli* (ATCC 8739) using fluorescent light and several acrylic paints formulations containing TiO_2 photocatalyst PC105 supplied by Millennium Inorganic Chemicals (UK).

The experimental method followed the JIS Z 2801:2000 (E). Bacterial growth / survival was determined by exposing a suspension of *E. coli* to the paint coated film for up to 96 hours, under constant intensity fluorescent light illumination. Cell viability was measured at intervals by total viable count.

The results showed that the presence of $CaCO_3$ reduces inactivation of bacteria from 100% to 20% after 96 h of fluorescent light exposure, indicating that $CaCO_3$ may exert a negative effect on photocatalytic activation of the photocatalyst in the inhibition of *E. coli*.

Results highlight the importance of the composition of paint in the activation of photocatalyst for *E. coli* killing under fluorescent.

EM 05 Effects of some cement constituents on growth of *Aspergillus nidulans*

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We are investigating how fungi respond to exposure to cement dust, an important pollutant in Libya, and its constituents. Incorporation of low concentrations of cement dust or powder into agar media stimulates fungal growth, whereas higher concentrations inhibit growth and spore formation. The involvement of constituents of cement, in particular Ca, Si, and Al, in these effects was investigated using the model fungus *Aspergillus nidulans*. Calcium chloride at concentrations up to approximately 1000 mg/l stimulated growth and calcium silicate at concentrations above approximately 5000 mg/l inhibited growth. Calcium silicate increased spore numbers at 1250 mg/l and reduced them at 5000 mg/l and above. Aluminium oxide had little effect on growth or numbers of spores. The results suggest that growth promotion by Ca ions and inhibition by silicate contribute to the effects of cement dust.

EM 06 Mercury bioremediation: a multi-faceted approach

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Mercury poses an ecological and human health risk due to its volatility, solubility and mobility in the environment. A promising avenue for mercury remediation involves the combination of unique sorbent materials with the mercury fixation capacity of sulfate-reducing bacteria (SRBs) to enhance natural transformation and immobilization processes in the environment. Current laboratory studies focus on: establishing the sorption potential of mercury by nanoparticles of zero-valent iron (nZVI) and charcoal compounds, and exploring the potential of combining SRBs and sorbent materials to capture and stabilize mercury within the environment. Initial results show that nZVI does not significantly retard bacterial growth at concentrations of up to 1g/L. Also, following the initial removal of Hg^{2+} ions from solution by nZVI, there was no indication of increasing Hg^{2+} in solution due to bacterial growth and metabolism. A bi-lateral approach that utilized sorbent materials as an innovative support matrix for sulfate-reducing bacteria would enhance the overall potential for mercury immobilization and could ultimately be incorporated into a long-term site solution.

EM 07 Comparative analysis of an anaerobic community degrading aromatic compounds

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Aromatic hydrocarbons are widely distributed throughout the environment and, due to the relative stability of the benzene ring backbone, often persist for long periods of time. This fact coupled with the toxicity of certain aromatics has resulted in a significant interest in the degradation of these compounds both aerobically and anaerobically. The object of this investigation is to understand the reductive nature of anaerobic micro-organisms, using molecular studies, and use this information as a template to propose new factors for process optimization in the field of anaerobic degradation.

Anaerobic populations were set up using benzoate as a sole carbon source, destructively sampled over the course of the experiment and probed using gene specific primers to gain an insight into a model system which can be adapted for other compounds.

During the experiment we observed an increase of key degradative genes, associated with anaerobic degradation, within the microbial consortium.

EM 08 Understanding the fate of phenolic compounds in anaerobic microcosms

Osadolor Osahon[1,2], Mike Larkin[1,2], Christopher Allen[1,2] & Leonid Kulakov[1,2]

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This research is based on the use of molecular techniques, natural sediments from the river bed of the river Lagan and industrial polluted soils to study molecular ecology. We will investigate the potential attenuation of pollutants including benzene and phenol. Soils sediments from the river Lagan were used as inocula in setting up Winogradsky columns as well as aerobic and anaerobic phenol enrichments. After, incubation times of 7 and 14 days, our results – colorimetric and molecular analysis – show the removal of phenol by more than 80% from aerobic and anaerobic studies. These results were consistent with the presence of genes associated with degradation of phenols in our enrichments. We suggest that application of these methods may offer new insights that can be applied to the challenges of sustainable environmental and waste remediation.

EM 09 Naphthalene dioxygenase of *Rhodococcus* sp. NCIMB 12038: breaking the mould

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A *Rhodococcus sp.* NCIMB12038, initially isolated from pesticide contaminated soil, was found to be able to use naphthalene as sole carbon and energy source. We were able to isolate and characterize the catalytic component of this naphthalene dioxygenase (NDO) which led to further studies into the electron transfer system and in to the genetics of this enzyme. Unlike the model NDO found in *Pseudomonas putida,* studies into the electron transfer system did not result in the identification of a ferredoxin or NADH:reductase associated with the *Rhodococcus sp*. NDO. Studies into the regulation of the *Rhodococcus sp*. NDO resulted in the identification of genes expressed during growth on naphthalene. A comparative study of these regions with other Rhodococci resulted in the elucidation of a cassette of genes expressed during growth on naphthalene. In order to test whether the proteins associated with the upregulated genes were involved with the *Rhodococcus sp*. NDO the genes were cloned and expressed in *E. coli*. Using the catalytic component isolated from *Rhodococcus* sp. NCIMB12038, NDO assays were performed with cell free extracts containing the cloned proteins. The results suggest that the proteins identified are involved with NDO activity and that the NDO present in *Rhodococcus sp*. NCIMB12038 could operate a greatly reduced levels NAD(H).

EM 10 Metagenomic approaches for analysis of bacteriophages ecology in wastewater treatment plants

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Bacteriophages are known to be the most abundant biological entities on Earth. They are natural bacterial predators and therefore they are essential for maintaining of natural diversity of bacterial populations by controlling their size in natural environments. Moreover lytic bacteriophages are agents of lateral gene transfer since approximately 1% of offspring carry host DNA. Due to the lack of cultivation of their bacterial host, actual natural diversity has been studied only recently thanks to metagenomics methodologies.

Wastewater treatment plants contain vast and important microbial populations. The aim of this work is to study ecology of phage populations present in municipal wastewater treatment system and assess the role of transductional gene transfer in this environment. To achieve that we isolated total phages fraction from municipal wastewater and purified it from contaminating DNA. Metagenomic phage DNA was isolate and is purity assessed using a series of control experiments. PCR analysis of the phage metagenomic DNA using universal 16S rRNA primers showed that transduction of bacterial 16S rRNA genes takes place in municipal wastewater environment.

EM 11 Directed evolution of oxidoreductases for biocatalytic applications

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The oxidative degradation pathways of aromatic compounds have been identified for many microbes. In aerobes, the first step of the degradation is often dioxygenation of an aromatic ring by a multicomponent dioxygenase – composed of ferredoxin reductase, ferredoxin, and terminal oxygenase components. The terminal oxygenase component catalyzes the stereospecific dioxygenation of aromatic compounds using dioxygen and two electrons. To date, many dioxygenases have been identified in various degradation pathways of aromatic compounds, with naphthalene dioxygenase (NDO) and toluene dioxygenase (TDO) from *Pseudomonas sp*. being intensively studied. Biochemical studies have revealed their substrate specificity, regiospecificity, and enantiospecificity. From a biotechnology perspective, these enzymes are interesting because they catalyse stereo- and region-selective oxidations which are difficult to accomplish by organic synthesis.

The goal of directed evolution is to accumulate improvements in activity through repetitions of mutation and screening processes. Thus, iterative point-mutation-based approaches are generally limited to improvements made in small steps.

Here NDO & TDO related random mutagenesis is undertaken. We develop new screening methods to facilitate study of these enzymes, and select for improved mutants with specifically required oxidative abilities. The library of mutants for each enzyme is created using two specific methods: error-prone PCR & gene shuffling.

EM 12 The development of assaying methods using dioxygenase/dehydrogenase enzymes from soil bacteria

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There is intense demand for chiral precursors for use in various sectors of industry and, till recently, organic chemistry was the favoured means of producing them. However, improved knowledge and manipulation of biological catalysts offers a potentially cleaner, easier, and (in some cases) cheaper alternative to produce these much desired chemicals. The enantioselectivity of compounds in certain cases makes this route of synthesis more desirable, especially for drug synthesis. Currently, the full exploitation of these biological catalysts has been hindered, partly due to the laborious assaying methods applied to study them. The work here presents the application of alternative assaying methods to these enzymes, to allow for their potential use in screening products in large scale biotransformations, as well as aiding in studying their classical biochemistry.

EM 13 The characterization of a bacterial community capable of biodegrading MTBE and its related fuel oxygenates

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A mixed bacterial culture originating from a site contaminated with BTEX and MTBE has been found to utilize MTBE as is sole carbon and energy source. When the mixed culture was provided with additional fuel oxygenates (ETBE and TAME) or benzene the culture was found to preferentially degrade both ETBE and benzene before utilizing MTBE as its substrate, despite the culture being grown on MTBE for a prolonged period of time. Only once the benzene and ETBE were completely degraded was MTBE used as a carbon and energy source. The microbial community was characterized using 16s rDNA sequence analysis which identified two dominant species similar to *Polaromonas* (JS666) and *Methylibium petroleiphilum* (PM1).

EM 14 Capability of some white-rot fungi to decolorize textile effluent

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Wastewater released from textile and dye industry can cause serious environmental effects due to the presence of toxic dyes and dark coloration. Colour from this effluent makes the receiving water bodies unaesthetic affecting its water transparency and gas solubility. The study has been carried out to know the capability of white rot fungi to decolorizing the textile industry effluent. The simulated effluent with the varying final conc. of 50mg/l and 100mg/l of dyes was used. The fungi were provided with sufficient nutrients to grow in the effluent using submerged liquid fermentation technique. Experiments were conducted both at static and shaking incubation conditions maintaining the optimum pH for the growth of the fungi. Also the percentage of liquid fungal inoculation was varied to get greater percentage of decolourization at the maximum wavelength of absorbance of the dye sample. Preliminarily eleven species of fungi were tested of which four gave good results and were screened for further test on the simulated effluent. *Trametes hirsuta* achieved maximum decolorization under shaking conditions.

EM 15 The effects of ultra-violet radiation on soil microbiota

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Soil microbial communities are complex and important determinants of several physical, chemical and biological properties of soils. In this work we investigated the molecular fate of soil microbiota under ultra-violet (UV) radiation. The microbiota of a light clay-loam agricultural soil was cultured on a standard heterotrophic media, and the resulting biomass was freeze-dried and degraded under UV radiation (300–350 nm) at 25°C. Samples were collected at 0, 6 and 14 weeks post degradation and characterized by Fourier Transform Infrared (FT-IR) Spectroscopy and Gas Chromatography-Mass Spectrometry (GC-MS). FT-IR indicated a significant increase in the lipid content of the degraded biomass with a strong contribution from low branching polymethylenic chains, and noticeable decreases in the proteinaceous and carbohydrate fractions of the biomass. GC-MS analyses of the combined free lipid extracts revealed compounds of

the classes: *n*-alkanes, *n*-alkanoic acids, *n*-alkenoic acids, *n*-alkanedioic acids, monoacylglycerols, hydroxyalkanoic acids, and saccharides. The molecular biosignatures of the soil microbiota indicated good preservation of the recalcitrant lipid fraction but a reduction in labile proteinaceous and carbohydrate fractions during the degradation process.

EM 16 Shifts in quantitative community structures of methanogens in anaerobic bioreactors treating different wastewaters

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Although the microbial communities in anaerobic digestion processes have been extensively studied, little quantitative information on how methanogenic communities evolve and grow in anaerobic processes is yet available. Therefore, this study aimed to quantitatively examine changes in methanogenic community structures associated with changes in chemical profiles. Three anaerobic bioreactors treating different wastewaters (i.e., synthetic glucose wastewater, whey permeate, and liquefied sewage sludge) were operated in batch mode. The quantitative community structure of methanogens, assessed using real-time PCR, significantly varied over time in each reactor and such variations were well correlated with performance data. Biphasic production of methane, with the successive rises in aceticlastic (mainly *Methanosarcinaceae*) and hydrogenotrophic (mainly *Methanomicrobiales*) methanogens, was observed in all trials. The non-metric multidimensional scaling (NMDS) analysis of the quantification data showed that the communities in three reactors shifted totally differently. Given the identical operating conditions except substrates, these differences were probably the effect of different substrate compositions. This implies that wastewater could directly affect the shaping and evolving of methanogenic communities in anaerobic digestion processes. Our results are contrary to the conventional wisdom that methanogenic community structure changes little in diversity (i.e., qualitatively), suggesting the need for quantitative as well as qualitative approaches.

EM 17 Microbial colonization of the biofuel crop oil seed rape

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There has been increased concern over heavy metal pollution in soils not only because of their toxicity, but also due to their persistent in the environment and the fact that they are not biodegradable. Phytoremediation, the use of plants to clean up polluted soils, water or air is becoming a realistic metal remediation option. However, due to the long period of time required to decontaminate soils, it is argued that the method is only considered feasible if biomass is produced during the process. In this study, the phytoprotective abilities of various bacterial strains in protecting plants against toxic effects of heavy metals such as As, Cu, Zn, Cr, Te and Hg were examined. The heavy metal tolerance levels of three targeted bacterial strains, two endophytes, VM1450 and VM1453, and one rhizospheric strain, F113rifPCB was established. The endophytic strains displayed high tolerance levels towards Cu and As, while the rhizospheric strain showed high resistance towards Te. The microbial phytoprotection properties and improved seed germination capacity of microbial inoculated oil seed rape in the presence of heavy metals was also examined, as were the actual microbial plant colonization levels in the presence of target metals. This research aims not only to help in clearing pollutants from contaminated areas, but also to provide phytoprotection in the germination and growth of this environmentally significant crop on polluted sites, by enhancing phytoprotection through microbial inoculation.

EM 18 Mutagenesis of Cys10 and His106 in BphKLB400, a bacterial glutathione transferase

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The *bphK* gene in *Burkholderia xenovorans* LB400 encodes a bacterial glutathione transferase (GST), $BphK^{LB400}$, which has been shown to dehalogenate a number of toxic chlorinated organic compounds, including PCB metabolites and pesticides, thus rendering them less toxic. This detoxification ability is achieved through the attack of the thiolate form of glutathione on the electrophilic centres of these toxic compounds. However, little is known about the specific amino acids in $BphK^{LB400}$ involved in this catalytic reaction. In a recent report of crystalline ternary complexes of $BphK^{LB400}$, Cys10 and His106 were reported to be involved in the dehalogenation of PCB metabolites. In the present study a number of $BphK^{LB400}$ mutants were generated *in vitro* yielding Cys10Phe, Cys10Trp, Cys10Tyr, His106Gly, His106Pro and His106Val. GST activity of these mutant proteins was investigated using a number of PCB metabolites and pesticides as substrates so as to determine the effect of these mutations on the catalytic activity of $BphK^{LB400}$ and the importance of Cys10 and His106 in the active site of $BphK^{LB400}$.

Acknowledgement This work is funded by Embark Initiative Postgraduate Research Scholarship

EM 19 Bacterial endophytes isolated from *Miscanthus*, a potential bioenergy crop in Ireland

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This project aims to investigate the role endophytes may have in bioremediation and plant growth enhancement in terms of nutrient uptake and pathogen fate.

Endophytic bacteria were isolated randomly from the bio-energy plant *Miscanthus* (Elephant grass). The bacteria were identified by ribosomal RNA sequencing and were shown to belong to the following groups *Pseudomonas, Acinetobacter, Bordetella, Bacillus, Exiguobacterium, Janthinobacterium, Rahnella, Pantoea, Sphingobacterium* and *Flavobacterium*.

Isolates were characterized with respect to plant protective and growth enhancement properties, i.e. resistance to heavy metals, antibiotics, degradation of organic compounds, nitrogen fixation, phosphate solubilization, phytase activity, siderophore production, biocontrol activity against fungal and bacterial pathogens.

To investigate the possible effect of these endophytes in planta, *gfp* labelled endophytes were constructed using *pUT* plasmid containing *mTn5-gusA-pgfp21* insert. Plants will be inoculated with labelled endophytes to investigate their colonization and location within plants by fluorescent microscopy.

Acknowledgement This work is funded by the Department of Agriculture and Food- Research Stimulus Fund 2005–Sustainable Agricultural Production Research

EM 20 The influence of the bacterial secondary metabolite 2,4-diacetylphloroglucinol on induced systemic resistance in *Hordeum vulgare*

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Inoculating roots of plants with certain bacteria can lead to the establishment of Induced Systemic Resistance (ISR) in which aerial plant parts are 'primed' to resist pathogen challenge. One bacterial metabolite which is known to trigger ISR is the antifungal compound 2, 4-diacetylyphloroglucinol (DAPG) produced by *Pseudomonas fluorescens*. The mechanism(s) through which DAPG exerts these effects is not fully understood. The aim of our work was to investigate the

molecular basis of the influence of DAPG through analysis of plant gene expression. The DAPG-producing wild type strain *P. fluorescens* F113 and a DAPG-deficient derivative were inoculated into soil into which seeds of barley were planted. Seedlings were then challenged with the fungus *Fusarium culmorum*. Plants inoculated with wild type *P. fluorescens* F113 showed reduced disease symptoms compared to those inoculated with the DAPG non-producing mutant. RT-PCR analysis of extracted RNA showed significant changes in expression of a number of genes including those encoding members of the Myb family of transcription factors.

EM 21 The elucidation of the biofertilization mechanisms in microbial inoculants

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In order to investigate alternatives to chemical fertilizers and to direct agriculture towards sustainability an investigation of the modes of action of Plant Growth Promoting Bacteria (PGPB) was carried out. Phosphate, iron and sulfate are all essential nutrients for plant growth but most forms found in soil cannot be utilized by plants for nutrition. Phosphate solubilizing bacteria can transform organic phosphate into soluble forms. Siderophores are iron chelating compounds produced by micro-organisms that sequester iron making it readily available to the plant. Sulfate mobilization by bacteria transforms sulfate esters or carbon-bonded sulfur into inorganic sulfate which is utilized by the plant for growth.

In this study, the plant growth promoting abilities of 15 isolates were investigated. Through the use of plate assays it was established that eight strains had phosphate solubilizing ability and 14 showed siderophore production. The ability to utilize ethanesulfonate as a sulfur source was assessed though broth assays.

The work carried out to date indicates strong biofertilization potential in the bacteria examined.

EM 22 Low-input agricultural regimes produce an enhanced inorganic phosphate-solubilizing microbial community

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Microbes are the main drivers of ecological processes in a range of soils but our understanding of the diversity and function of such microbial systems is limited, especially with respect to phosphate cycling. Most phosphate forms within soil are not directly available for plant growth but can be made available through solubilization of mineral phosphate – a microbially mediated process. Given the advanced nature of investigative tools now available it is becoming increasingly possible to study the phosphate-solubilizing microbial communities of soils, and also to research the impact of altered agricultural inputs upon microbial communities and their functions in soils. In this project, culture-based methodologies were applied to study the diversity of microbial inorganic phosphate-solubilizers in key managed agricultural sites. We determined that isolates selected from the low-input sites show a greater efficiency to solubilize inorganic phosphate than the high-input isolates, an indication that the microbial community has evolved towards an enhanced phosphate-solubilizing population.

EM 23 Microbial functional diversity in low-input agricultural soil systems

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High agricultural yields are strongly dependent on the addition of chemical fertilizers, which can seriously damage the natural environment. Because of this, there is considerable interest in developing sustainable practices that take advantage of activities naturally present in microbial communities. This strategy is limited, however, by a lack of knowledge of how these microbial communities function in different agricultural systems. From a long-term experiment (15 years) assessing the impact of high and low fertilizer inputs on crop yields, soil samples were used to assess the microbial functional diversity in these systems. Initially, we used the Ribosomal Intergenic Spacer Analysis (RISA) method to compare the diversity of 150 isolates recovered from relevant sample sites. We are currently sequencing representatives of the major phylotypes to identify the dominant species and the next step will be to carry out functional analysis of bacteria to generate an integrated understanding of how the different agricultural inputs impact both on the diversity and the function of the microbial community.

EM 24 Biodegradation of sheep dip wastes as an environmentally safe method of disposal

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The agri-food sector contributes 10% of Irish GNP and 11% of employment, but it is also the main producer of organic and chemical wastes in Ireland. In upland and some lowland grassland areas, sheep production is significant and sheep dips are widely used to control a wide range of animal parasites. Until recently, most sheep dips have been based upon a range of organophosphate chemicals but these have been banned because of their high mammalian toxicity and health problems associated with their use. Sheep dips are now largely formulated from synthetic pyrethroids (pyrethrin), an insecticidal ester originally derived from the flower heads of certain Chrysanthemum species. Although these compounds exhibit a much lower mammalian toxicity than their organophosphate counterparts concentrations as low as 10ng/L have the potential to eradicate invertebrate life in rivers and lakes. The causes of this pollution include loss from the dipping tank, run-off from recently dipped sheep and/or from soil which was used for disposal. The wash from woollen mills in the textile industry is also a potential source of this toxic waste.

At present information regarding the biodegradation of synthetic pyrethroids in the environment is incomplete. The focus of this project is to identify and isolate, using both conventional and molecular approaches, micro-organisms which break down synthetic pyrethroids. Several bacteria were isolated from both uncontaminated soil and soil previously exposed to synthetic pyrethroid sheep dip using submerged fermentation. Extraction and analytical methods were developed to detect this compound in aqeous based systems. Isolates were then screened for their ablility to degrade the synthetic pyrethroid, cypermethrin in submerged fermentation.

EM 25 Response of microbial community structure to anthropogenic activity and environmental factors in Irish peatlands

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Peatlands are unique ecosystems supporting important and rare wildlife habitats. They cover more than 4×10^6 km^2 (approx. 3%) of the earth's total land and freshwater area and are responsible for 40–60% of global methane emissions of which around 74% is derived from microbial activity. Atmospheric concentrations of methane are increasing globally by approximately 1% yr $^{-1}$. The objectives of this study are to assess diversity of the main microbial groups active in Irish peatlands, including bacteria, archaea and specifically methanogenic archaea and methanotrophic bacteria. Such analyses will endeavour to relate microbial community structure to peatland type, human activity and methane fluxes. Molecular community analysis approaches such as terminal restriction fragment length polymorphism

(TRFLP) are being applied, using specific functional and universal PCR primer sets to fingerprint target communities and specific microbial diversity. Organisms associated with methane production (methanogenic and methanotrophic communities) are being targeted by using the functional molecular markers, *mcr* (methyl-coenzyme M reductase) and *mmo* (methane monooxygenase) respectively. The effects of environmental parameters and human influences on peatland microbial community structure will be discussed.

EM 26 Fungal degradation of synthetic pyrethroids

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Synthetic pyrethroids, derived from the natural insecticide pyrethrin, are a group of ester-containing insecticides that were developed as alternatives to more environmentally toxic compounds such as organophosphates. Synthetic pyrethroids are used in the control of sheep scab and a wide range of crop pests. They are also used as prophylactics against the spread of blue tongue virus, are formulated as head lice shampoos and are found in residues from the wool and carpet manufacture industries. Although synthetic pyrethroids exhibit high insecticidal activity and low mammalian toxicity compared with other insecticides they are toxic to fish and aquatic organisms and leaching of these compounds into watercourses is a major environmental issue. Microbial degradation is considered to be the principal route for pyrethroid removal in the environment. Although a number of bacteria have been isolated and characterized that are capable of detoxifying these compounds there are few reports to-date of pyrethroid biotransformation by fungi. The aim of this study was to examine the ability of selected soil fungi to degrade the synthetic pyrethroid, cypermethrin. Based on the results of an initial screen, *Trichoderma longibrachiatum* was selected for further investigation as this fungus removed >50% of cypermethrin added to a submerged fermentation after 10 days. Conditions for cypermethrin degradation by this fungus were optimized and a pyrethroid hydrolase involved in degradation was isolated and basic properties of the enzyme determined.

EM 27 Analysis of changes in gene expression in soil exposed to 2,4-dichlorophenol using RNA arbitrarily primed PCR

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2,4-Dichlorphenol, a by-product of 2,4-D metabolism, is a recalcitratant and highly toxic chemical widespread in the terrestrial and aquatic environment. With its carcinogenic and mutagenic properties it is not only of concern in the environment but also to human health. The addition of xenobiotics to the environment is hypothesized to strongly influence soil community dynamics. Although much is known about 2,4-DCP degradation in pure culture systems, relatively little is known about *in situ* biologically-mediated degradation of 2,4-DCP, particularly in terms of the micro-organisms functionally involved. The purpose of this study was to profile differential gene expression in a soil exposed to 2,4-DCP. A microcosm was set up with soil artificially contaminated with 100 mg/kg of 2,4-DCP and degradation was monitored over a period of 21 days. Total RNA was extracted at selected at selected time points. Differential expression of mRNA in the treated soil was assessed using the RNA fingerprinting method arbitrarily primed PCR (RAP-PCR) where no prior knowledge of the target genome is required. Changes in RNA fingerprints were observed over the time course using a range of primer sets. A wide variety of differentially expressed genes were detected, with a number of novel nucleotide sequences identified. Relative abundances of a number of genes identified in this study were subsequently quantified by Real-Time PCR and indicated different levels of expression at each time point.

EM 28 Effect of mixtures of polycyclic aromatic hydrocarbons on microbial communities in soil

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Creosote is a persistent chemical mixture composed of approximately 85% Polycyclic Aromatic Hydrocarbons (PAHs), 10% phenolic compounds and 5% *N*-, *S*- and *O*-heterocyclics. It has been extensively used as a commercial timber preservative in wood-treatment plants, leading to widespread contamination of soils and groundwaters underneath wood treatment plants and in adjacent areas. Bioremediation is a potentially cost effective and safe approach for the rehabilitation of PAH contaminated sites. However, it remains an unpredictable technology due to a lack of understanding of the microbial communities involved in bioremediation and the factors affecting these communities. PAH contaminated environments typically contain a mixture of low and high molecular weight PAHs but little is known about the factors influencing microbial degradation of complex mixtures in soil . The aim of this study was to examine the effect of mixtures of PAHs on degradation and microbial communities in a soil. Soil used in the experiment was obtained from a former creosote manufacturing facility. A microcosm experiment was set up containing soil amended with a 3 ring (phenanthrene), a 4 ring (fluoranthene) and a 5 ring (benzo[a]pyrene) PAH in various combinations. The rate of disappearance of individual PAHs was measured by gas chromatography. The influence of the PAHs on microbial community structure was assessed using both culture dependent and culture independent methods (ARISA, TRLFP). Dehydrogenase activity was used as an indication of general microbial activity in the soil. Results indicated that microbial communities in the soil readily removed phenanthrene and fluoranthene. The level of benzo[a]pyrene didn't change significantly during the course of experiment but this PAH did not negatively influence removal of other PAHs.

EM 29 Investigation of the potential for low pH induced, phosphate removal from high nutrient load, synthetic dairy industry waste water, by an activated sludge from a dairy processing plant aeration tank

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Industrial groups generating high nutrient wastewaters face continually tightening legislation with regard to permissible outputs of inorganic nutrient effluent loads, (e.g. nitrogen and phosphorous), to prevent pollution of receiving water bodies. Approaches to the remediation of phosphate from such waste waters is often based on precipitation with metal salts, generating metal contaminated sludge requiring redirection to landfill. This study investigates application of the relatively novel biological approach of low pH induced, activated sludge phosphate accumulation, to the remediation of phosphate in high nutrient load, dairy industry wastewaters. A sequencing batch reactor system was seeded with activated sludge from a dairy food ingredients processing plant aeration tank and exposed to a synthetic feed at pH 6.0, containing increasing COD:P:N ratios up to a maximum of 2000:100: 30 mg/L, respectively. Under these conditions, removal of 65–80mg/L phosphate was achievable, following a 4week period of acclimation. Further reductions in pH to 5.4 and 4.5, as well as increased sludge retention times were subsequently investigated, but only resulted in reduced P removal capacities. Based on the stability and reproducibility of the system in our studies to date, it seems that this approach may offer a feasible biotechnological approach to phosphate remediation of industrial, high phosphate load waste streams.

Acknowledgement Funding for this work was provided under the FIRM initiative of the Department of Agriculture, Fisheries and Food

EM 30 Genetic manipulations of the *sty* operon in *Pseudomonas putida* CA-3 to enhance the rate of styrene metabolism

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Pseudomonas putida CA-3 is a bioreactor isolate capable of degrading the toxic pollutant styrene. In addition, under appropriate growth conditions, this strain also has the ability to redirect styrene catabolism to the production of poly-hydroxyalkanoates, a potential source of biodegradable plastics. Here we seek to enhance the ability of *P. putida* CA-3 to degrade styrene by applying recombinant DNA approaches to increase the flux of styrene through the pathway and eliminate potential bottlenecks. The *styABCDE* operon encodes the genes necessary for the conversion of styrene to phenylacetic acid and analysis of the promoter region has identified the presence of three separate transcriptional regulator binding sites which, under different growth conditions, are involved in the transcriptional activation or repression of the *sty* operon. One site located downstream from the transcription start site of the *sty* operon is the target of negative regulatory influences, including catabolite repression. Site-directed mutagenesis has been used to disrupt this inhibitory binding site and the effect of these base pair changes on the transcription of the catabolic genes and on overall styrene degradation is currently being examined under mixed substrate growth conditions. In addition we have previously demonstrated the role of a *styE* gene encoded active transport protein in styrene uptake and increased transcriptional activation of the *sty* operon. We are currently investigating the effects of random mini-Tn*5* transposon based chromosomal introduction of a p*styA-styE* construct on pathway activity in the presence of styrene. Finally, the effects of increased *styABCD* gene copy numbers will also be examined.

EM 31 The microbial ecology of low-pH induced biological phosphate removal from synthetic dairy wastewaters in lab-scale sequencing batch reactors

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A current area of significant research in the field of bioremediation is the microbiology of biological phosphate removal (BPR) from wastewaters. A major focus involves attempts to correlate successful and unsuccessful BPR with the sludge microbial community structure to allow identification of physiologically relevant organisms and the conditions under which they proliferate. In the current study, a lab scale SBR was operated at a low pH and was supplied with a synthetic dairy waste influent. Several isolated periods of high phosphate removal were observed and were demonstrated to be reproducible. The temporal stability of the microbial structure of the sludge throughout was studied using DGGE analysis of the 16S rRNA gene. More detailed resolution of the microbial ecology of the sludge during successful BPR was obtained by generating 16S rRNA clone libraries. It was observed that periods of successful and stable BPR were associated with stable microbial sludge consortia.

EM 32 The nitrilase gene of *Rhodococcus erythropolis* AJ270 is carried on a mobile genetic element

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Nitrile metabolism has been extensively studied in numerous bacterial species, due to the potential application of the associated enzymes for biotransformation and bioremediation. Four isolates, *Microbacterium* sp. AJ115, *Rhodococcus erythropolis* AJ270, AJ300 and ITCBP, are known nitrile-metabolizers, and harbour both a two-step nitrile hydratase/amidase enzyme system and a one-step nitrilase system. The isolates contain identical nitrilase genes. Cloning and expression of the nitrilase gene from strain AJ270 in *E. coli* has allowed determination of nitrilase activity. The enzyme appears to be very substrate specific with phenylacetonitrile the only substrate identified to date.

Real-time PCR analysis has indicated that the nitrilase gene is carried on an unstable, variable copy number mobile genetic element. Sequences showing homology to integrases, IS elements and antibiotic resistance genes have been identified on this element also. Restriction enzyme analysis using PFGE is currently being used to determine the size of the element. Preliminary metagenomic analysis of soil samples using Real-Time PCR suggest that this nitrilase element may be widespread in the environment.

EM 33 *Abstract withdrawn*

EM 34 Biodegradation of toxic pollutant – *N,N*-dimethylformamide

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Background N,N-dimethylformamide (DMF) is widely used in the chemical industry as a versatile solvent, a solubilizing agent or a reaction medium. For this reason it is commonly found in industrial effluents, leading to environmental pollution. DMF is toxic to human beings and other organisms (hepatotoxicity, embryotoxicity, teratogenicity) and it shows a slow rate of degradation in the environment.

Results We found that plasmid pAMI2 of *Paracoccus aminophilus* (*Alphaproteobacteria*) carries a phenotypic module responsible for degradation of DMF. This module is composed of three genes: *luxR* (encoding a regulator), *dmfA1* and *dmfA2* (encoding subunits of *N,N*-dimethylformamidase – DMFase). We constructed a mobilizable, broad-host-range vector pDMF, carrying a functional DMFase cassette. This vector was introduced into several strains of *Paracoccus* spp. It appeared that one of them (*P. alcaliphilus*) gained the ability to degrade DMF at very high concentrations.

Conclusions We constructed a modified strain of *P. alcaliphilus* which can be used for rapid and efficient degradation of DMF. This strain might be a convenient tool for biotreatment of DMF contaminated industrial wastewaters.

EM 35 Bioremediation of organic-rich copper tailings at neutral or slightly alkaline pH by indigenous micro-organisms

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Polish copper industry produces 25 millions tones of tailings per years which contain depending on the origin 2.7–3.5 millions tones of black shale. This organic fraction contains considerable reserves of noble and valuable metals which occur in sulfide mineral particles and metalloorganic complexes merged with sandstone and dolomite.

In present study the role of indigenous micro-organisms in bioremediation of copper wastes was evaluated. Micro-organisms were isolated from black shale ore, copper tailings and middlings. They belonged to γ-Proteobacteria, Firmicutes, Actinobacteria and Flavobacteria.

All indigenous bacteria are active in biogeochemical cycles and their activity may strongly affect the environmental conditions as well as they can be useful in bioremediation. Micro-organisms are able to use black shale ore as a carbon and energy source. The biodegradation of organic matter is accompanied by release of metals. A part of micro-organisms can sorb metal and accumulate metalloporphyrins. Exopolymer produced by micro-organisms can modify the mineral surface, solubilize metal sulfides and create ecological microsites around bacteria enhancing indirect bioleaching.

EM 36 *Abstract withdrawn*

EM 37 Electricity generation from organic acids by microflora enriched from thermophilic methanogenic sludge

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Electricity generation by micro-organisms has been attracting attention as a new way of biological energy conversion. In order to investigate the mechanism of electricity generation by microflora, a fuel cell type cultivation apparatus (125 ml) was set up. Methanogenic microflora was continuously cultivated at hydraulic retention time (HRT) between 1.8 and 7.2 day at temperature of 55 centigrade. The medium contained acetate and butyrate as electron donors. The electrical circuit was composed by the connection of 560 ohm resistance between anode and cathode made of graphite. During 180 days of operation, electricity generation gradually increased and maximum output of 1.1 W/m^3 was observed at 1.8 day of HRT. In the clone library analysis targeting on 16SrDNA, it was found that some kinds of micro-organism were enriched on the anode. Most of them were affiliated to unidentified micro-organisms that are related to syntrophic micro-organisms, although hydrogenotrophic methanogens were not detected. These findings imply that these unidentified micro-organisms are involved with electricity generation, and that unidentified mediator material may exist in the culture system. This work was supported by NEDO.

EM 38 *Abstract withdrawn*

EM 39 *Abstract withdrawn*

Posters

Environmental Microbiology Group

ET 01 Demonstration of microbial interactions in undergraduate laboratory classes

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The vast majority of the planet's micro-organisms exist attached on surfaces. Attached cells may multiply to form biofilms.

Within a relatively constrained undergraduate curriculum, it is not always easy to explore biofilms, especially if lab classes are run for 3–4 hours on a weekly basis. However, interactions between micro-organisms are key to the development and existence of, the biofilm community. Such interactions can be demonstrated within relatively short time periods, assisting student appreciation of the complexity of the biofilm lifestyle.

(i) Adhesion. Immersion of a substratum in standardized cell suspensions for one hour will enable adhesion to occur. Attached cells can be stained and enumerated.

(ii) Co-adhesion. The attachment of a second micro-organism to those cells already attached illustrates a route for secondary colonization.

(iii) Autoaggregation. Some culture suspensions autoaggregate in the presence of an external facilitating agent (eg *Streptococcus mutans* with sucrose, but not with glucose).

(iv) Coaggregation. Mixing suspensions of two different genera and observation of a drop in optical density due to cell-cell interaction and the formation of aggregates, illustrates coaggregation.

(v) Biofilm formation. Using microtitre plates, cultures in wells can be stained, and the density of stain, related to the amount of biofilm, measured using ELISA plate readers.

All of these methods are derived from published papers, and are easily modified. Students can investigate the effect of external factors on these properties, and relate findings to the literature.

ET 02 Undergraduate laboratory investigations into the microbial contamination of frequently used items

Joanna Verran

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These laboratory classes have been carried out with final year undergraduates. The aim is to demonstrate contamination of frequently handled inert items, and to thus consider sources of contamination, and potential for cross-contamination.

(i) Mobile phones. Students pressed their mobile phones onto large agar plates before and after wiping with an antibacterial wipe, during three consecutive weekly classes. In the first session, students also swabbed their own skin (eg ear, cheek, hand). If students did not wish to use their phones, then alternative inert objects were used (eg laptop keys, pencil). The range of contaminant species/colonies were noted, and presumptive staphylococci were subcultured from phone and skin plates, and identified. Antibiotic sensitivity profiles were also determined.

(ii) Toothbrushes. Students brought their toothbrushes to the laboratory. Contamination of the toothbrush head was determined by immersion in diluent, followed by sonication and vortex mixing, and the resultant suspension was plated onto a range of culture media, incubated both aerobically and anaerobically. The range of contamination was compared with the cultivable flora of dental plaque. Students were given replacement toothbrushes (and toothpaste) after the class.

ET 03 Undergraduate research and the biofilms online exercise collection

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Few approaches have been more productive in engaging students than enabling them to carry out laboratory projects of their own design. These experiences can be the first steps on the path to careers in science. Research studies suggest that students who engage in undergraduate research learn more, have increased academic retention and enhanced completion of graduate degrees.

The Biofilms Online Collection is a useful 'toolbox' of protocols for enabling students to design their own research programs in biofilm microbiology. It is intended to be useful to instructors in locating resources for inquiry-based labs to motivate students through undergraduate research experiences? The exercises presented are derived from research protocols. They are inexpensive, and use readily available materials.

Hits on the web site and interviews with teachers and students who have used the materials suggest the collection is having the effect desired in encouraging independent student investigations.

The materials described were developed under NSF Grant 0618744, and will appear as a component of *Biofilms: The Hypertextbook*.

ET 04 Biofilms as biobarriers

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There is growing recognition of the importance of Biofilms in the undergraduate curriculum. Much of the emphasis has been on the clinical implications of biofilms. This emphasis, while important, does not detail the significance of biofilms over all. In this exercise, Biofilms and Biobarriers, students simulate an important tool in the hands of environmentalists attempting to control the spread of hazardous materials in ground water. Biofilms composed of harmless micro-organisms, introduced into porous media such as sand, can substantially reduce the rate of dispersal of potentially hazardous materials. Such Biobarriers have demonstrated their effectiveness in laboratory, and field plot tests and in the containment of hazardous materials in ' real life' incidents.

In practice, a column, partially filled with a porous 'soil simulating' material such as sand, is charged with a BSL1 organism and 'fed' a medium that stimulates EPS production. Flow rate of the medium through the biofilm is recorded, and plotted over time. This exercise has been approved for inclusion in a collection of Biofilm educational exercises.

Website www.biofilmsonline.com

Acknowledgement Developed under NSF Grant 0618744

ET 05 *Abstract withdrawn*

Posters

Eukaryotic Microbiology Group

EukM 01 Chromatin-mediated silencing of LTR retrotransposons

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Long terminal repeat (LTR) retrotransposons are mobile genetic elements that are present in the genomes of all eukaryotes. They mobilize via an RNA intermediate and are functionally related to retroviruses. Eukaryotic cells must restrict the expression of these elements because their mobilization may disrupt or alter the expression of host genes. One cellular defence mechanism is to package these elements in repressive chromatin in order to limit their expression and spread. Indeed we have found that the Tf2 LTR retrotransposons of *S. pombe* are associated with a distinct form of silent chromatin. In contrast to the centromeric repeats and the mating type locus, the silencing of Tf2 elements does not depend upon histone H3 lysine 9 methylation or the heterochromatin protein 1 (HP1) homologue, Swi6. Furthermore, Tf2 expression is not affected by loss of the RNAi machinery. Instead, Tf2 silencing is dependent upon the HIRA histone chaperone as northern analysis showed that disruption of these complexes led to a large (>10-fold) increase in Tf2 mRNA levels. However, as the 13 full-length Tf2 elements are a homogenous group, it was not clear whether this increase resulted from the de-repression of a subset or all the Tf2 elements. Therefore the Tf2 elements were systematically marked with a reporter gene. Analysis of these reporters revealed that their basal level of expression is dependent upon chromosomal context and that all elements are subject to HIRA-mediated repression. Although the Tf2 retrotransposons are the only full-length retroelements in the *S. pombe* (972) genome, there are 274 solo LTR elements that are the remnants of extinct retrotransposons. Initial analysis has suggested that in cells lacking HIRA at least a proportion of these solo LTR elements function as strong RNA polymerase II promoters. This suggests that the HIRA histone chaperone complex is required to silence both intact retrotransposons and also solo LTR elements.

Posters

Fermentation & Bioprocessing Group

FB 01 Production of succinic acid from cellulose employing the cellulolytic bacterium, *Fibrobacter succinogenes* S85

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Succinate is an important bulk chemical employed in a wide range of industrial applications. It is used as a surfactant, ion chelator, and as an additive in the pharmaceutical and food industries. Traditionally, succinate is synthesized petrochemically from maleic anhydride. Recently, several industrial plants have been established to produce succinate from glucose, taking advantage of succinate-overproducing bacteria. Bio-based succinate is more cost-competitive compared to the petroleum-based one, but is still limited by the high feedstock price. However, cellulose is the most abundant carbohydrate in the world and several cellulolytic bacteria are known to be excellent succinate producers, among them *Fibrobacter succinogenes* S85 is best known. In the present study, the effects of media composition on the performance of *F. succinogenes* S85 were investigated in anaerobic culture tubes. The process has been successfully scaled up to 1 L, in a modified stirred tank bioreactor, in which the effects of bioprocess parameters, such as pH and redox state of the media, on the performance of fermentation were examined. Our results demonstrate, for the first time, that it is viable to produce succinate from cellulose in a more cost-efficient and more sustainable way.

FB 02 Keratinase production by proteolytic micro-organisms in activated sludge and farmyard waste

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Thirty-two microbial strains from activated sludge and farmyard waste were isolated on feather-meal agar. Five of these isolates demonstrated significant proteolytic activity raising prospects of biotechnological application. The keratinolytic activity was assessed by casein-agar plate assay and further confirmed by keratinase assay using keratin azure as substrate. One of the five isolates (a Gram-positive cocci bacterium) produced the highest keratinase activity within 24h at a cell density of 5.2 x 10^8 CFU/mL under the optimized conditions of pH 10, 37°C and substrate concentration of 0.4 g/L. The crude extract gave an activity of 14 U/mL. Addition of carbon (starch) and nitrogen (yeast extract and peptone) sources caused a decline in enzymatic activity. The culture supernatants was purified by ultra filtration, concentrated by Amicon Ultra-15 centrifugal filter device and further purified by HiTrap affinity chromatography column. The resulting fraction has a purification factor of 108.7 and specific activity of 2000 U/mg. The molecular weight of the enzyme was determined by Matrix-assisted laser desorption/ionization (MALDI) to be 27.9 KDa.

FB 03 Biomass to biofuel: generation of cellulose-based biomass degrading strains of brewery yeasts

James Fitzpatrick, Ursula Bond & T.C. James

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As the world enters the post-fossil fuels era, there is now the need for environmentally sustainable energy sources. One such potential energy source is the use of biofuels derived from biomass or known more specifically as lignocellulose. Cellulose, the main component of lignocellulose, is made up of thousands of repeating units of the disaccharide cellobiose. Consequently, cellulose represents a major reservoir of sugar that could be potentially converted to alcohol and used as a fuel source. The most characterized micro-organism known to ferment simple sugars to ethanol are yeasts belonging to the *Saccharomyces stricto sensu* group, however *Saccharomyces* species do not possess the enzymes needed to degrade cellulose. These enzymes are known as cellulases. There are three major types of cellulases: endoglucanases (EG), cellobiohydrolases (CBH) also known as exo-glucanases and β-glucosidases (BGL). The filamentous fungi *Trichoderma reesei* is known to have five EG genes, at least two CBH genes and two BGL genes.

The aim of this project is to combine the fermentative capacity of *Saccharomyces* species with the cellulolytic ability of *T. reesei*. Thus far, we have successfully cloned three cellulolytic genes of *T. reesei* separately into a haploid strain of *S. cerevisiae* S150 and also into the polyploid *S. pastorianus* strains CMBS and C10B-51. Saccharomyces pastorianus strains possess a high fermentative capacity and convert sugars to ethanol more efficiently than *S. cerevisiae* strains. The cellulase genes were transformed into the target organism from genomic DNA via *in vivo* homologous recombination cloning. We are currently examining the expression of these genes by RT-PCR and Western blotting.

FB 04 Identification of pathways and proteins involved in tolerance to weak organic acids in *Kluyveromyces marxianus*

Melanie M. Lane & John P. Morrissey

Microbiology Dept, University College Cork, Ireland

Kluyveromyces marxianus is a homothallic ascomycetous yeast with multiple applications in industry. It is exploited for the production of biomass, ethanol, enzymes, single cell protein and heterologous proteins. Like other food microbes, sensitivity to organic acids can impair growth and efficacy of yeasts. We therefore undertook a study of organic acid and pH sensitivity of *K. marxianus*. We determined that *K. marxianus* was specifically sensitive to organic acids, rather than low pH. Variation in tolerance to different organic acid within the species was also observed. Some *K. marxianus* strains were tolerant to elevated concentrations of citric acid but sensitive to lactic acid, whereas others displayed the opposite trend. Current work is focused on understanding the mechanistic basis of tolerance to different organic acids using both targeted and global approaches. Targeted genes are those that have been shown to be key to organic acid stress response in other yeasts. In addition, proteomic analysis in *K. marxianus* is being employed using 2D gels to identify key proteins that mediate tolerance to weak organic acid.

FB 05 Maximizing biohydrogen production by *Escherichia coli*

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Escherichia coli is a facultative anaerobe that, under anaerobic conditions and in the absence of any exogenous respiratory electron acceptors, can perform a mixed acid fermentation. The main products of this process are ethanol, acetate, lactate, and formate. All of these are secreted out of the cell and, under closed batch culture conditions, the fomate levels reach a critical extracellular threshold before being taken back up and disproportionated into CO_2 and H_2 gas. This reaction is catalysed by the formate hydrogenlyase (FHL) complex comprising a molybdenum-dependent formate dehydrogenase and a Ni-dependent hydrogenase. Thus, hydrogen production by *E. coli* is strictly dependent on both cellular formate metabolism and the activity of FHL. Biological approaches to hydrogen production (so-called 'biohydrogen') are growing in importance as fossil fuel resources verge on the limits of economical extraction, and the environmental impact of carbon

emissions gains long-overdue recognition. This project involves genetically altering the metabolism of *E. coli* so that it produces more hydrogen than normal and uses a wide variety of carbon sources for that production, which will be of interest to the energy industry, waste-management businesses, and other environmental biotechnology companies. By tinkering with native gene regulation, enzyme maturation pathways, and formate transport systems, as well as designing and building synthetic enzymes for expression in this amenable host organism, a library of synthetic 'superstrains' will be developed that are enhanced for biohydrogen production.

FB 06 The effect of using different techniques for increasing cell density on biological hydrogen production by *Escherichia coli* HD701

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E. coli HD701, a hydrogenase up-regulated strain has the potential for industrial-scale H_2 production on an energetically self-sufficient basis. To make this an industrially capable and energetically efficient process an increase in cell density is needed. The techniques used to increase cell density are; the alteration of the medium to promote higher cell growth and the change from batch to Fed-batch cultivation. These changes while increasing cell density have a knock on effect on cell metabolism, which in turn will determine the cells ability to produce hydrogen. The trade off between conversion efficiency (on a per mol. Substrate basis) and energetic efficiency (on a per mole of hydrogen produce per mole of hydrogen required basis) will be assessed.

Posters

Food & Beverages Group

FdBev 01 A comparison of industrial and analytical methods used for the detection of food soils and cells on stainless steel

Kathryn A. Whitehead & Joanna Verran

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The preparation of food results in the transfer of soil and cells to the substratum. The origin of the food soil will affect the efficiency of cleaning regimes. Inappropriate or inefficient cleaning may result in accumulation of soil on a surface, which may affect the retention of micro-organisms. The nature of the soil and cells deposited is determined by the food product; however the composition of the retained soil is usually unknown. As a result inappropriate cleaning regimes may be used leading to a continual soil buildup on a surface which may in turn enhance or retard potential pathogenic cell attachment. Further, the buildup of soil may provide a nutrient source and protection for attached cells and result in subsequent food contamination in downstream processing. In order to fully understand the nature of soil deposition at the soil: substratum interface a range of rapid analytical and industrial methods was used to compare the detection of soil on stainless steel. Chemical methods included Energy Dispersive X-ray (EDX) and Fourier Transform Infrared Spectroscopy (FTIR), whilst surface physicochemical methods included contact angle, surface free energy, dispersive and polar measurements. Microscopy methods *in vitro* included Scanning Electron Microscopy (SEM), and epifluorescence microscopy, including a differential staining method whilst rapid industrial methods included ATP (adenosine triphosphate) bioluminescence and an ultra violet (UV) light detection method. This diverse range of techniques demonstrated that although rapid industrial methods can detect soil *in vivo,* at lower concentrations of soil they were not always effective. The use of more complex analytical methods allows insight into the nature and effect of soil on surface properties and on subsequent cell attachment and retention. The nature of soil should be defined to ensure effective cleaning regimes contribute appropriately towards maintenance of hygienic status of food contact surfaces.

FdBev 02 Factors affecting the colonization of food processing surfaces by *Listeria monocytogenes*

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A major concern in the food industry is the contamination of preparation surfaces by pathogenic micro-organisms, and those involved in spoilage. *Listeria monocytogenes* is a human pathogenic bacterium which may contaminate food and food preparation surfaces. The ability of *L. monocytogenes* to attach to, and be retained on, food contact surfaces is important for survival in food processing plants, where surfaces are continually being cleaned and disinfected. In addition, residual organic soil (ie food material) on the surface may reduce cleanability and the effectiveness of disinfection, as well as affecting the retention and survival of the attached micro-organisms. It is therefore important to control surface contamination and cross-infection by implementing effective cleaning and disinfecting protocols, to ensure optimum product safety and plant hygiene.

The aim of this work was to differentially assess (using staining and culture techniques) the retention of *L.monocytogenes* and surface soil (fish extract) on stainless steel following a number (0–30) of cleaning cycles in the presence and absence of industrial cleaning products. In general, organic soil was less easy to remove, and some indication of accumulation was evident. The presence of residual soil may interfere with the subsequent hygienic status of the surface due to interactions with contaminating micro-organisms and/or cleaning products/components. It is therefore valuable to assess the presence of both soil and micro-organisms on surface before and after cleaning.

FdBev 03 Effect of duck meat on the adhesion of *Listeria monocytogenes* serotypes to glass and solvents

Ogueri Nwaiwu, H.T. Lawrence Wong & Catherine Rees

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Reports from manufacturers have indicated that *Listeria monocytogenes* is often associated with products containing cooked duck meat, and is often found in the environment from areas in factories where duck meat is processed. As the bacterium is not expected to survive the temperatures used to cook the meat product, this suggests that the presence of the bacterium in the product is occurring as a post-process contamination, possibly because the meat itself supports growth of the organism.

In this study laboratory strains of *Listeria monocytogenes* were compared with isolates recovered from one such factory environment. Cultures were prepared using a minimal medium (D10), a rich medium (BHI) and also the D10 medium supplemented with duck meat extract. Growth in the BHI medium was fastest, but significant increases in growth were seen when D10 was supplemented with 10% duck meat extract. One feature of cells that become persistent in factory environments is their ability to form biofilms that are resistant to cleaning and disinfection. Therefore microbial attachment to solvents (MATS) assays and the blot succession method were both used to measure the hydrophobicity and adhesive properties, respectively, of the different test strains grown in the different media.

Variation in the surface properties of different isolates were seen, but generally results indicated that adhesion was highest in cells grown in minimal D10 medium with duck meat supplement. This suggest a change in the surface properties of the organism occurs that relates to the presence of nutrients provided by this supplement, and that this change is not related to limited nutrients or growth rate *per se*. In addition, dramatic differences in the MATS assay results were obtained depending on the volume of the solvent used, suggesting that different forms of emulsion may be produced that influence this type of study.

FdBev 04 Investigation on the microbial spoilage of sweet potato (*Ipomea batatas*)

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The spoilage of sweet potato (*Ipomea batatas*) is a significant problem in Nigeria and constitutes the object of this study. Sweet potato samples were stored at three different temperatures, 13°C, 21°C and 29°C for a period of four weeks. Samples were collected over the storage period and viable counts determined on potato dextrose agar (PDA) and tryptone soya agar (TSA). Aerobic microbial counts were obtained from the samples as well as from swabs taken from the storage environment. Bacterial counts on TSA at 13°C ranged between (log 10) 6.037 and 4.580 cfu/ml, at 21°C counts ranged between 7.991 and 3.519 cfu/ml, while at 29°C counts were between 4.857 and 3.491 cfu/ml. The swab counts were (log 10) 5.049 on TSA, and 4.987 on PDA. Sub-culturing was carried out, and axenic cultures were Gram-stained. All the bacteria isolates were Gram-positive. Biomolecular methods which include DNA extraction, the polymerase chain amplification of rDNA sequences as well as sequence determination were employed in the characterization and identification of the bacteria isolates. Results revealed nine (9) different bacteria. These

include *Bacillus fusiformis*, *Arthrobacter woluwensis*, *Klebsiella pneumoniae*, *Lysinibacillus fusiformis*, *Staphylococcus sciuri*, *Staphylococcus gallinarum* NT-S, Marine sediment bacterium ISA-7256, *Staphylococcus kloosii* and *Bacillus pumilus*. Fungi were recovered which included yeasts and filamentous fungi that were discriminated on the basis of phenotypic observations. Filamentous fungi included *Penicillium* sp., *Aspergillus* sp., *Mucor* sp. and *Rhizopus* sp. Further characterization is underway using DNA methods.

FdBev 05 Production of reduced ethanol beer by metabolic engineering of the brewing yeast *Saccharomyces cerevisiae*

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Increasing health and societal concerns have raised awareness of the dangers of consumption of excessive quantities of alcohol. As such, the production of reduced alcohol beverages has recently attracted much attention. Current methods to reduce ethanol content include distillation, interrupted fermentations and molecular sieving but these methods have the unfortunate side effect of negatively influencing the organoleptic properties (taste, viscosity, etc.) of the final product. The brewing yeast *Saccharomyces cerevisiae* is a Crabtree positive yeast, i.e. in the presence of oxygen and elevated external glucose concentrations, glucose metabolism produces energy, CO_2 and ethanol. We are attempting to alter the metabolic pathways of this yeast using metabolic engineering in order to reduce ethanol production without significantly altering the organoleptic profile of the resulting beer. Three strategies are being employed, namely the overexpression and/or deletion of genes encoding enzymes at critical points in the metabolic machinery, the replacement of key metabolic enzymes with those from a Crabtree negative yeast (*Kluyveromyces lactis*) and the limitation of the import of maltose, the predominant sugar in beer-making wort.

FdBev 06 *Cronobacter* and the risk to infant health

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Background *Cronobacter* is a recently proposed genus that accommodates novel species previously referred to as *Enterobacter sakazakii*. These organisms are implicated in neonatal infections. A survey of *Cronobacter* in infant drinks and a review of cases of infections in children were undertaken.

Methods Infant drinks were collected in Europe during March 2008. Samples were pre-enriched in BPW, 100µl incubated overnight in *Cronobacter* Screening Broth, 10µl streaked onto TSA and DFI agar and presumptive positive isolates confirmed by real-time PCR. All isolates were identified biochemically and characterized using PFGE and rep-PCR.

Results 470 samples were tested, 16 products were found to contain Gram-negative organisms. *Cronobacter* was found in two brands of cereal-based infant drinks. Of the >123 documented cases, only 4 occurred in children >3 months (1x tonsillitis, 1x intradural dermoid cyst and 2x bacteraemia).

Conclusion Considering the diversity of food and drink that infants consume from 4 months and the low incidence of infection in infants >3 months, the health risk posed by *Cronobacter* in follow-on infant drinks seems negligible.

FdBev 07 Antimicrobial peptides preventing beer spoilage

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Antimicrobial peptides (AMPs) unlike many antibiotics are broad-spectrum in their activity. In innate immunity they act as a first line of defense against hostile bacteria. Human beta-definsin 3 (HBDEF3) is perhaps the most active AMP known to date, displaying antibacterial activity against a broad range of bacterial species such as *S. mutans, L. acidophilus, P. gingivalis* and *H. pylori*.

Beer has been recognized for hundreds of years as a safe beverage. It is difficult to spoil and has a remarkable microbiological stability. Beer is an unfavorable medium for many micro-organisms due to the high ethanol concentrations, high levels of carbon dioxide and low pH. However, a few micro-organisms, such as the lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus,* can infect beer fermentations. We have examined the capability of the AMP, HBDEF3, to kill beer spoilage bacteria and likewise have tested the efficacy of HBDEF3 to kill the yeasts. Our results indicate that Lactobacilli strains are highly susceptible to killing by beta defensin. Concentrations of < 1µg/mL of HBDEF3 led to 100% killing of beer-spoilage strains such as *L. brevis* and *L. brevismilis*. Lager yeasts, at cell concentrations encountered in a standard fermentation are not affected by beta defensins up to concentrations of 50µg/mL.

Using a series of Drag & Drop vectors we have integrated a copy of the HBDEF3 coding sequences, under the control of a GAL promoter, into a series both *S. cerevisiae* and *S. pastorianus* strains. The expression and secretion of the HDBEF3 peptide in these yeast strains has been confirmed using FACS, fluorescent microscopy and Western blotting. We are currently testing the ability of the expressed beta defensin to reduce bacterial contamination in a standard fermentation.

FdBev 08 *Abstract withdrawn*

FdBev 09 *Abstract withdrawn*

Posters

Microbial Infection Group

MI 01 The changing antimicrobial peptide profile in recurrent urinary tract infection following urinary diversion

Claire Townes[1], Ased Ali[2], Wendy Robson[2], Robert Pickard[2] & Judith Hall[1]

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Patients who undergo urinary diversion by ileal conduit suffer from a constant bacteriuria, however only a quarter of patients suffer repeated symptomatic urinary tract infections (UTIs). Why some patients with bacteriuria become symptomatic while others do not remains unclear. We hypothesize that the profile of cationic antimicrobial peptides (AMPs), synthesized as part of the bodies innate immune defences, may play a role. RT-PCR identified expression of genes encoding human alpha-defensin 5 (HD5) and beta-defensin 1 (BD1) in the ileal and ureter tissues of pre-diversion patients. Further RT-PCR analyses and quantitative Realtime PCR showed that both the number of patients expressing HD5 and BD1 and the level of gene expression differed significantly following diversion. In addition time-kill assays performed on *Escherichia coli* spp, isolated from the urine of patients with and without recurrent symptomatic UTIs, showed differing susceptibilities to recombinant HD5 and BD1. This data supports a role for AMPs in the differing susceptibilities of patients to UTI, following urinary diversion by ileal conduit.

MI 02 Potential novel autoregulatory compounds of *Aspergillus fumigatus*

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Background Candida albicans has been found to secrete a quorum sensing molecule called farnesol which controls dimorphic behaviour, i.e. yeast to hyphal transition. *Aspergillus fumigatus* undergoes similar morphological alterations to form multicellular structures.

Aims To identify secreted metabolites released from *A. fumigatus* whilst undergoing hyphal development into a multicellular structure to determine whether this organism also exhibits autoregulatory characteriztics.

Methods Supernatants from developing *A. fumigatus* biofilms were collected at several time points (8, 12 and 24 h), and secreted metabolites identified using GC-MS. Conidia and mature biofilms were then exposed to exogenous analogues of the putatively identified molecules, and their metabolic activity and biomass quantified.

Results Decanol, dodecanol, tetradecanoland, decanoic acid and octanoic acid were identified from the GC-MS analysis. All compounds exhibited a concentration dependent when exposed to conidia except tetradecanol. However the activity of decanol, dodecanol and octanoic acid was reduced against pre-formed biofilm structures.

Conclusions Hyphal development and population density of *A. fumigatus* may be regulated by the secretion of small chain fatty acids and alkanols.

MI 03 Regulation of type 1 fimbriae by *slyA*

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Type 1 fimbriae are phase variable, cell-surface structures responsible for mediating adhesion of *Escherichia coli* to mammalian cells. Production of FimB, the recombinase responsible for OFF-to-ON switching of type 1 fimbriation, is controlled at a transcriptional level by various activating and repressing proteins. The SlyA protein regulates several genes related to central metabolic processes in *E. coli*, and it is involved in the biosynthesis of amino acids and other important cofactors. Whilst SlyA also represses genes such as *ivy* (lysozyme resistance), it has been shown to activate K5 capsule (a virulence factor in urinary tract infection) and we have demonstrated that it both activates *fimB* transcription and enhances FimB recombination. SlyA is therefore likely to be crucial in regulating the delicate balance between *E. coli* and its host. We are currently studying the mechanism of SlyA's interaction with the *fimB* regulatory region (most likely involving an interaction with the abundant repressor, H-NS), as well as possible mechanisms of control of SlyA production itself, which may involve factors related to the host immune response or cellular metabolism.

MI 04 Antigen I/II family polypeptide adhesins in pathogenic and commensal streptococci

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Colonization of host tissues by streptococci involves primary interactions of bacterial cell surface adhesins with host proteins. The antigen I/II (AgI/II) family polypeptides (1310–1653 aa residues) are cell wall anchored proteins expressed by most indigenous oral streptococci. They interact with multiple host proteins including collagen, fibronectin, laminin, and also specifically recognize innate immunity glycoprotein gp-340 found at mucosal surfaces.

Proteins sharing significant amino acid sequence similarity (between 30–40%) with AgI/II family proteins are expressed on the surface of group A streptococci (GAS). They share the common NAVPC structure associated with AgI/II family members, with the alanine-rich (A) and proline-rich (P) regions supporting more divergent N and V regions, and a conserved C region. The V region of GAS AgI/II-like protein contains clusters of histidine residues that are characteriztic of metal-ion binding sites commonly associated with metalloproteinases and are absent from viridans AgI/II-like proteins. Using an *E. coli* expression system, recombinant fragments of the AgI/II-like protein of a fully sequenced group A strain (MGAS6180) have been overproduced. Functional and comparative assays have confirmed gp-340 recognition properties as well as binding activities unique from other members of the AgI/II protein family.

MI 05 Molecular analysis of the chymotrypsin-like protease complex genes of an ovine-derived treponeme

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Treponema bacteria are implicated in increasingly prevalent digital dermatitis diseases of hooves in cattle and sheep. Intriguingly, these bacteria are phylogenetically very similar to pathogenic treponemes associated with human periodontal disease. *Treponema denticola*, a well-characterized oral spirochaete, has strong chymotrypsin-like protease (CTLP) activity. Functional proteolytic activity requires expression an operon consisting of three genes encoding the protease and associated accessory proteins. An ovine isolate, UB1466, closely related to *T. denticola*, also has CTLP activity. Our aim is to understand expression and functionality, including adhesion to and destruction of host proteins, of the CTLP complex in treponemes isolated from digital dermatitis lesions. We have PCR amplified, cloned and sequenced contiguous fragments totaling 1493 bp from two genes within the CTLP complex operon from UB1466. The predicted peptide sequence

has 70% identity and 80% overall similarity to the *T. denticola* CTLP-complex *prcA* and *prtP* genes. The Asp, His, Ser subtilisin catalytic triad is present within the predicted UB1466 PrtP. In conclusion, the ovine treponeme CTLP-complex genes show high similarity with those of the oral pathogen, *T. denticola*.

MI 06 Cell surface interactions of *Candida albicans* and *Streptococcus gordonii* in biofilm communities

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Candida albicans forms biofilms comprised of blastospores and hyphae on a variety of surfaces. The presence of *Streptococcus* bacteria has been shown to stimulate hyphal development and enhance biofilm formation by *C. albicans*. Interaction of the oral bacterium *Streptococcus gordonii* with *C. albicans* involves surface proteins SspA and SspB, members of the streptococcal Antigen I/II family of polypeptide adhesions. Here we show that SspB expressed on the surface of *Lactococcus lactis* confers on these bacteria the ability to adhere to *C. albicans*. Adhesion is intimate, as revealed by atomic force microscopy, and localized to regions on the *C. albicans* cell surface. Als3p, a *C. albicans* hyphal specific surface glycoprotein, is required for mature biofilm formation. *S. gordonii* stimulates hyphal formation by *C. albicans als3*Δ/*als3*Δ but hyphae are unable to bind streptococci or form mixed species biofilms. The results suggest that AgI/II interactions with the *C. albicans* cell surface may involve receptors associated with Als3p expression.

MI 07 Identification of quorum sensing-regulated genes in the model mouse pathogen *Citrobacter rodentium*

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Citrobacter rodentium is a Gram-negative member of the Enterobacteriaceae which is used as a model organism to study attaching and effacing (A/E) lesion formation in its natural host, the mouse. Quorum sensing (QS) is a form of bacterial cell-to-cell communication using chemical signals to regulate gene expression in a cell density-dependent manner. Many Gram-negative bacteria synthesize and respond to *N*-acyl-homoserine lactone (AHL) signalling molecules. *C. rodentium* encodes an AHL synthase (CroI) which produces *N*-butanoyl-L-homoserine lactone (BHL) as its major product and also a LuxR-family protein (CroR), predicted to act as a BHL receptor and transcriptional regulator. A random mutagenesis screen was conducted using a promoterless *lacZ* (β-galactosidase) reporter to identify genes in the *croIR* regulon. A subset of *C. rodentium* genes, including known and putative virulence factors, was shown to be QS-regulated in the presence of BHL. The expression of selected genes identified in the screen was compared between the wild type and the *croI* mutant, throughout growth.

MI 08 The oxidative stress response of *Francisella*

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Francisella tularensis, the causative agent of tularemia, is a highly infectious obligate intracellular pathogen. The aim of this study was to use a DNA microarray to identify, *in vitro*, *Francisella* genes that were regulated upon exposure to stress conditions selected to represent the intracellular environment. RNA was isolated from *Francisella* cultured *in vitro* with hydrogen peroxide and was hybridized to the *F. tularensis* microarray in order to elucidate the transcriptome of *Francisella* cultured under oxidative stress. A number of genes were regulated in response to this condition and a detailed analysis of the data has provided insights into the oxidative stress response of *Francisella*, and thus some of the mechanisms that it may employ when encountering similar stresses *in vivo*. It is anticipated that the identification of genes that are regulated under intracellular conditions will aid future vaccine research.

MI 09 Invasion of oral keratinocytes by *Staphylococcus aureus*

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Preliminary work suggested that *Staphylococcus aureus* could adhere to and invade the oral keratinocyte cell line H357. However, *S. aureus* is not a common oral colonizer. Invasion of keratinocyte cell lines (H357, UP and Detroit 562) and primary oral keratinocytes by *S. aureus* strains Oxford, Newman and a clinical isolate, S-235, was studied using an antibiotic protection assay (gentamicin 100µg/ml 1h). Also, H357 and primary cells were 'coated' with saliva by exposure at an air-liquid interface. Intracellular bacteria were recovered by cell lysis and quantified by viable counting. The level of *S. aureus* invasion varied between the cell types studied. Strain Newman invaded poorly but in a fibronectin-independent manner. Bacterial invasion was increased by increasing cell confluence and increasing Multiplicity of Infection, suggesting receptor-density dependence. Presence of a salivary pellicle on the H357s, reduced invasion approximately 10 fold. The data indicate that invasion of oral keratinocytes by *S. aureus* is receptor mediated, saturatable and operates by at least 2 mechanisms. Presence of adsorbed salivary constituents inhibits *S. aureus* invasion, possibly explaining its relative absence from the oral environment.

MI 10 *Salmonella* invasion, persistence and immune responses in chicken macrophages

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Salmonella infections in humans are one of the leading bacterial food-borne zoonoses worldwide and transmission frequently occurs after consumption of poultry. Although *S.* Hadar and *S. infantis* are common poultry isolates where they cause little disease they can cause food poisoning in man. However little is known about their interaction with either the avian or human hosts or how this correlates with pathogenicity. The initial aim of this work is to determine the interaction of these serotypes with avian cells. Invasion and persistence of the *Salmonella* strains were determined by a gentamicin protection assay and nitric oxide production was used as a measure of immunity. Both *S.* Hadar and *S.* Infantis were detected in significant counts inside avian macrophages from 2 hours post invasion persisted for 24 hours. *S.* Infantis had a higher invasion rate than *S.* Hadar. Further studies will be carried out to compare these results to those observed with equivalent human cells.

MI 11 Genome wide analysis of gene expression in neisserial biofilms

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Understanding the mechanisms underlying commensal *Neisseria* and carriage meningococci biofilm formation will help towards understanding the initial nasopharyngeal colonization and carriage stages of meningococcal disease. Large-scale biofilm studies using a continuous flow fermentor system were carried out to identify the global gene expression profile of meningococcal or commensal Neisseriae. In the present study, we used microarray analysis to compare the transcriptomes of biofilm-grown acapsulated *Neisseria meningitidis (siaD* mutant*)* or *Neisseria lactamica* with the transcriptomes of logarithmic- and stationary-phase planktonic cultures of meningococcal or commensal Neisseriae, respectively.

In both neisserial species, a large number of genes were identified as up- or down-regulated into biofilms compared with planktonic cultures. Gene expression profiles were more different between biofilm and stationary-phase than with biofilm and logarithmic-cultures, suggesting that neisserial biofilms were more similar to exponentially growing bacteria. Global gene expression profile differences were either species-specific or common to both meningococcal and commensal Neisseriae. Quantitative RT-PCR confirmed a number of commonly up-regulated changes in the neisserial biofilms. In conclusion, we identified biofilm-associated global gene expression profiles with differentially expressed neisserial genes encoding for proteins which have similarity to biofilm-associated proteins of other bacterial pathogens. In addition, many other neisserial genes that may contribute to biofilm formation by carriage meningococcus or commensal *N. lactamica* were identified.

MI 12 The effect of environmental oxygenation on lethal photosensitization of wound-associated organisms using indocyanine green and near-infrared light

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The current worldwide increase in antibiotic-resistant bacteria reduces the ability to treat infected wounds. A promising alternative to antibiotics is photodynamic therapy. The PDT process is complicated by multiple factors including: photosensitizer, light and tissue oxygenation.

The effect of environmental oxygenation on the lethal photosensitization of *Staphylococcus aureus* and *Streptococcus pyogenes* using indocyanine green and near-infrared laser light was investigated.

Bacterial suspensions were irradiated with a light dose of 98 J/cm^2 under both aerobic and anaerobic conditions and the survivors were enumerated. Lethal photosensitization under aerobic condition, using an initial bacterial load of 10^7 cfu/ml resulted in 99.56% kills of *S. aureus* and 99.96% of *Strep. pyogenes*. Under anaerobic conditions, the kills were reduced to 96.77% and 71.62% for *Staph. aureus* and *Strep. pyogenes* respectively.

These findings imply that the level of tissue oxygenation is an important factor to consider during the eradication of bacteria from wounds.

MI 13 Evaluation of techniques to detect surface-associated pathogens

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The transmission of bacteria within hospitals poses an infection risk for patients and hand-touch surfaces are currently being considered more seriously as a transmission route for healthcare-associated infections. Current methods employed in hospitals to measure bacterial contamination include enumeration by viable counts, dipslides and touch plating. Luminometers are widely used in the food and cosmetics industries to measure bacterial contamination, but have rarely been applied to the hospital environment. Using this technique, results are available within 30 minutes, eliminating the need to incubate culture plates overnight. The aim of this work was to evaluate three luminometers, the Junior, UniLite NG and BioProbe and compare their detection against the viable count method. A series of glass surfaces were inoculated with *Staphylococcus aureus* or *Escherichia coli* and incubated at room temperature. The surface was then either sampled by the luminometer or swabbed and viable counts performed. The BioProbe luminometer detected *S. aureus* most accurately and the Junior luminometer, *E. coli*. All luminometers tested were unable to detect low levels of both bacterial species and performed better with higher bacterial inoculums. Luminometers can be used to rapidly indicate bacterial contamination but should be used alongside culturing for confirmation and species identification.

MI 14 Multiple approaches are required to model endodontic irrigation

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Designing a laboratory model of a microbiological system is a reductive process. As the model develops, spurious aspects are ignored or eliminated by standardization. A model that reproduces multiple facets of a system does so at the expense of accuracy, whereas a model that focuses on a single factor cannot reveal the interplay of multiple experimental variables. The case in point was demonstrated by a model of endodontic irrigation which used extracted human teeth that were infected with *Enterococcus faecalis* before being irrigated. This clinically relevant model showed that 1% sodium hypochlorite (NaOCl) achieved significant kill (1.75 log reduction; p=0.026), but could not reveal why a proportion of bacteria remained viable. Additional, simple models were required to determine the cause of disinfection failure. A filter-membrane model showed that although biofilms of *E. faecalis* possessed a limited intrinsic resistance to NaOCl, 1% achieved total disinfection. A further model which used biofilms grown within a section of narrow-bore tubing revealed that it is the inability of the irrigant to access the bacteria that causes endodontic disinfection failure.

MI 15 The role of *Burkholderia cepacia* complex (Bcc) lipopolysaccharide (LPS) in proinflammatory responses in CF lung disease

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Bcc is a group of opportunistic pathogens that have a significant impact on mortality in cystic fibrosis (CF). We and others have shown that Bcc induces a potent proinflammatory cytokine response from both epithelial cells and macrophages. We examined the role of the LPS component of these pathogens in the macrophage response and investigated the intracellular signalling pathways involved.

Purified LPS from a *Burkholderia multivorans* strain, was used to stimulate U937 macrophages *in vitro* in the presence or absence of MAPkinase pathway inhibitors. Real-time PCR and ELISA were used to determine cytokine gene expression and protein secretion respectively. Induction of TNFα and IL-1β was significantly upregulated in the presence of Bcc LPS and was greater than that stimulated by *P. aeruginosa* LPS. Inhibition of the JNK MAP kinase pathway significantly abrogated the cytokine response with only partial inhibition seen with inhibitors of the p38 and ERK MAP kinase pathways. These data further characterize the potent pro-inflammatory response stimulated by these pathogens and highlight potential targets for anti-inflammatory agents to combat these infections.

MI 16 Interactions of a novel cystic fibrosis pathogen genus *Pandoraea* with lung epithelial cells

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Cystic fibrosis (CF) is the most common hereditary disease in Ireland. Bacterial pathogens contribute considerably to morbidity in patients with CF. The genus *Pandoraea* has recently emerged as a novel pathogen in the Irish CF population, but little is known about its virulence. The genus comprises five species and we have previously shown that 19 strains across these species elicit a potent inflammatory response. The aim of this study was to further investigate the virulence of this CF pathogen. We demonstrated that *Pandoraea* strains are generally non-invasive and there was no difference between invasiveness of lung epithelial cells carrying the CF defect and those that are normal. The ability of *Pandoraea* strains to disrupt lung epithelial tight junctions was examined by monitoring the transepithelial resistance (TER) of Calu-3 cells and permeability of paracellular markers. Three out of four strains examined disrupted TER over four hours, however, only one strain showed an enhancement in

paracellular permeability. Further study is needed to determine the significance of these findings in the CF population.

MI 17 Proteomic analysis of *Pseudomonas aeruginosa* strains in response to bronchial epithelia

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Pseudomonas aeruginosa is an opportunistic human pathogen that typically infects immunocompromised individuals such as cystic fibrosis suffers and burn wound patients. During infection *P. aeruginosa* exerts tight regulatory control over gene expression to counteract host defense mechanisms and facilitate the establishment of infection. The post-transcriptional regulator RsmA plays a key role in controlling a number of traits associated with acute infection (Goodman *et al.*, 2004 ; Burrowes *et al.*, 2005). We demonstrated that RsmA mediates regulation of type III secretion with subsequent effects on invasion of and cytotoxicity towards epithelial cells in an *in vitro* infection model (Mulcahy *et al.*, 2006). RsmA is also involved in colonization and dissemination *in vivo* in a mouse model of acute pneumonia (Mulcahy *et al.*, 2008). To further characterize the global effects of RsmA during *P. aeruginosa*–host cell interactions, comparative proteomic analysis was carried out on wild-type *P. aeruginosa* PAO1 and an isogenic rsmA mutant strain in response to human airway epithelial cells. Using a functional genomics approach, we have identified genes that may play a role in *Pseudomonas*-epithelial cell interactions.

MI 18 HD-GYP domain proteins of *Pseudomonas aeruginosa* regulate functions associated with virulence

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HD-GYP is a protein domain involved in the hydrolysis of the bacterial second messenger cyclic-di-GMP. In this report we have investigated the role of HD-GYP domain proteins in the virulence of the human pathogen *Pseudomonas aeruginosa*. The genome of *P. aeruginosa* PAO1 encodes two proteins (PA4108, PA4781) with an HD-GYP domain and a third protein, PA2572, which contains the variant YN-GYP domain. Mutation of *PA4108* and *PA4781* led to an increase in the level of cyclic-di-GMP in *P. aeruginosa*, consistent with the activity of these proteins as cyclic-di-GMP phosphodiesterases. Mutation was also associated with reduced swarming motility and production of the virulence determinants ExoS and ExoT and differing effects on pyocyanin and pyoverdin levels. Mutation of *PA2572* had no effect on cyclic-di-GMP levels and did not influence swarming motility. However PA2572 had a negative influence on swarming that was cryptic and was revealed only after removal of an uncharacterized C-terminal domain. Importantly all three proteins contributed to the virulence of *P. aeruginosa* to larvae of *Galleria mellonella*.

MI 19 Detection of a non-coding small RNA in *Staphylococcus epidermidis*

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Non-coding small RNAs (nc-sRNAs) are regulatory elements that modify transcription, translation or stability of their target mRNAs. Here we describe a putative nc-sRNA in the nososcomial pathogen *Staphylococcus epidermidis* that was detected in a genome-wide search for transcripts in large intergenic regions. The transcript was verified by Northern blot analysis and RT-PCR. Size and polarity were determined and bioinformatic analyses revealed two putative promoters and a strong Rho-independent transcription terminator. Neither a ribosomal binding site nor an open reading frame was detectable. The element precedes a methionine-biosynthesis gene, and analysis of the met-sRNA sequence identified a conserved T-box motif. T-box systems are RNA-based transcription termination control systems of Gram-positive bacteria often governing tRNA synthetase and amino acid biosynthesis genes. Experiments with different *S. epidermidis* and *Staphylococcus aureus* strains indicated that the met-sRNA is conserved in these species, while transcription levels vary among strains and in response to environmental conditions. The role of the met-sRNA in metabolic adaptation of staphylococci to different ecological niches and its possible suitability as a novel antibiotic target are discussed.

MI 20 Purification and structural elucidation of a novel carbohydrate component of the biofilm matrix of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a gram-negative bacterium, which is responsible for chronic infections in the lungs of cystic fibrosis (CF) patients. There are strong indications that *P. aeruginosa* develops a biofilm in the CF lung. A key feature of mature *P. aeruginosa* biofilms is the presence of a matrix of extracellular polymeric substances (EPS) that encases the constituent cells and confers them an increased resistance to antibiotics, biocides, and host defenses. This matrix has been reported to contain a mixture of polymers, including nucleic acids, proteins, and polysaccharides. Identification of constituents of the EPS might enable the development of new therapeutic strategies aimed at disrupting biofilms. The exopolysaccharide alginate has traditionally been considered the major EPS of *P. aeruginosa* during CF pathogenesis by mucoid strains at the late stages of infection. It have been recently suggested that polysaccharides other than alginate may contribute to the biofilm formation in non-mucoid strains, which are first to colonize CF patients.

An analysis of the *P. aeruginosa* genomic sequence revealed four additional gene clusters which appeared to encode for exopolysaccharide biosynthesis. Two of these gene clusters, *pel* (*pel*licule) and *psl* (*p*olysaccharide *l*ocus) have been shown to contribute to the production of a glucose and mannose-rich matrix component, respectively.

In the present study, we investigated the carbohydrate polymers of the extracellular biofilm matrix of the non-mucoid, exopolysaccharide overproducing strain *P. aeruginosa* PAKΔ*retS*. Fractionation of the carbohydrate fraction of the crude biofilm matrix of this strain by gel-permeation chromatography revealed the presence of a family of glucose-rich polymers. Their structure has been elucidated using chemical analysis, one- and two-dimensional NMR techniques, and mass spectrometry.

MI 21 Mechanism of innate immune antiviral response induction during human parainfluenza virus-3 (HPIV3) infections

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Background HPIV3 causes severe respiratory tract infections in young children. Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I), Mda5 are essential for induction of interferon-α/β (IFN)/interferon regulatory factor-3 (IRF3) dependent innate antiviral response; however the specific receptor(s) utilized by HPIV3 is not known.

Results Ectopic expression of RIG-I and TLRs in 293 cells revealed that RIG-I and TLR3 is important for activation of IFN/IRF-3 upon HPIV-3 infection. Further studies in human lung epithelial cells demonstrated the ability of HPIV3 to induce expression of RIG-I and TLR3 in these

cells. These receptors are important since RIG-I silencing (by siRNA) or expression of dominant negative RIG-I resulted in failure of HPIV3 to activate IFN/IRF3 during early infection (2–12 h post-infection). In contrast, silencing of TLR3 expression diminished IRF3/IFN induction during late infection time frame.

Conclusion We demonstrated that during HPIV3 infection, RIG-I and TLR3 is required for induction of early and late innate antiviral response, respectively.

MI 22 Virulence properties of asymptomatic bacteriuria *Escherichia coli*

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In asymptomatic bacteriuria (ABU), bacteria colonize the urinary tract without provoking symptoms. Here we compared the virulence properties of a collection of ABU *Escherichia coli* strains to cystitis and pyelonephritis strains; specific UTI-associated virulence genes, hemagglutination characteriztics, siderophore production, hemolysis, biofilm formation, and the ability of strains to adhere to and induce cytokine responses in epithelial cells were analysed. ABU strains were phylogenetically related to strains that cause symptomatic UTI. However, the virulence properties of the ABU strains were variable, and dependent on a combination of genotypic and phenotypic factors. Most ABU strains adhered poorly to epithelial cells; however we also identified a subgroup of strongly adherent strains that were unable to stimulate an epithelial cell IL-6 cytokine response. Poor immune activation may represent one mechanism whereby ABU *E. coli* evade immune detection after the establishment of bacteriuria.

MI 23 Assessing contact lens disinfection through stand-alone and regiment tests

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The aim of this work was to compare the disinfection levels obtained through stand-alone test and regimen test on conventional and silicone hydrogel contact lenses (CLs).

Tests were made in accordance to the international guidelines for soft CL disinfection, ISO 14729:2001. The multipurpose solutions (MPS) Opti-Free® Express® and ReNu Multiplus® and Complete® were tested against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The CLs materials etafilcon A, polymacon, balafilcon A and lotrafilcon A were assayed in this study.

All MPS passed the stand-alone test since a 3-log reduction was obtained for every bacterial strain. However, regimen test results were not as satisfactory since a 4 to 5-log reductions was not always achieved.

All MPS meet stand-alone test, but fail the regimen test in some combinations bacteria/lens material. It seems that the lens material and the adhered bacteria affect the performance of the MPS. It is strongly suggested that CL disinfection should be firstly tested according to the regimen test instead of stand-alone test.

MI 24 Growth inhibition of pathogenic *Vibrio* species in a restaurant's tank by the cupric ion diffused from brass 'Bangzza'

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The aim of this study is finding potential effect from a brass that alloyed with the special composition of 78% copper and 22% tin and called *Bangzza*' in Korea, as an antibacterial agent. The antibacterial activity determined viability of *V. parahaemolyticus* in TCBS agar and the amount of cupric ion was measured by ICP. When the brass (300?300?2 mm) was applied in a restaurant's tank, *V. parahaemolyticus* were detected at less than 10CFU/mL from 10^5CFU/mL in seawater at 24hr. And the viability of *V. parahaemolyticus* (~10^5CFU/mL) in each organs of contaminated raw fish and shellfish were rapidly decreased to <10 CFU/ mL or not detected while concentration of cupric ion increased ~0.13mg/ mL at 48hr. However, almost diffused cupric ions were adhered to the polyester and sand as filters with impurity materials. Therefore, the present study identified antibacterial activity of cupric ion diffused from brass against the pathogenic *Vibrio* spp. in the restaurant's tank.

MI 25 *Abstract withdrawn*

MI 26 *Abstract withdrawn*

MI 27 *Abstract withdrawn*

MI 28 *Abstract withdrawn*

MI 29 *Abstract withdrawn*

MI 30 *Abstract withdrawn*

MI 31 Inactivation of pathogenic bacteria using pulsed UV-light and its application in water disinfection and quality control

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The lethality of pulsed ultra-violet (UV) rich light for the inactivation of pathogenic bacteria has been investigated. A low pressure xenon filled flash lamps that produced UV intensities have been used. The pulsed operation of the system enable the release of electrical energy stored in the capacitor into the flash lamp within a short time and produces the high current and high peak power required for emitting the intense UV flash. The flash frequency was adjusted to one pulse per second. Several types of bacteria were investigated for their susceptibility to pulsed UV illumination. The treated bacterial populations were reduced and determined by direct viable counts. Among the tested bacteria *Pseudomonas aeruginosa* was the most susceptible to the pulsed UV- light with a 8 log10 cfu/ml reduction

after 11 pulses, while the spores of *Bacillus megaterium* was the most resistant and only 4 log10 cfu/ml reduction achieved after 50 pulses of illumination. The results of this study demonstrated that pulsed UV-light technology could be used as an effective method for the inactivation, of pathogenic bacteria in different environments such as drinking water.

MI 32 *Abstract withdrawn*

MI 33 *Abstract withdrawn*

Posters

Physiology, Biochemistry & Molecular Genetics Group

PBMG 01 Experimental evolution of *Escherichia coli* lacking *rpoS* in a high osmolarity environment

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The alternative sigma factor RpoS is involved in adaptation of *Escherichia coli* to stresses like stationary phase or growth in hyper-osmotic conditions. Despite the importance RpoS in responding to a variety of stresses, mutations in *rpoS* are rapidly selected in laboratory populations, and *rpoS* null alleles are found in natural isolates at measurable frequencies. To understand how *E. coli* evolves in response to the loss of this global regulator, we serially transferred five $rpoS^+$ and five $rpoS^-$ lines for 250 generations in hyper-osmotic media, allowing all 10 lines to increase their fitness in this environment.

While all 10 lines increased their growth rate under these conditions, the $rpoS^-$ lines did so in a more uniform manner than the $rpoS^+$ lines. Additionally, the increase in fitness of the $rpoS^-$ lines was more tightly correlated to the increase in growth rate than for the $rpoS^+$ lines. Expression microarrays were used to explore how the difference in *rpoS* status effected the evolution of global regulatory networks.

PBMG 02 The plasmid pSf-R27 encodes a homologue of the thermosensing protein TlpA

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The large IncHI1 plasmid pSf-R27 is thermosensitive for conjugative transfer. It encodes a protein, named TlpAR27, which is a structural homologue of the thermosensing protein TlpA that is expressed by the virulence plasmid, pSLT, of *Salmonella* Typhimurium. TlpA forms DNA-binding homodimers at 30°C, but dissociates into non-DNA-binding monomers at 37°C. It is also regulates the transcription of its own gene, *tlpA*. Hence it was of interest to investigate if TlpAR27 possessed such a thermosensing function.

Activity of the *tlpAR27* promoter was monitored at 30°C and 37°C, in strains containing the wild-type plasmid (pSf-R27) and a plasmid containing an insertion mutation in the *tlpAR27* gene (pSf-R27*tlpAR27*::Spc^R). Mutation of the *tlpAR27* gene resulted in a large increase in activity (181%), compared to wild type levels, but only at 30°C. The competitive fitness of the *tlpAR27* mutant was compared with that of the wild type, with the mutant having a greater fitness at 37°C, but a lower fitness at 30°C compared with that of the wild type.

PBMG 03 Biasing switching outcomes in the *Escherichia coli fim* site-specific recombination system through DNA supercoiling and nucleoid-associated proteins (NAPS)

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The promoter for the *Escherichia coli fimA* gene is located on an invertible element (*fimS*) that controls phase variable expression of type I fimbriae. DNA inversion is catalysed by a site-specific recombinase (FimB) that binds at inverted repeats (IRs) that flank *fimS*. The inversion reaction is sensitive to changes in DNA supercoiling such that inhibition of DNA gyrase activity and the associated relaxation of DNA causes *fimS* inversion to become biased towards the ON phase, with the promoter reading into *fimA*.

Maintenance of this ON- phase bias requires binding of integration host factor (IHF) at a site external to *fimS* (IHF1) near the left IR (IRL) and is independent of IHF binding within *fimS* at a site (IHF2) that is proximal to the right IR (IRR) in the phase ON orientation. Removal of the IHF1 site reversed the switch bias, with IHF1 mutants becoming biased to the OFF phase in response to DNA relaxation, whereas IHF2 mutants maintained a bias towards the ON phase. Removal of the histone-like nucleoid structuring protein (H-NS), which binds near the IHF1 site, caused a bias towards the ON phase that was not affected by altering DNA supercoiling through inhibition of DNA gyrase. These results demonstrate a role for IHF, H-NS and local DNA topology in inversion biasing of *fimS*.

PBMG 04 Mechanisms of how a bacterial pathogen develops resistance/tolerance to host cationic antimicrobial peptides

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Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen, is one of the main causes of nosocomial infections and also known to infect cystic fibrosis patients. *P. aeruginosa* has also received attention due to its resistance to a wide variety of conventional antibiotics resulting from an intrinsically impermeable outer membrane and multi drug resistance efflux pumps.

This has lead to an increased interest in cationic antimicrobial peptides (CAMPs) as potential therapeutic molecules. CAMPs are ubiquitous in nature, and are key components of the innate immune response. LPS modification is known to be a primary mechanism by which cells become resistant to colistin. To further understand the interaction between CAMPs and bacteria we utilized DNA microarray technology to investigate the influence of sub-inhibitory colistin on the transcriptome of *P. aeruginosa*. Analysis of this 'colistin signature' reveals transcriptional changes in several functional classes of genes in response to sub-inhibitory colistin; these global changes implicate biofilm formation, transport, virulence and defence in the bacterial response to colistin.

PBMG 05 Characterization of a phage abortive infection system of *Erwinia carotovora* subsp. *atroseptica*

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Bacteria possess many strategies to resist bacteriophage (phage) infection, including abortive infection (Abi) systems. We identified a cryptic plasmid of the Gram-negative phytopathogen *Erwinia carotovora* subsp. *atroseptica* (*Eca*) containing a gene with sequence similarity to an Abi system. Microbiological characterization confirmed that this *Eca* homologue acted as an Abi system and could protect very efficiently against multiple phage. We have begun analysis of the molecular genetics of this system. It is comprised of two components, which have features suggesting action as a toxin and an antagonistic antitoxin. The ecological and physiological roles of 'toxin–antitoxin' systems remain under debate. Our work shows that a highly effective abortive infection mechanism relies upon a toxin–antitoxin module to provide protection against multiple phage in this enterobacterial phytopathogen.

PBMG 06 Sensitivity of *Erwinia* and *Serratia* quorum sensing systems to non-cognate N-acyl homoserine lactone signalling molecules

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Quorum sensing (QS) is important in the regulation of various phenotypes in Gram-negative bacteria, including the biosynthesis of a carbapenem antibiotic in *Erwinia carotovora* subsp. *carotovora* (Ecc) and the red tripyrrole pigment prodigiosin in *Serratia* ATCC 39006 (S39006). In QS systems, a homologue of LuxI synthesizes an N-acyl homoserine lactone (AHL) signalling molecule, which varies in chemical structure between bacterial species. These AHLs interact with a cognate LuxR-type transcriptional regulator in a highly specific manner. This study investigated the sensitivity of the *Erwinia* and *Serratia* quorum sensing systems to a range of different AHL molecules. Response to the non-cognate AHLs was measured by assaying carbapenem (Ecc) and prodigiosin (S39006) production, and by measuring expression of the relevant biosynthetic operons. Bioassay results showed an extremely specific response to the native AHL molecule in both systems, and a gradation of responsiveness to a range of non-cognate AHL molecules, dependent on the chemical structure of the signal.

PBMG 07 Effects of a *rhlAB* mutation on the membrane proteome of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium, which is well known for its colonization of the lungs of cystic fibrosis sufferers and its prevalence in nosocomial infections. *P. aeruginosa* produces a range of secondary metabolites that are regulated by quorum sensing, including detergent molecules called rhamnolipids. Rhamnolipids are virulence factors that promote paracellular evasion of epithelial cells. They are also important in biofilm formation and architecture as well as cell motility. In industry much interest has been shown in the efficacy of rhamnolipids for bioremediation and use in pesticides. However, the natural function of rhamnolipids is poorly understood. In order to characterize the effects of rhamnolipids on cellular physiology, the membrane proteome of wild type PA01 was compared with that of a *rhlAB* mutant, which is deficient in rhamnolipid production. The mutant was altered in several phenotypes including siderophores, pyocyanin production, motility and protein secretion. These results were validated using phenotypic assays and could provide information on the secretion and natural function of rhamnolipids.

PBMG 08 Structural studies of capsule saccharide biosynthesis in *Burkholderia pseudomallei*

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The bacterium *Burkholderia pseudomallei* causes melioidosis, a common cause of community acquired infection in Thailand and North-East Australia. This bacterium has been identified as a potential bioterror agent, as it requires complicated treatment and shows high mortality in patients presenting with severe infections. Consequently, there is a strong desire to prepare a vaccine for melioidosis. Current research suggests that the strongest vaccine candidates are conjugates of major polysaccharides with key protein virulence factors.

This study examines the proteins involved in the biosynthesis of the capsule polysaccharide of *B. pseudomallei*, one of the two major polysaccharides of this organism. The polysaccharide is a homopolymer of 2-*O*-acetyl-6-deoxy-β-D-*manno*-heptopyranose. This saccharide is synthesized from sedoheptulose-7-phosphate, an intermediate in the ubiquitous pentose phosphate pathway.

Key point: the nucleotide specificity of the pathway has been determined by analysing the affinity to the five nucleotide triphosphates. This specificity has been confirmed by demonstrating that the downstream enzymes in the pathway share this specificity. These proteins are being crystallized to determine the structural basis for nucleotide specificity and mechanism in this pathway.

PBMG 09 Investigation of the mechanism of sialic acid binding to the extracytoplasmic solute receptor, SiaP

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Sialic acid utilization plays an important role in the growth and persistence of the human respiratory pathogen *Haemophilus influenzae*, which takes up sialic acid via a tripartite ATP-independent periplasmic (TRAP) transporter. These possess an Extracytoplasmic Solute Receptor (ESR), which binds the substrate in the periplasm and delivers it to the specific membrane permease. The ESR contains two globular domains, which close around the substrate upon binding. Here, the mechanism of sialic acid binding is investigated using site-directed mutagenesis of residues in the ligand binding site of the ESR, SiaP. We report data demonstrating that stabilization of the carboxylate group of sialic acid by Arginine-147 is critical to ligand binding.

PBMG 10 Analysis of the transport of a Tat dependent substrate in *Haloarcula hispanica*

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The Twin-arginine translocase (Tat) is a system for protein translocation that is found in the thylakoid membranes of chloroplasts and the cytoplasmic membrane of prokaryotes. It has the unique ability to transport proteins in a fully folded conformation. In most prokaryotes, the Tat system only plays a minor role in protein secretion, with the bulk of the proteins (>90%) being secreted by the Sec pathway. A notable exception is found in halophilic archaea, in which most secretory proteins are predicted to be Tat-dependent. This is probably an adaptation to the highly saline conditions in which haloarchaea live; the cytoplasm of haloarchaea can contain up to 5 M KCl, and in these conditions haloarchaeal proteins may fold very rapidly due to the salting-out effect. If so, secretory proteins could fold before translocation, explaining the dominant role for the Tat pathway. Strikingly, the Tat pathway is essential for viability in haloarchaea, providing further evidence its vital role. Here we show the characterization of the secretion of an alpha-amylase from *Haloarcula hispanica*. We demonstrate that the protein Tat dependent and analyse the bioenergetics of its translocation using *in vivo* and *in vitro* translocation assays.

PBMG 11 Biochemical and physiological studies of *Escherichia coli* TorD: a twin-arginine signal peptide binding protein

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The export of proteins across the cytoplasmic membrane is an essential function of all bacterial cells. In *Escherichia coli*, most proteins are exported via the general secretory pathway, however a subset of extracellular enzymes are transported into the periplasm by the twin-arginine translocation (Tat) apparatus. Such proteins contain distinctive N-terminal signal peptides exhibiting a tripartite structure comprising a polar n-region, a relatively hydrophobic h-region, a polar c-region, and a highly conserved SRRxFLK twin-arginine motif. One of the most heavily exploited bacterial Tat signal peptides is that of the trimethylamine *N*-oxide reductase (TorA) of *E. coli*. Prior to export, the TorA signal peptide (ssTorA) is bound tightly by a dedicated cytoplasmic chaperone (TorD), possibly to prevent export until the maturation of TorA is complete in a process described as Tat Proofreading. This function is in addition to the established role of TorD in facilitating the cytoplasmic loading of the molybdenum cofactor into TorA. TorD was recently reported to bind weakly to the guanosine moiety of GTP and the role of GTP in Tat proofreading is under investigation. Additionally a facile expression system has been

developed allowing the purification of ssTorA as a fusion with maltose binding protein. Interactions between the MalE:ssTorA fusion protein and monomeric and multimeric forms of TorD have been assessed using liquid chromatography and calorimetric techniques. This work has provided new insights into the biological significance of higher order oligomers of TorD.

PBMG 12 The role of CspAs in the regulation of gene expression in *S.* Typhimurium

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Persistence of *Salmonella enterica sv.* Typhimurium through food processing and storage, places this organism as one of the major causes of food-borne disease. Adaptation to and multiplication at low temperature requires the multigenic cold shock response, involving coordinated gene expression.

Gene expression is the basis for cellular adaptability and is regulated from initiation of transcription to post-translational control. The CspA family of RNA binding proteins is essential for adaptation and growth at low temperature. Microarray studies of *S.* Typhimurium, using mutants in which all of the *cspA* gene paralogues have been deleted and the isogenic parental strain, have shown significant changes in gene expression during incubation at low temperature. However such data requires validation.

Using Northern analysis and the quantitative reverse transcription polymerase chain reaction, further insight into the regulation of gene expression has been gained. The direct and indirect roles played by *cspA* paralogues in regulating RNA species during the cold shock response have also been examined.

PBMG 13 Timely initiation of DNA replication in the deep-sea bacterium *Photobacterium profundum* SS9

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Initiation of DNA replication has not been previously investigated in deep-sea bacteria. To gain further insights, we investigated two *Photobacterium profundum* SS9 mutants, FL23 (*pbpra3229*::m-Tn*10*) and FL28 (*pbpra1039*::m-Tn*5*). Pbpra3229 is 75% identical to *E. coli* DiaA (stimulator of DNA replication) and 45% identical to *E. coli* GmhA (essential for lipopolysaccharide core biosynthesis), whereas Pbpra1039 is 55% identical to *E. coli* SeqA (repressor of DNA replication). Interestingly, both FL23 and FL28 show growth defects whereas in *E. coli* neither *diaA* nor *seqA* mutants have growth defects. The growth defect in FL23 was not due to a lipopolysaccharide alteration. Instead, both *pbpra3229* and *E. coli diaA* complemented the growth defect of FL23 and both restored the timing of DNA replication in an *E. coli diaA* mutant. Combined, these findings show that Pbpra3229 is a DiaA homologue and not a GmhA homologue. The functional homology of Pbpra1039 and SeqA is currently under investigation.

PBMG 14 Biogenesis and function of the small conductance mechanosensitive channel from *Escherichia coli*

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Mechanosensitive (MS) ion channels protect bacteria against the rapid increase of pressure caused by a hypoosmotic shock. The Small Conductance Mechanosensitive Channel (MscS) from *Escherichia coli* is a homoheptamer with three transmembrane helices and a complex architecture. We are investigating MscS biogenesis and structure–function relationships. The periplasmic N-terminal region was not resolved in the crystal (residue 1–27). We have made defined deletions and mutations in the periplasmic region to define residues required for stability of MscS. Hypoosmotic shock causes a rapid increase pressure within the cell and in the absence of MscS causes cell lysis. We have investigated the effect of hypoosmotic shock on cell integrity. Under transmission electron microscopy a mutant strain containing no functional MS channels showed significant numbers of cells with no cell contents, a much rounder shape and significant differences in size. There is a considerable level of heterogeneity following downshock and demonstrates hypoosmotic shock is a catastrophic event.

PBMG 15 Detection and analysis of genes coding for arsenite oxidation in chemolithoautotrophic *Sinorhizobium* sp. M14

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Sinorhizobium sp.M14 isolated from gold mine is able to growth chemolitoautrophically in minimal medium using arsenite as a source of energy as well as in minimal medium supplemented with yeast extract. Arsenite oxidase gene (*aoxB*) of M14 was identified using specific oligonucleotide primers designed to conserved regions of the large subunit of arsenite oxidase from previously described arsenite oxidase sequences. Southern hybridization experiments using *aoxB* probes showed that this gene is encoded in one copy on a ~150kb plasmid. Sequence analysis of the DNA flanking *aoxB* gene allows identified gene cluster essential for arsenite oxidation, including those coding for regulatory controls. This cluster contained six genes with the same orientation, in the following order *aoxS* (transduction signal gene), *aoxR* (response regulator), *aoxAB* (structural genes for arsenite oxidase), *cyt*-c2 (c-type cytochrome) and *chlE* (molybdopterin biosynthesis gene). The predicted amino acid sequences of all of the gene products are homologous (91–100% identity) to the amino acid sequences of the arsenite oxidation genes products of *Agrobacterium tumefaciens* strain 5A.

PBMG 16 *Abstract withdrawn*

Posters

Plenary

PS 01 Role of Antigen I/II family polypeptide adhesins in biofilms formation by *Streptococcus pyogenes*

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Streptococcus pyogenes M28 strains have been shown to be highly associated with puerperal sepsis, and to have horizontally acquired a 37.4 kb locus. Within this locus is encoded a protein (Spy1325) with homology to oral streptococcal antigen I/II (AgI/II) family polypeptides. These polypeptides are expressed by most indigenous oral viridans streptococci and are involved in adherence to host factors and in biofilm community interactions. The objective of this study was to determine the role of AgI/II-like proteins in biofilm formation by M28 Group A *Streptococcus* (GAS). Four M28 GAS strains were evaluated for biofilm formation. The AgI/II genes in two of these M28 strains were inactivated by allelic replacement with *aad9* (spectinomycin resistance). The various M28 GAS strains formed architecturally distinct biofilms on saliva-coated surfaces. Ag I/II-deficient mutants were reduced in adherence to salivary agglutinin glycoprotein (gp-340) and showed alterations in biofilm biomass and structure compared with respective wild-type strains. The results suggest that expression of AgI/II family proteins influences development of M28 GAS biofilms, and that AgI/II may therefore contribute to colonization and persistence of GAS.

PS 02 Identification of *Candida albicans* receptors for *Streptococcus* in mixed biofilm communities

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Streptococcus bacteria are primary colonizers of oral cavity surfaces and pave the development of complex biofilm communities. *Candida albicans* is a fungus carried by ~40% of the human adult population, colonizing mucosal and dental surfaces together with bacteria. *Streptococcus gordonii* attaches to *C. albicans*, promotes hyphal formation, and influences development of *Candida* biofilms. To identify *C. albicans* receptors for streptococci, we have utilized biochemical and genetic approaches. Biotinylated proteins from *C. albicans* were adsorbed to *S. gordonii* cells and a major polypeptide band (~90 kDa), identified as enolase complex, was found to be a potential *Streptococcus*-binding receptor. A range of *C. albicans* mutants were screened for adherence and biofilm formation with streptococci. Two banks of transposon mutants in cell surface proteins or transcription factors identified Orf19.5412 (369 aa) and Orf19.2476 (1684 aa), orthologues to *S. cerevisiae* ECM9 and ECM5 respectively, as influencing *S. gordonii* interactions and biofilm formation. In addition, a screen of glycosylation-deficient mutants showed that *MNT1*, encoding a mannosyltransferase involved in O-linked glycosylation, was necessary for *S. gordonii*-*C. albicans* recognition. These results show that cell wall biogenesis and glycosylation defects modulate interactions between streptococci and *C. albicans* in mixed-species biofilms.

PS 03 An expression model for functional studies of the biofilm linked accumulation associated protein of *Staphylococcus epidermidis*

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Staphylococcus epidermidis is a leading cause of nosocomial infection and biofilm formation is one of the main virulence factors. The polysaccharide intercellular adhesin (PIA), which induces biofilm formation, is found in many clinical isolates. However, the accumulation associated protein (Aap) also induces biofilms independently of PIA. Full length Aap is a 280-kDa, cell-wall-anchored fibrillar protein with a modular domain organization. The N-terminal A-domain is followed by a variable number of B-repeats and a LPXTG cell-wall-anchoring motif. In order to study the functions of Aap independently of other staphylococcal polysaccharides and proteins, Aap with 6 B-repeats was expressed on the surface of *Lactococcus lactis*, a strain lacking surface fibrils and without biofilm forming ability. Aap expressing *L. lactis* formed a thin biofilm indicating that Aap retained biofilm inducing activity in this background. Versions of Aap with engineered domain organizations are also being expressed in surrogate hosts and the importance of numbers of B-repeats and A-domain truncation investigated. Preliminary results suggest that Aap induces biofilm formation independently of other staphylococcal proteins or polysaccharides.

PS 04 Upregulation of biofilm specific genes in *Pseudomonas aeruginosa* PAO1 exposed to polyhexamethylene biguanide (PHMB)

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Pseudomonad biofilms are found in many environments such as swimming pools and in manufacturing processes. They are difficult to control as biofilms are more tolerant to biocides than planktonic cells. In this study the genetic response of biofilms to polyhexamethylene biguanide (PHMB) was investigated. DNA microarrays were used to compare gene expression of both planktonic and biofilm grown *P. aeruginosa* PAO1 cells, before and after exposure to PHMB. The model biofilms were grown in silicone tubing and a total of 117 genes were upregulated in the biofilms in response to PHMB. Of these 117 genes, 73 were biofilm-specific as they were not upregulated in planktonic cells exposed to PHMB and these included 30 genes that code for pyocins. The specific upregulation of genes in biofilms may therefore play a role in biofilm resistance to biocides, however, full characterization and understanding of the roles such genes play in resistance is necessary.

PS 05 Quantification of biofilm on glassy coke support material for expanded bed bioprocesses

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We have developed novel technology for bioprocess intensification, based on immobilization of cells on inert support particles and operation as an expanded bed. The best material for these particles is glassy coke, which is carbon foam (+10% ash) that has interconnecting pores. Cells enter these pores, attach to the coke and grow as a biofilm. However, quantification of the immobilized biomass is difficult because of the nature of the support material.

We have investigated the use of a muffle furnace to incinerate biofilm and thus determine the biomass associated with the coke support particles.

We have demonstrated that biofilm mechanically recovered from coke can be fully ashed (> 80% mass loss) at temperatures <400°C. However, biofilm still associated with coke did not become ashed until the temperature exceeded 400°C but was fully ashed at 420°C. In contrast, only 6% of the coke was ashed at 420°C and significant combustion only occurred at temperatures >500°C.

In conclusion, we have developed a technique for the quantification of biomass immobilized on support materials such as glassy coke. This then allows specific rates of reaction to be calculated for bioparticles, based on the amount of biofilm associated with the support material.

PS 06 Intestinal biofilms in health and neoplastic disease

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Background The gut microbiome plays a significant role in the health of the host. Intestinal dysbioses is an imbalance of the gastrointestinal tract flora and is linked with inflammatory conditions and cancer. In previous studies we have demonstrated the uniqueness and conserved nature of intestinal microbiota.

Methods Faeces was collected from T-cell deficient nude mice to examine the influence of neoplastic disease and irradiation. Human tumours, (breast, MDA-231: colonic, HCT-116: lung, calu-6) for cancer studies and colonic (SW-620) for irradiation, were implanted dorsally. Eubacterial communities were analysed via Q-PCR and PCR-DGGE.

Results Whilst overall microbial richness was unaltered during neoplastic disease, DGGE profiles from control, MDA, HCT and calu-6 implanted mice revealed marked differences in community composition. Irradiation therapy caused transient shifts during and post-treatment. Q-PCR revealed significant differences also occurred in 16s ribosomal DNA copy numbers.

Conclusion Establishment of tumours distal to the GI tract and dorsal irradiation, produced profound changes in microbial flora. This could impact on drug metabolism, host nutrition and both cancer and general treatment.

PS 07 Microbial stability in oral biofilm microcosms

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Introduction This investigation explores the micro-ecological and host-derived factors that influence ecological and metabolic homeostasis within oral microbial communities.

Methods MSDs (multiple sorbarod devices) support sessile growth on cellulose filters. Biofilms can be analysed, together with released cells in eluted medium (perfusates). Two separate MSD oral microcosms were established using saliva from two separate volunteers as inocula. Developed communities were monitored over 20d using viable counting and PCR-DGGE. A reciprocal swap of perfusates (20ml) between the two fermenters was done after 11d to challenge community stability. MSDs were also run with and without the addition of human beta-defensins 1 and 2.

Results Aerobes, anaerobes and Gram-negative species were unaffected by immigrant consortia, whilst in both fermenters, streptococci significantly increased with concomitant reductions in lactobacilli. Cluster analyses showed that once established, DGGE fingerprints were not significantly altered by immigration of foreign consortia.

Conclusion The immigration of dental plaque consortia derived from a different individual into pre-established plaques caused changes in population densities that could not be explained based on simple augmentation of bacterial numbers but did not markedly alter overall species composition.

PS 08 Ecological analyses of pair-wise interactions amongst bacteria derived from individual oral biofilms

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Background Each person's oral microbiota is both unique and highly stable. The specificity and strength of coaggregation has been previously used to examine inter-species interactions in early plaque development. In the current study, we tested the hypothesis that the frequency of positive bacterial interactions will be higher between bacteria derived from the same mouth (self/self) than from different mouths (self/non-self).

Methods Bacteria were isolated from four individuals using enrichment techniques to maximize recovery. All possible pair-wise interactions were then determined amongst 49 isolates using a modified cross-streak method which reveals positive (growth stimulation), negative (growth inhibition) or neutral interactions (no effect compared to the control) among paired strains.

Results From a total of 1176 pairings, 52% were neutral, 42% were negative and 6% were positive. No statistical difference in the frequency of positive or negative interactions was apparent between self/self and self/non-self pairings (mean 6.7 and 36.7 vs. 5.2 and 38.6, respectively).

Conclusions The majority of inter-strain interactions among oral microbiota are negative or neutral and there was no evidence for bias in the outcome of bacterial interactions amongst self/self or self/non-self pairings.

PS 09 Analyses of surface-associated bacteria in the nasal cavity of healthy adults

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The microbial ecology of the human nasal cavity is poorly understood. In the current investigation, the inferior turbinates of four healthy volunteers were analysed using differential culture and PCR-denaturing gradient gel electrophoresis (DGGE). A hierarchal dendogram was constructed to compare DNA fingerprints derived from each volunteer, along with fingerprints similarly constructed from concomitantly collected saliva samples. Dominant organisms were identified by exhaustive sequencing of DGGE bands. All samples were colonized by staphylococci, corynebacterium and propionibacterium. Aerobic cell counts ranged between 33 and 321 cfu/cm^2 (mean 142 cfu/cm^2) and anaerobes between 28 and 130 cfu/cm^2 (mean 52 cfu/cm^2). DGGE-based estimates of diversity were markedly higher than those based on isolation (22 phylotypes *vs.* 13, respectively). Concordance between nasal DGGE fingerprints ranged between 52 and 72% whilst salivary fingerprints were at least 50% different and clustered separately. Sequencing of DGGE bands identified little homology between oral and nasal microbiotas. Site-specific surface colonization of the nasal cavity has significant implications for health through colonization resistance, pathogen carriage and infection.

PS 10 Effect of specific growth rate on the biocide sensitivity of planktonic and biofilm-derived *Pseudomonas aeruginosa*

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To investigate the effect of specific growth rate (μ) on biocidal kill rates, *Pseudomonas aeruginosa* was grown in planktonic (chemostat) and biofilm (perfusion system) at a range of growth rates. Growth rates were controlled by adjusting the dilution rate (in the chemostat) and the flow rate (in the biofilm). Cell samples were removed from each condition and exposed to 3 biocidal agents; chlorhexidine, NaOCl and H_2O_2, and kill rates determined by viable count. The sensitivity of planktonic cells was strongly dependant on μ since kill rates differed significantly across growth rates. The relationship between μ and cell sensitivity to biocides was also observed at high and low μ for biofilm-derived cells in otherwise identical conditions. When comparing the sensitivity of growth rate matched planktonic and biofilm-derived cells, no significant differences in kill rates were observed. This study suggests that there is no special planktonic or biofilm phenotype that is expressed to give biocide sensitivity/resistance (for those agents tested) and that growth rate is by far the most important predictor of sensitivity of *P. aeruginosa* to biocides.

PS 11 Performance and microbial community dynamics of microbial fuel cells fed with sucrose

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Much remains unknown about the role of the micro-organisms within anodic biofilm communities in a microbial fuel cell (MFC). Understanding how microbial populations evolve within a MFC is an essential step towards achieving better performance. The aim of this study is to determine the microbial community composition dynamics by DGGE in MFCs inoculated with anaerobic sludge.

A maximum power density of 1.0 W m^{-3} was obtained in a continuous-mode MFC with sucrose as substrate. A shift in the bacterial biofilm community composition was observed over time. When a biofilm sample, taken from a well-performing sucrose-fed MFC inoculated with anaerobic sludge, was used as inoculum, no further improvement in power density was observed. However, the microbial community composition differed when these different types of inocula were used.

Our results show that in sucrose-fed MFCs, the composition of the microbial community changes over time. However, the nature of the inoculum affects the composition of the microbial community, but does not have a significant effect on power generation.

PS 12 Role of oxygen and carbon dioxide in biofilm formation in *Campylobacter jejuni*

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Background The microaerophilic pathogen *Campylobacter jejuni* is a leading cause of human enteric disease worldwide. It is still unclear how *C. jejuni* is able to survive exposure to stresses like atmospheric oxygen during transmission from poultry to man, and biofilm formation in *Campylobacter jejuni* is thought to contribute to environmental survival.

Objective to identify mechanisms involved in biofilm formation by *C. jejuni.*

Results and conclusions Incubation of static cultures of *C. jejuni* in atmospheric oxygen resulted in increased biofilm formation when compared to cultures incubated at microaerobic conditions (10% CO_2, 5% O_2). Incubation of *C. jejuni* at 10% CO_2 / 90% air however did not result in increased biofilm formation, suggesting that lack of CO_2 may be an important stimulus in initiating biofilm formation. Microarray analysis revealed the transcriptional changes during static culture under these different combinations of (micro) aerobic/CO_2 conditions. Responses included a downshift in genes encoding respiratory pathways, while stress-resistance systems were up-regulated. We are currently investigating the role of the essential orphan response regulator Cj0355c in control of this biofilm-associated lifestyle.

PS 13 Biofilm formation by *Francisella tularensis*

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Francisella tularensis infects an unusually wide range of wildlife and can survive in many different ecosystems. It is not fully understood how it persists in the environment. Many *F. tularensis* subsp. *holarctica* disease outbreaks have been associated with water and it is well documented the organism can survive for very long periods in cold water. The object of this research was to explore the hypothesis that *F. tularensis* persists in aquatic environments as a biofilm.

Biofilm development by *F. tularensis* occurred in distinct steps and exhibited typical biofilm properties such as increased antimicrobial resistance. There was no evidence for the formation of an extensive biofilm matrix by electron microscopy although a thin capsule-like material was evident. Biofilm formation occurred between 30–37°C but not at 20°C suggesting that biofilm formation may not play a role outside a mammalian host or arthropod vectors. The up-regulation of a chitinase gene and chitin binding protein indicates biofilm formation might be consistent with a role in the colonization of insects.

PS 14 Biofilms of *Pseudomonas aeruginosa* secrete an altered spectrum of virulence factors compared with their planktonic counterparts

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Pseudomonas aeruginosa is an opportunistic human pathogen which readily forms antibiotic-insensitive biofilms on biotic and abiotic surfaces. This resistance to antibiotic intervention means that biofilms are often associated with chronic, recalcitrant bacterial infections such as those found in the lungs of cystic fibrosis patients. *P. aeruginosa* also produces a wealth of secreted proteinacious virulence factors. We have investigated whether the spectrum of secreted proteins differs depending on the lifestyle (growth mode) of the bacterium. In particular, we have compared the profile(s) of secreted protein from biofilms and planktonic cells of *P. aeruginosa* using quantitative 2D-DiGE proteomic analysis. The secretome fractions were different between these growth modes, and many of the differences were discernable shortly after biofilm establishment. This approach has enabled us to identify novel potential virulence factors, including one that is a homologue of vascular endothelial cell apoptosis-inducing protein from rattlesnake venom.

PS 15 Biofilms and type III secretion are not mutually exclusive

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Pseudomonas aeruginosa is an opportunistic pathogen that causes chronic infections in immunocompromised individuals. These infections are difficult to treat, partly due to the formation of highly antibiotic-tolerant biofilms. This tolerance has often been attributed to nutrient limitation and low metabolic activity, and gene expression in biofilms has been shown to resemble that of planktonic cells in stationary phase. Furthermore, biofilms are often considered to be passive reservoirs for more virulent planktonic cells. In support of this, mechanisms have been identified that inversely regulate biofilm formation and type III secretion. However, we have found that biofilms in our growth conditions are metabolically active structures that produce a different spectrum of secreted virulence factors compared with planktonic cells. Furthermore, our results show that the type III secretion system is induced in biofilms but not in planktonic cells. Biofilm formation and type III secretion are therefore not mutually exclusive, and biofilms could play a more active role in infection and virulence than previously thought.

PS 16 Role of mutation in the development of bacterial biofilm communities

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Biofilms generate discrete 3-dimensional growth foci – called microcolonies – which are densely packed and antibiotic tolerant proliferations of bacterial cells. Despite the ubiquity of the microcolony-based architecture of biofilms, it remains to be fully understood how a subset of bacteria proliferate to form microcolonies in biofilms; and others do not. Here we examined whether mutation and genetic heterogeneity in biofilms may play a role in microcolony initiation. To study mutation *in situ* within biofilms, we transformed *Pseudomonas aeruginosa* cells with a plasmid harbouring the green fluorescent protein gene containing a premature translation-termination codon. Transformed *P. aeruginosa* cells are non-fluorescent unless the stop codon reverts. Fluorescence-inducing mutations were observed in microcolony structures, but not in other biofilm cells, or in planktonic cultures of *P. aeruginosa*, suggesting microcolony-specific increases in mutation frequency. We also explored whether mutator phenotypes can enhance microcolony-based growth of *P. aeruginosa*.

For a range of *P. aeruginosa* strains defective in DNA error repair mechanisms, we found that microcolony initiation and growth was correlated with the mutation frequency of the organism. We suggest that microcolony-based growth can involve mutation and subsequent selection of mutants better adapted to grow on surfaces within crowded-cell environments.

PS 17 Co-operation and group structure in bacterial biofilms

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A key problem in understanding major transitions in evolution is the evolution of cooperation: how are mutants that exploit the benefits of cooperation without paying the costs (cheats) suppressed within populations? Biofilms, which display properties of both single cell and multicellular organisms, provide an excellent model system to address this question. Biofilms exhibit grouped population structure – they exist primarily as dense aggregates of cells called microcolonies. We aim to test the hypothesis that cell-grouping displayed by microcolonies in bacterial biofilms provides a mechanism to suppress cheats within the biofilm population. We are using the co-operative trait of siderophore production (an extracellular iron-chelating molecule) within *Pseudomonas aeruginosa* biofilms to investigate cooperation in biofilms. Under iron-limited conditions, production of siderophores enhanced wild type growth, but microcolonies containing GFP-tagged, pyoverdin-mutant 'cheats' developed poorly. In iron-rich conditions, cheats are favoured as siderophore production is costly. With mixed strain biofilms, cheats are dependant on the wild type for successful growth. We suggest that, if cheats reduce colony size, thus self-limiting their global population, microcolony-based group structures may be an important (potentially evolved) mechanism to suppress cheats in bacterial populations.

PS 18 *In vitro* production and composition of extracellular polymeric substances formed by ruminal bacteria on perennial ryegrass

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To understand more about the role of biofilm formation during attachment of ruminal bacteria to forage, the temporal production and composition of extracellular polymeric substances (EPS) was measured post-attachment of rumen bacteria to fresh perennial ryegrass (PRG). Serum bottles containing Van Soest media, 10% strained rumen fluid and 5% fresh PRG (6-week old) were incubated under rumen-like conditions. Colonized plant material was harvested at intervals up to 24 h. EPS was extracted with 3% glutaraldehyde prior to extraction of DNA from microbes attached to forage. EPS production increased up to 2 h, decreasing thereafter. At all times EPS contained mainly carbohydrates (25–62% of EPS) with proportionally less protein (9–14%) and DNA (3.2–5.6%). Although proportionally low, the DNA concentration within EPS was similar to that of the attached microbes at all times. A high diversity in 16S rRNA was seen within DNA extracted from EPS and attached microbial fractions, harvested following 2h of incubation, by 454-pyrosequencing. Some proportional differences were also evident between both fractions at a Family level. These data indicate that DNA is an integral component of EPS produced by ruminal bacteria attaching to fresh perennial ryegrass although the source of DNA in EPS remains to be identified.

PS 19 Biofilm analysis and management in a novel no-touch tap system

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Large scale water distribution systems are subject to biofilm formation, and may harbour potential pathogens. Studies are being carried out at the University of Plymouth, in partnership with Dart Valley Systems, Paignton, Devon, on a novel water distribution system 'test rig', in order to assess the effects of stagnation, high- temperature flushing, and different materials on biofilm formation. The test rig is designed to simulate conditions in a large scale system, such as may be found in a hospital situation. Analysis of biofilms is being carried out using a wide variety of culture-based, molecular, and microscopy techniques. These include SEM, confocal microscopy (FISH, LIVE/DEAD analysis), ATP analysis, and PCR-DGGE. Preliminary data suggests that biofilm formation may be curtailed by periods of stagnation in a high pH environment, such as has been observed in this system. It is assumed that this is likely to significantly affect the nature of any biofilm formed.

PS 20 Bacterial arsenite oxidation in cold environments

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Arsenic is toxic to living cells and its two soluble inorganic forms, arsenite and arsenate, have caused mass poisoning in East Asia. In addition, arsenic contamination due to anthropogenic activity (e.g. mining) is becoming increasingly important in regions across the globe. Despite arsenic's toxicity, some prokaryotes have mechanisms to gain energy by either oxidizing or reducing it, and are involved in the cycling of arsenic. Prokaryotic arsenic metabolism has been detected in various environments but has never been reported in cold environments. Here we report the first detection and isolation of aerobic psychrophilic arsenite-oxidizing bacteria from two sites in Canada; a biofilm in an abandoned underground gold mine, storing large amounts of arsenic trioxide and sediments from the creek draining this mine. We have compared the diversity of arsenite-oxidizers in two sub-samples of the biofilm that vary in arsenic speciation. We have also determined that the arsenite oxidizers have a broad temperature range, and could be ideal candidates for use in arsenic bioremediation in cold-moderate climates.

PS 21 Determination of sources of bias in molecular characterization of oral bacterial communities

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Molecular ecology studies of the oral microbiota have shown that the high G+C Gram-positive phylum *Actinobacteria*, which includes many oral bacteria associated with oral health and disease, is under-represented in 16S rRNA gene libraries compared with culture. The ability of 16S rRNA primers including 27F, 1492R, 63F, 1387R, 61F, a shortened version of 63F, and a novel primer, 39F, to amplify DNA from oral representatives of *Actinobacteria* and *Firmicutes* was assessed. A 1:1 mixture of *Streptococcus sanguinis* (*Firmicutes*) and *Actinomyces naeslundii* (*Actinobacteria*) was prepared and 16S rRNA genes amplified using Thermoprime *Taq* or PhusionHS, designed for high G+C templates. Inserts cloned into TOPO PCR2 were identified by restriction digestion. Primer sequence had a greater influence on library composition than the polymerase. In libraries prepared with 27F /1492R, *A. naeslundii* was under-represented (*Taq* – 12.0% of total clones, PhusionHS – 8.0%). In contrast, with primers 61F / 1387R *A. naeslundii* made up 73.9% and 41.3% of the libraries respectively. In conclusion, choice of 'universal' primer is an important consideration in 16S rRNA-based molecular analyses.

PS 22 Iron availability influences biofilm formation in *Escherichia coli*

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Low iron concentration induces free-living forms among bacteria, while high iron concentration stimulates biofilm formation. This research

investigates effect of iron availability on biofilm formation by 6 strains of *Escherichia coli* to enhance understanding of their biofilm-forming potential. Biofilm formation by these isolates was tested at 37°C using modified Luria Bertani broth without NaCl (LBNS), iron depleted LBNS (dLBNS) and dLBNS separately supplemented with: ferric ammonium citrate, sodium citrate, ammonium citrate and citric acid. Three independent experiments were conducted in triplicate. In general, a decrease in biofilm formation was observed under iron-limited conditions ($p < 0.05$) in all isolates; however, upon replenishing iron, biofilm formation was restored in all isolates in a dose dependent manner. The concentration at which biofilm formation was restored varied slightly among different isolates. No change was observed on addition of control compounds to iron-depleted medium ($p > 0.05$). It is therefore concluded that *E. coli* biofilm formation is stimulated by availability of iron.

PS 23 Anaerobic induction of bacteriocin (pyocin) production in *Pseudomonas aeruginosa* biofilms

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The pathogenic bacterium *Pseudomonas aeruginosa* is known to reside as a static biofilm population within the thickened airway mucus in the lungs of individuals with Cystic Fibrosis. As this is a heterogeneous environment with regions of anaerobicity, a transcriptomic comparison of mature (2 day) *P. aeruginosa* PAO1 biofilms grown under both aerobic and anaerobic conditions was performed. Amongst the genes that were up-regulated under anaerobic biofilm conditions were those that encode the R-, F- and S-type pyocins (bacteriocins) of PAO1. Increased bacteriocidal activity in supernatant from anaerobic biofilms was verified experimentally using pyocin overlay and planktonic growth assays. In addition, biofilm co-culture of PAO1 and a pyocin sensitive clinical *P. aeruginosa* isolate showed that pyocin production can have a significant impact on bacterial populations within a biofilm under anaerobic conditions. Our data suggests that the induction of pyocin production in anaerobic biofilms could play an important role in niche establishment and maintenance by *P. aeruginosa*.

PS 24 Biofilm evidence in horse wounds

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Equine wound healing is notoriously problematic, specifically when biofilms are evident. Biofilms are naturally recalcitrant towards antimicrobial agents, and the host's immune system, highlighting their possible significance to chronic wounds. This study aimed to identify biofilms, characterize the microbial flora of equine wounds and evaluate their antibiograms. Thirty equine wounds were swabbed according to standard protocols. Aerobic and anaerobic bacteria were cultured and identified using traditional microbiological culture techniques. A swab sample was also frozen and processed for microbial identification using Denaturing gradient gel electrophoresis (DGGE) and PCR. Similarly, intact skin, representative of the wound site, was swabbed and the bacteria identified. Biopsy samples were obtained and processed for evidence of biofilms using standard histological dyes. Analysis of the culturable microflora identified numerous bacterial species. In conclusion this study provided evidence that biofilms exist in chronic horse wounds and that the microflora of wounds is diverse.

PS 25 Microbial interactions of cystic fibrosis pathogens

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Background Aspergillus fumigatus is a ubiquitous mould found in up to 57% of CF respiratory secretions. However, little is known about interactions between *A. fumigatus* and the bacteria present in the CF lung.

Aims To characterize the interactions between *A. fumigatus* and *Pseudomonas aeruginosa*, *Burkholderia cenocepacia* or *Staphylococcus* species.

Methods Representative clinical strains of *P. aeruginosa*, *B. cenocepacia* and *Staphyloccocus aureus* were co-cultured with conidial suspensions and pre-formed *Aspergillus* biofilms, with resulting fungal biomasses quantified after 24h. The influence of quorum sensing molecules was assessed using *P. aeruginosa* Δ*las* mutants, and a Transwell™ system to provide physical separation.

Results A. fumigatus hyphal development was severely inhibited the direct interaction of Gram-negative bacteria, however little inhibition and alteration of biofilm structure was noted with pre-formed biofilms. Indirect bacterial/fungal interaction reduced filamentation by 60–80%. Mutant *lasI/R* strains exhibited reduced capacity to inhibit hyphal development compared to the wild type.

Conclusions Presence of bacterial CF pathogens and their secreted products can detrimentally influence the colonization and development of *A. fumigatus* in the CF lung.

PS 26 A functional AcrAB-TolC efflux system is required for biofilm formation in *Salmonella enterica* serovar Typhimurium

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Background and objectives To investigate the role of the AcrAB-TolC efflux system of *S.* Typhimurium in biofilm formation.

Methods Biofilms of *S.* Typhimurium 14028 and isogenic derivatives lacking or over-expressing AcrAB-TolC were grown in defined minimal media in microtitre trays or transwell trays and quantified by staining with crystal violet or ruthenium red.

Results Mutants lacking either *acrAB* or *tolC* were compromised in their ability to form biofilms compared to 14028. Co-incubation of mutants lacking *acrAB* or *tolC* and 14028 separated by a membrane which acts as a barrier to cells but not solutes showed no rescue of biofilm formation. The addition of efflux pump inhibitor molecules was able to reduce/prevent biofilm formation by 14028S in a concentration-dependent fashion. Expression of *acrB* and *tolC* was increased in biofilm cells compared to planktonic cells but artificial over-expression of AcrAB-TolC did not increase biofilm formation.

Conclusion A functional AcrAB-TolC system is required for efficient biofilm formation in *Salmonella*. Molecules which inhibit this system are able to prevent biofilm formation and may have clinical/commercial potential.

PS 27 Biodegradation of fluorobenzoate in a *Pseudomonas* sp. B13 biofilm

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Here we describe our investigations on the degradation of fluorinated compounds in planktonic and biofilm cultures of *Pseudomonas* sp. B13. A planktonic growth rate was established after adapting the strain to 10 mM 4-fluorobenzoate. Biofilm formation characteriztics and morphology were examined using a glass cover-slip method combined with fluorescence microscopy. A continuous flow biofilm reactor was employed to compare reaction rates between biofilm and planktonic cultures. The degradation of the substrate was monitored using fluoride ion selective electrode, HPLC and ^{19}F NMR spectroscopy. Oxygen uptake rates and biofilm thickness were measured throughout three-week cultivations. Comparison of specific substrate utilization rates showed that the biofilm was more effective than the planktonic culture in terms of removal efficiency. Further investigation of this comparative advantage is being investigated quantitatively by examination of the temporal and spatial distribution of the cell cycle in the biofilm using flow cytometry.

PS 28 Correlation between biofilm forming capacity and expression of the major autolysin gene, atl, in *Staphylococcus aureus* 8325-4

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The *Staphylococcus aureus* cell wall protein Atl plays multiple roles in autolysis, cell separation and peptidoglycan turnover. In *S. epidermidis*, the Atl homolog is required for primary attachment and therefore biofilm formation. However, the role of Atl in *S. aureus* biofilm phenotype has not yet been fully described, in part because the pleiotrophic impact of an *atl* mutation on the cell surface. To address this we sought to genetically manipulate *atl* gene expression in order to gain further insights into both Atl regulation and the role of Atl in biofilm formation. Allele replacement was used to construct an insertion mutation in the putative transcriptional regulator *atlR* in *S. aureus* 8325-4. Autolysis and lytic activity were unaffected by the *atlR*::Tc^r mutation. However biofilm forming capacity on polystyrene was significantly increased in the *atlR*::Tc^r mutant. Consistent with this RT-PCR analysis revealed a significant increase in *atl* expression in the *atlR*::Tc^r mutant. These data indicate that AtlR acts as a repressor of *atl* expression and that activation of *atl* transcription correlated with increased biofilm forming capacity.

PS 29 A methacrylate polymer is bactericidal to biofilm producing *Staphylococcus epidermidis*

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Bacterial biofilms have a major role in infections associated with implanted biomaterials. One approach for treatment is prophylaxis, such as incorporation of antimicrobials as anti-adhesive and bactericidal coatings. One potential example is the mucoadhesive cationic polymer, poly(2-(dimethylamino ethyl)methacrylate)) (pDMAEMA). pDMAEMA inhibits adhesion of *S. typhimurium* to human intestinal monolayer cultures and is bactericidal. The aim of this study was to investigate the antimicrobial effects of pDMAEMA against biofilm-producing bacteria. Antimicrobial activity was assessed by agar diffusion assays and by determination of MICs. Mutant strains of *S. aureus* were also used for investigation of the polymer's mode of action. Results showed that pDMAEMA kills *S. epidermidis* efficiently, but not *S. aureus*. However the mutant *S. aureus* strain, SA113 Δ*mpr*F, was susceptible to pDMAEMA. In contrast to wild type, the mutant does not modify phosphatidylglycerol in its cell membrane with L-lysine. The data suggest that formulated pDMAEMA may potentially be useful as a preventative treatment against *S. epidermidis* infections. It may act by interacting with the negatively charged surface of the bacteria since the more positively charged wild type *S. aureus* was less susceptible than the negatively charged mutant, SA113 Δ*mpr*F.

PS 30 Regulation of *icaADBC*-dependent biofilm development by a GGDEF domain protein in *Staphylococcus epidermidis*

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Cyclic di-GMP is a bacterial secondary messenger synthesized by proteins with a conserved GGDEF domain. Staphylococci encode only one predicted protein with a conserved GGDEF domain, which we designated GdpS (GGDEF domain protein staphylococcus). Inactivation of *gdpS* in *Staphylococcus epidermidis* impaired biofilm formation, whereas *gdpS* overexpression complemented the *gdpS* mutation and enhanced wild type biofilm development. GdpS activated *icaADBC* transcription and production of the *icaADBC*-encoded biofilm exopolysaccharide. Heterologous diguanylate cyclases (c-di-GMP synthases) failed to complement the mutation and mutagenesis of the 'GGDEF' motif essential for diguanylate cyclase substrate binding did not impair GdpS-mediated biofilm activation. Purified GdpS domain showed no diguanylate cyclase activity *in vitro*. Also, the N-terminal membrane spanning domain of GdpS alone complemented the *gdpS* mutation. The *gdpS* gene from *S. aureus* also complemented the *S. epidermidis gdpS* mutation suggesting that the role of GdpS in biofilm regulation is conserved in staphylococci. These data suggest that GdpS functions as a novel, c-di-GMP independent regulator of *icaADBC*-mediated biofilm development in *S. epidermidis*.

PS 31 The ecophysiology of anaerobic granular biofilm formation at 15°C

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Anaerobic granulation describes the self-immobilization of methanogenic consortia into dense, granular biofilms, underpinning the operation of high-rate, methanogenic, wastewater treatment bioreactors, typically operated at 20–45°C. Recent research has focused on potential low-temperature (3–20°C) applications. However, the biotic and abiotic factors influencing granulation at low-temperatures are poorly understood. Granular biomass from a full-scale bioreactor was crushed and used to inoculate 12 bioreactors. Four trials (circa 300 days each) were conducted, treating glucose-, volatile fatty acids- or peptone-based wastewaters. Each trial consisted of a control (37°C±1) and two low-temperature (15°C±1) bioreactors. Organic loading rates of 1–5.8 kg chemical oxygen demand (COD) m^{-3} d^{-1} were applied with COD removal efficiencies of ⩽80% at 15°C. Low-temperature granule formation was successfully demonstrated and quantified by particle size and density distribution analysis. 16S rRNA gene cloning, denaturing gradient gel electrophoresis and Real-Time-PCR, highlighted, the selective advantage of *Methanomicrobiales*. Activity assays, corroborated the dominance of hydrogen-mediated methanogenesis. Comparative metaproteomics, identified changes in gene expression associated with adaptation to low-temperatures and granular biofilm formation.

PS 32 Lactoferrin iron saturation affects the ability of *Burkholderia cepacia* complex (Bcc) to invade lung epithelial cells and form biofilms

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The *Burkholderia cepacia* complex (Bcc), are Gram-negative, opportunistic pathogens in Cystic Fibrosis (CF) patients. Previously we have shown that recombinant human lactoferrin (rHL) could enhance susceptibility of Bcc strains to antibiotics and also inhibit biofilm formation. This study focussed on whether the iron saturation level of rHL influenced the biofilm forming ability and invasive activity of Bcc strains. Results showed that in the presence of iron depleted apo-rHL, biofilm formation was 50% less than without lactoferrin. Furthermore, the ability of Bcc strains to invade cultured A549 cells was significantly enhanced in the presence of apolactoferrin. However, this activity was also dependent on the lactoferrin iron saturation level. In particular, the invasive ability of some strains was 25–30 times greater in the presence of apo-rHL. These findings demonstrate a significant relationship between the iron saturation level of lactoferrin and both the invasion efficiency of Bcc strains and their ability to form biofilms *in vitro*.

PS 33 Interspecies signalling mediated by the diffusible signal factor of *Stenotrophomonas maltophilia* influences biofilm formation and stress tolerance in *Pseudomonas aeruginosa*

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Stenotrophomonas maltophilia and *Pseudomonas aeruginosa* occur ubiquitously in the environment and can be found together in diverse niches including the rhizosphere of plants and the cystic fibrosis lung. In mixed species biofilms, *S. maltophilia* substantially influenced the architecture of *P. aeruginosa* structures, which developed as extended filaments. This effect depended upon the synthesis of the diffusible signal factor (DSF) by *S. maltophilia* and could be mimicked by addition of synthetic DSF. This response of *P. aeruginosa* to DSF required the sensor kinase PA1396. Mutation of *PA1396* or addition of DSF to *P. aeruginosa* led to increased levels of a number of proteins with roles in stress tolerance, including those implicated in resistance to cationic antimicrobial peptides. Synthesis of DSF or DSF-like compounds has been demonstrated not only in xanthomonads but also in other bacteria such as *Burkholderia cenocepacia*. Furthermore, homologues of PA1396 also occur in a number of pseudomonads suggesting that DSF-mediated interspecies signalling may be a phenomenon that occurs widely.

PS 34 Identification of a novel target, *hdfR*, required for both biofilm formation and colonization in *Photorhabdus luminescens* TT01

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Photorhabdus is a genus of Gram-negative bacteria which belongs to the family Enterobacteriaceae. The *Photorhabdus* life cycle involves alternating pathogenic and mutualistic relationships with invertebrate hosts. Species of *Photorhabdus* form specific mutualistic associations with entomopathogenic nematodes of the family *Heterorhabditis*. Together the nematode and associated bacteria are virulent pathogens of larval stage insects. Continuation of the mutualism with the nematode depends on successful colonization of the infective juvenile (IJ). The objective is to investigate whether a link exists between biofilm formation *in vitro* and colonization. A total of 8 mutants affected in colonization were identified from a transposon mutant library. Notably 5 mutants were also affected in biofilm formation. The majority of mutants were identified with transposon insertions in genes involved in lipopolysaccharide biosynthesis. However, one of the mutants involved a transposon insertion affecting *hdfR*, encoding a LysR-type transcriptional regulator. In this study we elucidate the role of *hdfR* in both biofilm formation and IJ colonization in *Photorhabdus*.

PS 35 The role of extracellular polysaccharide in the pathogenicity and lifestyle of *Photorhabdus luminescens*

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Photorhabdus is a Gram-negative, bioluminescent entomopathogenic bacterium which infects its insect hosts via a symbiotic association with *Heterorhabditis* spp nematodes. The bacteria colonize the gut of the nematode symbiont which actively seeks out and infects the insect host. The nematodes then regurgitate the bacteria into the open blood system of the insect, which is quickly killed by a bacterial septicemia. During this time both the bacteria and nematodes undergo several rounds of replication, after which the nematodes re-acquire *Photorhabdus* and emerge from the insect carcass. These worms then seek new hosts, thus completing the cycle. *P. luminescens* produces extracellular polysaccharides (EPS) giving colonies a 'sticky' phenotype. EPS has been shown to be important in the virulence of various plant and animal pathogens, but its role has not been investigated in *P. luminescens*. We present an investigation into the role of EPS by screening a knockout mutant library for biofilm defects. We genetically characterize these mutants and assess the impact of various mutants on pathogenicity. Implications are discussed.

PS 36 Mechanism of precise IS256 excisions from insertion sites in *Staphylococcus epidermidis*: impact on biofilm phase variation and switch to PIA-independent biofilm formation

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The mobile element IS*256* creates regularly eight-base pair target site duplications (TSDs) upon transposition. IS*256* is also known for its capacity to excise precisely from an integration site, including the removal of the TSD and restoration of the original DNA sequence. Notably, in *Staphylococcus epidermidis* PIA-mediated biofilm formation undergoes phase variation by reversible inactivation of the *icaADBC*-operon. Here we show that precise IS*256* excision is a host-driven process that occurs independently of a functional transposase and relies instead on the integrity of the TSD. IS*256* excision from the *ica*ADBC genes is a rare event (10^{-11}). Obviously to compensate for the impaired PIA-production, *icaC*::IS*256* insertion mutants were found to switch spontaneously to proteinaceous, Aap-mediated biofilm formation, highlighting the importance of the biofilm lifestyle for that organism. Atomic force microscopy revealed remarkable differences in PIA and Aap biofilm substructures.

PS 37 Improving imaging of biofilms with multiphoton laser scanning microscopy

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Imaging is an important tool in studying biofilm formation, dispersal and eradication, and visualizing living biofilms allows processes to be followed over time. This is frequently done using single photon confocal laser scanning microscopy. However, multi-photon laser scanning microscopy (MP-LSM) can allow deeper imaging with greater sensitivity and resolution, and inflict less biological damage. Here we describe a systematic study on the application of MP-LSM to image biofilms. We show increased resolution and significantly increased sensitivity with MP-LSM leading to images with resolution of single bacteria at depths up to 150 µM. We show that light scatter due to the biofilm biomass limits sensitivity, and since this is wavelength-dependent this can bias BAClight LIVE/DEAD stain results. MP-LSM can also be used as a manipulation tool. Examples include bleaching to follow cell motility, and localized ablation that to examine biofilm damage and repair. We describe hardware adaptations, which enable the use of high magnification, high numerical aperture objectives with flow cells. Subsequent to these adaptations time lapse imaging of biofilm growth in a flow cell at high resolution can be obtained. Thus MP-LSM should be considered for use as both an imaging and manipulation tool in the study of biofilms.

PS 38 Social cheating in bacterial biofilms

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It is generally assumed that the function of quorum sensing (QS), is to coordinate behaviours at the population level. However, evolutionary theory predicts that (1) the stability of QS could be threatened by social cheats who exploit the cooperation of others and (2) that the fitness advantage of a cheat changes with respect to population structure and spatial heterogeneity. Recent research has shown that in planktonic cultures of the opportunistic pathogen *Pseudomonas aeruginosa,* QS is a social trait that can be exploited by social cheaters. QS has been

shown to be important for biofilm formation in *P. aeruginosa* so here we assess whether a *P. aeruginosa* QS cheat can invade a biofilm. We used two experimental biofilm approaches, a peg-lid microtitre plate assay and a continuous flow cell culture combined with confocal scanning laser microscopy (CLSM) to investigate whether biofilms are vulnerable to invasion by QS cheats. We will report on (1) the ability of a QS cheat to invade a biofilm and (2) the effect of cheating load on biofilm formation and structure.

PS 39 The *Bacillus subtilis* transition state regulator Abh is part of a complex regulatory network that controls biofilm formation

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In the natural environment bacteria are found as part of a multicellular community known as a biofilm. The undomesticated *Bacillus subtilis* strain 3610 is an excellent model for analysis of biofilm development as when grown on a solid surface it produces a colony of complex morphology which is covered with aerial structures containing spores. Cells within a biofilm become highly differentiated, leading to the existence of multiple cell states, each with a specific role to perform, such as the production of the 'protective' polymeric matrix or spore formation. The 'complex colony' phenotype is utilized to assess various biofilm mutants as its development is dependent on the production of both a protein and polysaccharide component of the extracellular matrix. The transition state regulator Abh activates biofilm formation and our work is aimed at addressing the molecular basis of activation. Abh is predicted to be a member of the overlapping SigMWX regulons that are activated upon sensing external stress and are required for biofilm formation. Current analysis indicates that Abh is downstream from *sigW* since artificial induction of *abh* in a *sigW* mutant complements the biofilm deficient phenotype, suggesting Abh is part of a regulatory network that 'senses' external stress leading to the development of a stress resistant biofilm.

PS 40 The protein tyrosine phosphatase PtpZ inhibits biofilm formation by *Bacillus subtilis*

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Over 20 transcriptional regulators control biofilm formation by *Bacillus subtilis* but how the regulatory network fits together is largely unknown. In this study we investigated the interaction between the pathways controlled by the response regulator DegU, which activates biofilm formation, and the transcription regulator SinR, which inhibits biofilm formation using epistasis analysis. We determined that SinR and DegU are predominantly part of separate regulatory cascades that control biofilm formation but, in addition they have a small subset of genes that they jointly co-repress during biofilm development. These genes were identified as uniquely up-regulated in the absence of both *sinR* and *degU* through DNA microarray analysis. Based on the subsequent characterization of this novel set of genes we describe for the first time the identification of three novel repressors of biofilm formation by *B. subtilis,* PtpZ, YomI and YueH. These findings add a further level of complexity to the regulatory network that underpins biofilm formation to demonstrate that biofilm formation is controlled at the post-translational level through a protein-tyrosine phosphatase, PtpZ, as well as at multiple points at the transcriptional level.

PS 41 Dormancy, persister cells and antibiotic sensitivity

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Bacteria in normally susceptible populations that survive antibiotic or antimicrobial treatment are known as 'persister' cells and are thought to underpin biofilm survival. Persister cells are dormant or exhibit a substantially decreased growth rate but their underlying physiological status is often poorly defined. We have constructed a (null) mutant of *S. enterica* serovar Typhimurium, lacking all cold shock protein (*cspA*) paralogues required for growth following cold shock to 10°C. This strain is unable to multiply at low temperatures, persists for several weeks without a significant decline in viability and is capable of full recovery at 37°C. Thus, they represent conditional persister cells. We have discovered that wild type and 'null' cells exhibit different antibiotic killing patterns, particularly with respect to the antibiotic ofloxacin and low temperature. Using confocal microscopy and survival assays, we explore both population level/single-cell dynamics of planktonic and biofilm cultures at both 37°C and 10°C, using *S. typhimurium*. This will provide insights into whether all dormant cells behave uniformly.

PS 42 Polymer induced bacterial aggregation

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Bacterially derived polymers, such as polysaccharides, play an important role in the ability of bacteria to accumulate into aggregates and biofilms. Polysaccharides in particular are often ascribed a structural role in terms of providing the 'glue' that sticks cells together in a process known as bridging flocculation. To further understand the role of polysaccharides in aggregate formation we are utilizing *Sinorhizobium meliloti*, an agriculturally important legume symbiont that fixes nitrogen for a plant host, as a model system. The polysaccharides produced by *S. meliloti* are well characterized both in terms of the structure of the polysaccharides and the biosynthetic pathways, with a suite of mutants available affected in polysaccharide biosynthesis. We have found that a mutant that overproduces polysaccharide has enhanced aggregation and sedimentation. Adding exogenous polysaccharide to the parent strain also results in enhanced aggregation. Rather than bridging flocculation we believe the polysaccharide causes formation of aggregates as a result of a novel mechanism of depletion attraction, a hitherto underappreciated force in bacterial multicellular organization.

PS 43 Biofilm formation by the periodontal pathogen *Tannerella forsythia*

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Tannerella forsythia is a major contributor to the globally important disease of periodontitis. These bacteria commonly exist within the dental plaque biofilm *in vivo* but there is limited information on the genetic and physiological mechanisms employed during biofilm formation by *Tannerella*. We are employing a combination of RT-PCR, proteomics and reverse genetics to examine the genetic basis of the adaptions *Tannerella* makes during its switch to a biofilm lifestyle. Expression of putative virulence genes identified from the *Tannerella* genome sequence, such as *uspA, ustA, ompA,* and the sialidase genes *siaH1* and *TF0035* were assessed by RT-PCR using specific oligonucleotide primer sets developed in house with the housekeeping 16s rRNA gene as a control. Differential expression of a range of these known and putative virulence genes was established and the physiological basis of their possible involvement in biofilm formation is being investigated further. In parallel experiments proteins were extracted from biofilm-grown cells to develop proteomic protocols for this organism. Initial proteomic experiments have revealed several differentially expressed proteins that are being identified to reveal novel target genes for future studies.

PS 44 Presence, diversity and biofilm forming capacity of bacteria in drinking water

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Biofilms form at interfaces within aquatic environments, including oligotrophic environments such as drinking water systems. As microbial cells detach and are dispersed from biofilms, these cells exist in planktonic forms either as individual cells or as aggregates of cells shed as clumps into the bulk fluid. In this study, the community structure, diversity and biofilm formation potential of planktonic bacteria present within drinking water has been investigated via cultivation and assessment of biofilm formation (crystal violet assay) and metabolic activity (XTT-reduction assay) both of single cells and consortia; with additional molecular comparison of community composition.

Planktonic isolates from drinking water differed in both their ability to form biofilms and in their metabolic activity. Moreover, combinations of different isolates promoted biofilm formation. Improving our knowledge of the diversity and synergistic and antagonistic interactions within drinking water biofilms will improve our ability to maintain the safety of drinking water.

PS 45 Anaerobic gene expression and elemental heterogeneity in bacterial communities

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Escherichia coli biofilms grow in the anoxic environment of the mammalian gut. This work used DNA microarrays to identify genes differentially-regulated in *E. coli* in response to biofilm growth in the absence of oxygen. Contrary to previously published data, it was found that *E. coli* forms biofilms anaerobically in minimal and complex media on a variety of solid surfaces. Genes significantly up-regulated in anaerobically-grown biofilm-associated cells were involved in flagellum synthesis, motility and transport, particularly of maltose. Cultivation of biofilms on glass wool inside custom-made chemostats allowed transcriptional profiling of biofilms during adaptation to anaerobiosis. As well as predictable gene expression changes in respiratory chain composition, other changes were detected including flagellum, phage and transport-associated genes. In order to examine heterogeneity in biofilms, the sensitive analytical method LA-ICP-MS was developed to measure variations in elemental distribution in *E. coli* colonies and biofilms. The resulting three-dimensional maps displayed significant differences in the spatial distribution of four biologically-significant metals.

PS 46 Inhibition of *Burkholderia cepacia* complex biofilm formation by quorum sensing inhibitors

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Burkholderia cepacia complex (Bcc) strains are opportunistic pathogens, causing life-threatening infections in CF patients. In this study we evaluated the quorum sensing (QS) inhibitory and anti-biofilm effect of several compounds on Bcc strains.

Inhibition of QS was assessed using various biosensors. The effect of the active compounds on Bcc biofilm formation was evaluated using Crystal Violet biomass and Cell-Titer Blue viability staining as well as by plating. Syto9 was used to stain biofilms for microscopy.

Several compounds (including antibiotics), used in Sub-MIC concentrations, interfered with the biofilm formation of Bcc strains. Our results suggest that they do not interfere with the initial attachment but affect later stages of biofilm formation, including maturation (i.e. the process of forming stable biofilms) and detachment. In addition, several QS inhibitors had a considerable impact on biofilm structure.

Our data indicate that several QS inhibitors had an effect on Bcc biofilm formation. These compounds may hold promise to treat Bcc biofilm related infections.

PS 47 Comparison of antibiotic resistance in planktonic and sessile *Burkholderia cepacia* complex cultures

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Burkholderia cepacia complex bacteria are opportunistic pathogens that can cause severe respiratory tract infections in cystic fibrosis patients. Treatment of infected patients is particularly problematic as these organisms are highly resistant to most antibiotics. The biofilm-forming capability of *Burkholderia cepacia* complex organisms may also contribute to the problem of resistance. The goal of the present study was to obtain a comprehensive overview of the bactericidal and bacteriostatic effects of six commonly used antibiotics on both planktonic and biofilm-grown *Burkholderia cepacia* complex cells.

The minimal inhibitory concentrations for planktonic cultures (MICs) and the minimal biofilm inhibitory concentrations (MBICs) were determined by using broth microdilution and a resazurin-based viability staining, respectively. The bactericidal effects on stationary phase planktonic and biofilm cultures were determined using traditional plate counting.

The extent of the growth inhibitory effect of the antibiotics examined is strain dependent, but in general, the MIC and MBIC data for a given strain are comparable. However, the bactericidal activity of the tested antibiotics is generally more pronounced for planktonic than for sessile *Burkholderia cepacia* complex cells.

PS 48 A putative symporter involved in biofilm formation by *Klebsiella pneumoniae*

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Klebsiella pneumoniae is an opportunistic pathogen commonly associated with nosocomial infections. The persistence and pathogenesis of this micro-organism is associated with its capacity to form biofilm.

A *K. pneumoniae* transposon mutant library was initially screened in a dynamic model to detect clones impaired in early stages of biofilm formation. Analysis of the insertion point of one attenuated mutant, YF9, revealed the transposon had inserted in a gene coding for a putative membrane transporter, a Na+/proline symporter. Transcomplementation of YF9 with the *symporter* encoding region including its putative own promoter restored the wild-type phenotype, as determined by measuring the biofilm biomass and by scanning electron microscopy (SEM) observation. An isogenic mutant of the putative *symporter* structural gene was created. No biofilm biomass attenuation was detected with this mutant and no modification was observed by SEM compared to the wild-type biofilm.

The creation of isogenic mutants with partial deletions of the *symporter* gene is in progress, to characterize the region involved in biofilm formation and its function.

PS 49 Influence of different environmental conditions upon *in vitro* biofilm-formation by staphylococci mastitis isolates

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Biofilm is an important virulence factor in mastitis staphylococci, allowing bacterial persistence in the udder. *Staphylococcus aureus* is a recognized mastitis pathogen, and *Staphylococcus epidermidis* is an emerging relevant agent.

The present work evaluated the influence of milk, milking shear forces and sub-inhibitory concentrations of oxytetracycline(OXT), penicillin(P), sulfamethoxazole/trimethoprim(SXT), gentamicin(GN), and enrofloxacin(ENR) in biofilm formation by *S. aureus* (n=21) and *S. epidermidis* (n=22) mastitis isolates. Biofilm quantification was

performed using a microplate Alamar Blue (AB) assay, under six conditions: bacterial suspensions in MHB and milk, incubated for 24h, 37°C, in the presence and absence of SubMIC, and in static conditions and under shear forces.

Mimicking milking shear forces did not influence *S. aureus* biofilm formation, but increased *S. epidermidis* biofilm. GN, P, SXT and ENR SubMIC significantly reduced biofilm in *S. aureus* MHB suspensions, and ENR in *S. aureus* milk suspensions. OXT did not influence biofilm formation in MHB, neither did GN, P, OXT, and SXT in milk. All antimicrobials tested decreased *S. epidermidis* biofilm.

In vitro biofilm expression by field isolates differs according to the environmental conditions. Results suggest a protective role of milk against antimicrobials SubMIC. Further studies are required to evaluate the milk effect upon biofilm growth inhibition.

PS 50 Invasive potential of biofilm-forming staphylococci mastitis isolates

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Staphylococcus aureus is frequently associated with chronic bovine mastitis, a condition associated with low cure rates, and leading to severe economic losses for dairy producers. Bacterial internalization and/or by biofilm formation are though to contribute to staphylococcal mastitis persistence.

Biofilm interference on the invasive ability of *S. aureus* subclinical mastitis isolates (n=6) was evaluated, using a bovine mammary epithelial cell line (BME). Confluent BME monolayers (≈$2x10^5$ cells/well) were infected with 1ml bacterial suspension (10^6 cfu/ml) and incubated (37°C, 5% CO_2, 30min). Monolayers were washed and lysed. Ten-fold dilutions of cell lysates were plated onto Plate Count Agar, for cfu counting. Invasive capability was confirmed in primary mammary cultures using a FISH protocol with fluorescent rRNA probe specific for *Staphylococcus* spp.

Two out of the three biofilm-producing isolates invaded BME cells, while only one of the three biofilm-negative isolates was able to invade those cells, suggesting that biofilm and invasive abilities are not related in *S. aureus* mastitis isolates.

Our results are in accordance with other authors, who showed that not all *S. aureus* bovine mastitis isolates have the ability of invading BME cells. The relation between biofilm production and establishment and duration of infection needs to be further assessed.

PS 51 Study of the role of virulence plasmid in the biofilm formation of *Salmonella enteritidis*

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In environmental settings, biofilm represent the common way of life of *S.* Enteritidis. Up to date, curli fimbriae, cellulose and the large surface protein BapA are three known components of this matrix. In our effort to achieve a better understanding of biofilm formation process, we decided to study the contribution of *Salmonella* virulence plasmid to this process.

Our studies revealed that virulence plasmid is dispensable for biofilm formation, but its instability due to a mutation in the *parAB*-encoded partition system impairs *Salmonella* multicellular behaviour. In our attempt to understand how plasmid missegregation was mediating biofilm inhibition, we have observed that, in the absence of *parAB, ytl2,* the ORF located just upstream of *parAB,* mediates the induction of membrane perturbations, SOS response activation and the delivery of a biofilm-inhibitory substance into culture supernatant.

Authors

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Authors

Physiology, Biochemistry & Molecular Genetics Group / Irish Branch Joint Session

Sealed membranes; the structural basis of transport and energetic processes

Structural insights into channels and pores

J.H. Naismith

University of St Andrews

The presentation will discuss insights gained from two structural studies of *E. coli* membrane proteins, Wza (outer membrane) and MscS (inner membrane).

These projects are collaborations with the labs of Chris Whitfield (Guelph) and Ian Booth (Aberdeen) respectively. The proteins have different structures and biological functions. There are interesting similarities in that both apparently allow essentially non-specific transport or at least any regulation is achieved by other proteins. Wza represents a novel class of protein and clearly undergoes significant conformational changes during its function. MscS is one of the few truly mechanical systems in biology (no chemical or electrical energy) and it too undergoes dramatic conformational changes during function. Recent results on both will be discussed, in particular how structure has guided functional studies.

Posters

Cells & Cell Surfaces Group

CCS 07 Investigation into the genetic control of the plasmid encoded toxin gene

Amanda Rossiter, Douglas Browning, Stephen Busby & Ian Henderson

University of Birmingham

Enteroaggregative *E. coli* exhibit pathogenicity due to their secretion of proteinaceous virulence factors. One of these factors, plasmid encoded toxin (Pet) is a prototypical member of the serine protease autotransporters of the *Enterobactericeae*. Although the function of this autotransporter has been well characterized, its regulation still remains elusive. Mapping of the pet promoter, using inverse PCR, has defined the minimal region required for Pet expression. Therefore, to investigate the genetic control of the pet gene, the minimal promoter region was fused to the *lacZ* gene in the plasmid pRW50, generating the fusion plasmid pAR1. A collection of mutants previously created by insertional mutagenesis, found 58 mutants to be defective in the secretion of Pet. Therefore, pAR1 was transformed into these mutants and β-galactosidase assays were performed. Here we show that both CRP and Fis are key regulators of pet transcription, yet further work is still required to define additional transcriptional regulators and to determine genes involved in the translation, translocation and secretion of Pet.

CCS 08 Identification and characterzation of the surface proteins of *Salmonella* secreted via the type V secretion pathway

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This study focuses on the characterization of the surface proteins of *Salmonella* which are predicted to play an important role in bacterial pathogenesis. From the genome sequence of *S.* Typhimurium serovar LT2 five proteins belonging to autotransporter family were identified. The Salmonella Reference collection A, B and C were screened by Southern blot analysis to study the prevalence of these genes in different serovars. The distribution of these proteins was found to be restricted to *Salmonella* and elated pathogenic species. Then single mutants of these proteins, namely SapA, SapB, ApeE and MisL, were created based on the one-step gene inactivation described by Datsenko & Wanner (2000). Previous studies have demonstrated MisL and ShdA to be adhesins essential for the intestinal colonization of mice and chickens. Several *in vivo* and *in vitro* experiments, such as adhesion and invasion assay in tissue culture cells, biofilm and autoaggregation assay; and survival assay in *Caenorhabditis elegans* are under progress to characterize them and identify their role in the pathogenesis of *Salmonella*.