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## Abstracts

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# Plenary session

## Intracellular life of microbes

### The intracellular environment and cellular activities

#### Intracellular inhibitors of innate immunity from *Vaccinia virus*

Geoffrey L. Smith

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*Vaccinia virus* (VACV) is a large DNA virus that replicates in the cell cytoplasm and is famous as the vaccine used to eradicate smallpox. The VACV genome encodes roughly 200 genes and approximately half of these are non-essential for virus replication in cell culture. Instead these genes encode proteins that affect virus host range, virulence and the host response to infection. The study of virus proteins that modulate the host response to infection provides a fascinating illustration of how the host and pathogen have co-evolved. For instance, just as the evolution of interferons has been driven by the threat of virus infection, so the threat of interferons has driven the acquisition of virus strategies to interfere with interferon. This is shown by the ten or so separate proteins encoded by VACV that restrict interferon production or antiviral activity. The lecture will describe two intracellular proteins encoded by VACV that inhibit intracellular signalling pathways leading to the induction of innate immunity. The findings will illustrate how the mechanism of action of a protein can be deduced from determining its 3-dimensional crystal structure, and how studying immune evasion strategies from poxviruses can uncover the function of a human gene that is essential for cell viability and conserved across eukaryotes.

#### Intracellular motility of bacteria

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Some pathogenic bacteria cause disease by invading host cells and moving through the cell cytosol. These organisms polymerize cellular actin into propulsive 'actin tails' at the bacterial surface. *Shigella* sp. specifically infect and spread through the colonic epithelium, leading to diarrhea and dysentery. The *Shigella* outer membrane protein IcsA recruits the cellular actin assembly protein N-WASP to the bacterial surface, whereupon N-WASP is activated. Activated N-WASP recruits and activates the Arp2/3 complex, which both nucleates actin polymerization and cross-links actin filaments. Actin tail assembly propels *Shigella* through the host cytoplasm to the cell periphery, enabling bacterial movement into adjacent uninfected cells and leading to spread of the bacterium through the cell layer.

We show that actin tail assembly by *S. flexneri* depends on the N-WASP activating factor Toca-1, providing the first evidence that Toca-1 is required for pathogenesis of an intracellular organism. Toca-1 is critically involved in the initiation of actin tail assembly by the bacteria, but has no significant effect on ongoing actin tail polymerization, indicating that Toca-1 is required for *S. flexneri* activation of N-WASP, but that once activated, the bacteria maintain N-WASP in an activated state independent of Toca-1. By extension, these findings suggest that under certain circumstances in mammalian cells, Toca-1 may function predominantly in the initiation of N-WASP activation and may be minimally involved in subsequent steps of actin polymer elongation.

#### Adapting the cell to optimise the intracellular niche for multiplication

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Virus replication and assembly are often confined to intracellular compartments called virus factories, viroplasm or viral inclusions. This involves extensive rearrangement of host cell membrane and cytoskeletal compartments to provide platforms to concentrate replicase proteins, virus genomes, and host proteins required for replication, and thereby increase the efficiency of replication. Interestingly, these same structures can recruit host components that are associated with cellular defences against infection and cell stress. Aggresome, for example, protect cells from the toxicity associated with protein aggregation, and are very similar to the virus factories produced by large cytoplasmic DNA viruses. Autophagosomes provide a defence against intracellular bacteria and parasites, and can be induced during the replication of picornaviruses and coronaviruses. This makes it possible that cellular defence pathways can be subverted by viruses to generate sites of replication.

#### Host factors involved in intracellular pathogenesis

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*Abstract not received*

#### Interferon 50 years on: detection of, and intracellular responses to, microbes

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2007 sees the 50th anniversary of Isaacs' & Lindenmann's observation that influenza-infected chicken cells produced a secreted substance that could protect naïve cells against infection. They termed this substance 'interferon', and soon established that interferon could be induced by most, if not all, types of virus examined, and furthermore that it effected protection against a broad range of unrelated viruses. We now know that the production of and response to virally-induced type I interferon (comprising α and β subtypes) comprises a major component of anti-viral innate immunity. Additionally, it is becoming clear that the production of type I interferon can be triggered by some bacteria and parasites. This review will concentrate on our current understanding of the interferon system and its role in antimicrobial defense. I will also discuss the importance to the invading microbe of an effective strategy to evade the interferon system using specific examples.

#### The intracellular niche: a safe house allowing *Yersinia* to arm itself *in vivo*

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Diseases initiated by *Yersinia enterocolitica* and *Y. pseudotuberculosis* are primarily enteropathogenic in nature as opposed to plague, a

severe systemic infection caused by *Y. pestis*. Nevertheless, all three yersiniae rely upon at least temporary residence within host cells in order to upregulate core features of a shared ~70-kb plasmid-mediated type three secretion system (TTSS). This plasmid is termed pCD in *Y. pestis* and pYV in the enteropathogenic yersiniae. Nonprofessional phagocytes serve as initial targets for the enteropathogenic species whereas macrophages fill the same role for plague bacilli; in all cases, chromosomally-encoded functions promote this initial host cell invasion. Once acclimated to the temperature and cation content of the host intracellular environment, the organisms emerge into extracellular spaces (especially of lymphoid tissue) and initiate an inflammatory process mediated by the TTSS. This phase is characterized by delivery to host cells of TTSS virulence effectors termed yersiniae outer proteins (Yops) via LcrV-dependent translocation and eventually becomes self-limiting for the enteropathogenic species. Cells of *Y. pestis*, however, continue to disseminate within the host to favored niches within visceral organs where they initiate a second systemic (and generally terminal) anti-inflammatory phase mediated by soluble LcrV. During both phases of infection, translocated Yops serve to inhibit local host cell signaling as well as actin-dependent scaffolding.

## The advantages and consequences of persistent infections

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The evolutionary dynamics between host and infective invader plays out its drama by balancing virulence with transmissibility. In sparse host populations or when opportunities for horizontal spread are few and far between, persistence of the pathogen is the name of the game. Thus many persistent infections are vertically transmitted, and where better to reside for long periods than inside host cells? As viruses are obligate intracellular parasites, their persistence will be discussed in relation to evasion of immune clearance and the molecular means of intrinsic host cell restriction of replication. Retroviruses are exemplars of ultimate persistence in becoming host Mendelian traits that may persist for thousands of host generations and yet regain an infectious life-style. Some 8% of human DNA represents fossil retroviral genomes. On the other hand, HIV-1, which is a retrovirus only recently acquired from another host, tops the current human toll due to infectious disease.

## Symbiosis: parallels between symbionts and pathogens

Mark J. Taylor

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*Wolbachia* are a diverse group of intracellular bacteria, which display a number of associations with their hosts ranging from parasitism to mutualism. In filarial nematodes, *Wolbachia* are mutualists and their presence is essential for normal embryonic and larval development and the long-term survival of adult worms – a feature exploited by the use of antibiotics as a new treatment for filarial diseases. Whilst the bacteria are beneficial for the nematode, their release into the blood and tissues results in inflammatory immune activation that leads to adverse events following anti-filarial treatment and contributes to the pathogenesis of river blindness and elephantiasis. In addition to stimulating innate responses, prior exposure to *Wolbachia* induces a state of tolerance to both itself and a variety of other microbial molecules, which may increase the susceptibility to opportunistic pathogens that are a feature of elephantiasis. Activation of innate immunity enhances adaptive immunity, which may trigger the progression to chronic inflammatory disease. The degenerate nature of the *Wolbachia* genome has resulted in the loss of many classical bacterial virulence factors. Recent studies, however, have identified a lipoprotein in the cell wall of the bacteria that appears to drive innate inflammation in a similar way to typical pathogens.

# Mechanisms of intracellular pathogenesis and gene regulation in the intracellular environment

## *Leishmania* survival within macrophages: the intracellular biology of *Leishmania* in macrophages and non-hematopoietic cells

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Infection with various species of the protozoan parasite *Leishmania* commonly leads to sub-clinical persistent infection but can also be responsible for a clinical spectrum of disease, ranging from localized self-healing cutaneous leishmaniasis to systemic, fatal kala azar. Although *Leishmania* are generally regarded as parasites which target monocytes and tissue macrophages, there is accumulating evidence that infection of other host cells, including neutrophils, dendritic cells and fibroblasts plays an integral part in the pathogenesis of leishmaniasis. This talk will focus on two related issues. First, it will review our current understanding of how *Leishmania* promastigotes and amastigotes establish infection in macrophages, remain relatively silent in terms of the induction of pro-inflammatory cytokine responses, and survive within the hostile niche of the phagolysosome. Second, it will compare and contrast this information with that gained from the study of atypical host cells, aiming to highlight the diversity of *Leishmania* –host cell interactions and also the varied means by which this parasite subverts immune function.

## *Salmonella* PhoQ recognition of the intracellular environment

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Salmonellae are Gram-negative bacteria that cause diverse diseases including diarrhea and the systemic disease enteric fever. Central to the ability to cause disease is the bacterial ability to survive and replicate within professional phagocytes such as macrophages. This is accomplished through sensing of the intracellular environment which signals cell surface remodeling to resist innate immune effectors such as cationic antimicrobial peptides and the assembly of type III secretion systems which function as protein transport devices to deliver bacterial proteins across the phagocyte membrane. Key to this environmental sensing is recognition of mammalian signals by a bacterial histidine kinase PhoQ. PhoQ is a membrane bound sensor kinase important for the pathogenesis of a number of Gram-negative bacterial species. PhoQ and its cognate response regulator PhoP constitute a signal-transduction cascade that controls inducible resistance to host antimicrobial peptides. *Salmonella typhimurium* PhoQ is directly activated by antimicrobial peptides and at low pH. A highly acidic surface of the PhoQ sensor domain participates in both divalent-cation and antimicrobial-peptide binding as a first step in signal transduction across the bacterial membrane. Identification of PhoQ signaling mutants, binding studies with the PhoQ sensor domain, and structural analysis of this domain can be incorporated into a model in which antimicrobial peptides displace divalent cations from PhoQ metal binding sites to initiate signal transduction. Sensing of acid pH appears to involve different components of the PhoQ sensor domain and a model can be generated in which antimicrobial peptides and pH act together as signal transduction is initiated by alteration of the PhoQ sensor domain. In contrast to Salmonellae, *Pseudomonas aruginosa* PhoQ functions largely as a sensor of divalent cations. Structural studies and analysis indicates that acquisition of the ability of Salmonellae PhoQ to respond to low pH and antimicrobial peptides could be part of the adaptation of Gram-negative bacteria such as Salmonellae to animals. Therefore bacteria sense small innate immune molecules to initiate a transcriptional program that promotes bacterial virulence.

## *Francisella tularensis*: a model intracellular pathogen

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*Francisella tularensis*, a facultative intracellular bacterium that replicates inside mammalian macrophages, is the causative agent of the disease tularemia. Tularemia outbreaks occur sporadically in northern latitudes, but in general the disease is no longer considered a significant clinical problem. The properties of fully virulent Type A *Francisella* were explored in early studies; however, most researchers who worked with virulent *Francisella* contracted tularemia, an issue that appears to have significantly diminished its popularity as a research problem. Nonetheless, using attenuated variants such as the Live Vaccine Strain (LVS), work on the organism as a general model for intracellular pathogens continued. The designation of *Francisella* as a potential Category A agent of bioterrorism, with accompanying new funding, provided renewed impetus to analyze the bacterium and host-pathogen interactions. The resulting data serve as a comparator for that from other models such as *Listeria*. In this presentation, both the common and unique features of the host response to following LVS vaccination will be discussed. Two major areas will be considered: the evolution of early innate cellular, chemokine, and cytokine immune responses following recognition of *Francisella* by Toll-like receptors (TLRs), and the mechanisms of the resulting *in vivo* protective immunity mediated by CD4, CD8, and 'double negative' T cell subpopulations.

## The complexities of eating bacteria from within: *Bdellovibrio* predation

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*Bdellovibrio bacteriovorus* is a small, Gram-negative, motile bacterium that has potential as a 'living antibiotic' because it preys upon and kills, other Gram-negative bacteria, including several known human pathogens such as *Salmonella*, *Pseudomonas*, *Serratia* and *Proteus*. *B. bacteriovorus* enters the periplasm of the host cell, where it grows, replicates and then lyses the host cell to release the progeny. *Bdellovibrio* do not usually replicate outside the prey bacterium, although they will grow slowly host-independently (HI) on rich media. Although *Bdellovibrio* predatory action was discovered and biochemically studied in the 1960s and 1970s, it is only with the advent of a genome sequence that we have been able to begin to unravel the complexities of how *Bdellovibrio* enter prey, and how they modify prey cells to osmotically stable 'bdelloplast' structures in which they consume prey nutrients and grow, without feeding the surrounding environment. *Bdellovibrio* is highly motile with a single, polar, sheathed flagellum. Recent work in which we have tested the roles of flagella and pili in prey entry will be discussed, along with new genomic data on some of the biochemical and regulatory events during early bdelloplast establishment.

## Molecular and *in vivo* insights into the role of protein secretion in the intracellular life of *Salmonella* and *Burkholderia* spp

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*Salmonella enterica* is a zoonotic diarrhoeal pathogen of worldwide importance. Targeted and genome-wide mutagenesis of *S.* Typhimurium has indicated that Type III secretion systems (T3SSs)-1 and -2 and their secreted effectors play key roles in intestinal colonisation and the induction of enteritis in cattle and pigs. Received wisdom on the role of these systems in systemic translocation of *Salmonella* derives from the murine typhoid model and dictates that T3SS-2 is induced in the intestinal lumen to facilitate bacterial translocation to the blood in $CD18^+$ phagocytes in a T3SS-1-independent manner. By using a lymphatic cannulation model, early systemic translocation of *S.* Dublin from the bovine terminal ileum was found to occur in a cell-free niche via efferent lymphatics in a manner dependent on T3SS-1, but not T3SS-2. *S.* Dublin is a natural systemic pathogen of cattle and ongoing studies using signature-tagged mutants continue to offer insights into the molecular basis of systemic Salmonellosis that cannot be obtained in surrogate rodent or cell-based assays.

A T3SS-1-like Type III secretion system (Bsa) also exists in the melioidosis pathogen *Burkholderia pseudomallei*. The Bsa apparatus facilitates invasion of non-phagocytic cells via injection of a SopE/E2-like guanine nucleotide exchange factor which activates Rho-family GTPases that regulate the subcortical actin cytoskeleton. In common with *Shigella*, *B. pseudomallei* uses its T3SS to lyse endocytic vesicles to gain access to the cytosol where the bacteria are then propelled by continuous polymerisation of actin at one bacterial pole. We recently identified a putative Type V secreted protein (BimA) required for actin-based motility of *B. pseudomallei*. BimA is localised to site of actin nucleation, binds monomeric actin and is capable of inducing actin polymerisation when expressed in eukaryotic cells or *in vitro*. BimA orthologues in related *Burkholderia* spp. are functional, despite varying in primary sequence. Both BimA and the Bsa T3SS play key roles in the pathogenesis of melioidosis in murine models.

## Observing and controlling *Mycobacterium tuberculosis* gene expression in macrophages

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Nearly one third of the world's population is estimated to be infected with *Mycobacterium tuberculosis*. While infections are effectively contained in most individuals they are rarely cleared. Instead, the pathogen often persists indefinitely even in the face of an intact immune response. Survival of *M. tuberculosis* during infections of humans and animals depends on a number of processes that include the interaction of the pathogen with its primary host cell, the macrophage. Our functional genomics work has focused on this interaction and defined – together with the work of other research groups – parts of the regulatory network that mediates transcriptional adaptation of the bacterium to intraphagosomal environments. This work suggests phagosomes to be nitrosative, oxidative, functionally hypoxic, low in carbohydrates and iron, and capable of perturbing the pathogen's cell envelope. More recently we also developed approaches to conditionally inactivate *M. tuberculosis* gene expression during infections. We are using these to identify *M. tuberculosis* genes that are essential for growth and persistence in macrophages and animals.

## Interacting with the host immune response: pathogenesis of *Burkholderia pseudomallei*

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*Abstract not received*

## Living inside the human erythrocyte

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Not only do infectious diseases such as malaria affect the health of countries in which they are endemic but they also increase global poverty. While there are effective treatments for many infectious diseases, including malaria, more research is clearly needed. First of all, microbial resistance has made some of the most effective and inexpensive drug regimes unreliable and dangerous to use on severely ill patients. Second, many existing antimicrobial or antiparasitic drugs show toxicity or are too expensive for countries where per capita income is on the order of hundreds of US dollars per year. Because drug development is expensive our laboratory is using and developing high-throughput genome-wide approaches to create functional catalogs for malaria parasite and host proteins. For example, we are using tiling arrays to identify parasite proteins that are evolving quickly in the parasite's response to drug and immune pressure, we are defining transcriptional regulatory networks through comparative genomics and expression profiling and we are identifying host genes involved in malaria susceptibility through characterization of different inbred mouse strains' survival after parasite infection. These data allow us to construct models that can be used to predict the likelihood that an uncharacterized gene is essential to parasite viability, involved in the symptomatic stages of malaria infection, involved in drug resistance or is involved in immune evasion. Our long-term goal for these data is to discover proteins that can most likely be targeted by small molecules or vaccines, or which are likely to be the targets of uncharacterized small molecules with antiparasitic activity.

# Cells & Cell Surfaces Group session

## Microbial transport systems



### Evolutionary overview of protein secretion in bacteria

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In contrast to other organisms, Gram-negative bacteria have evolved numerous systems for protein export. Eight types are known that mediate export across or insertion into the cytoplasmic membrane while eight specifically mediate export across or insertion into the outer membrane. Three of the former secretory pathway (SP) systems, ISP (ABC), IIISP (Fla/Path) and IVSP (Conj/Vir), can export proteins across both membranes in a single energy-coupled step. A fourth generalized mechanism for exporting proteins across the two membrane envelope in two distinct steps (which we here refer to as type II secretory pathways (IISP)) utilizes either the general secretory pathway (GSP or Sec) or the twin-arginine targeting translocase (Tat) for translocation across the inner membrane, and either the main terminal branch (MTB) or one of several protein-specific export systems for translocation across the outer membrane. We here survey the various well-characterized protein translocation systems found in living organisms and then focus on the systems present in Gram-negative bacteria. Comparisons between these systems suggest specific biogenic, mechanistic and evolutionary similarities as well as major differences.

### Pseudomonas aeruginosa pyoverdine receptors contribute to pyocins sensitivity

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*Pseudomonas aeruginosa* produces the high-affinity peptidic siderophore pyoverdine (PVD). Three different PVDs can be produced by *P. aeruginosa* and the sequences of the corresponding three PVD receptors are known, allowing via multiplex PCR, to determine the PVD specificity of a given strain. Strains with type II PVD receptor (FpvAII) are killed by the soluble bacteriocin, pyocin S3. Two other soluble pyocins, S1 and S2 are produced by some *P. aeruginosa* strains. Via multiplex PCR we identified the PVD receptor of 81 *P. aeruginosa* strains and in parallel determined their sensitivity to pyocins S1, S2, and S3. Thirty-two strains had type I *fpvA*, 31 had type II, and 15 had type III. Three isolates were *fpvA* and PVD-negative, and one strain produced type II PVD although no receptor gene could be amplified, suggesting that this receptor deviated from the known FpvAII receptors. S1-sensitive strains were found in all three groups, suggesting that the S1 receptor is not a PVD receptor. All S3-sensitive strains had the *fpvA*II gene, but 4 *fpvA*II positive strains were S3-resistant, three of them because of the presence of the S3 immunity gene. All seventeen S2-sensitive strains had the *fpvA*I gene. Among the S2-resistant *fpvA*I strains, nine had the S1/S2-immunity gene, and were consequently also S1-resistant. The other six S2-resistant *fpvA*I strains had all a mutation changing a valine at position 46 of FpvA to an isoleucine. The *N*-terminal part of the receptor protein is in the periplasm and interacts with the membrane-bound FpvR anti-sigma factor. When pyoverdine binds the receptor, a conformational change takes place and the sigma factors PvdS and FpvI can activate the transcription of the pyoverdine genes and the receptor gene, respectively. Analysis of *fpvR* sequences from S2-resistant, *fpvaI*-positive strains also revealed some substitutions. We therefore suggest that FpvAI is the receptor for S2 pyocin, but that mutations in the N-terminal part of the receptor and/or FpvR are enough to cause resistance to S2 pyocin while maintaining pyoverdine siderophore utilization. Finally, comparison of *fpvA* sequences suggests a more rapid evolution of type II pyoverdine receptor genes.

### A cryptic, acid-induced, $Fe^{2+}$ transporter (FtrABC) in *Escherichia coli* K-12

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Previous analysis of global-iron regulation by Fur (ferric uptake regulator) in *E. coli* revealed the *ycdNOB* gene cluster (*ftrABC*, ferrous transport) predicted to encode a new type of bacterial iron transporter. FtrA is homologous to the iron transporter of yeast (Ftr1p); FtrB is periplasmic; and FtrC is a Tat-secreted periplasmic haem-peroxidase-like protein. Deletion of *ftrABC* gave no growth defect under iron restriction in a range of backgrounds. The natural frame-shift mutation in the *ftrA* gene was therefore corrected, which resulted in a major growth advantage under iron restriction. This effect was enhanced by low pH and by ascorbate ($Fe^{3+}$ reductant). The *ftrABC* operon (lacking any *ftrA* defect) of *E. coli* O157 conferred a similar growth advantage. Expression analysis confirmed the $Fe^{2+}$-Fur regulation and demonstrated maximum induction during log phase. A ~14-fold increased expression was seen at low pH which was dependent on CpxAR (acting as an alkaline repressor). All three *ftr* genes were required for full Ftr activity. Thus, *ftrABC* specifies a three-component, acid-induced, ferrous iron transporter that is cryptic in K-12 but apparently functional in O157.

### Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*

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The biosynthesis and export of capsular polysaccharides in *Escherichia coli* provides a fascinating biological problem. How is a large negatively charged macro-molecule synthesised and then exported across two membranes and the intervening peptidoglycan. In this presentation I will review latest literature on the different biosynthetic and export mechanisms used by *E. coli* to express cell surface capsular polysaccharides. Specifically I will compare and contrast the processes involved in expression of the *E. coli* K5 and K30 antigens, highlighting conserved themes but also crucial differences in the way these capsular polysaccharides are synthesised and exported. I will suggest ways in which we can use this information for polysaccharide engineering and the development of new anti-microbials.

### Caught in a TRAP: sialic acid uptake by bacterial pathogens

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Disease causing bacteria have evolved numerous mechanisms to evade the host's immune system, one of which involves 'molecular mimicry'

by the placement of a host molecule, sialic acid, on the bacterial cell surface. The human respiratory pathogen *Haemophilus influenzae* uses a novel transporter that allows it to utilise host sialic acid for this purpose and also for its own nutrition.

I will describe our recent work to characterise this transporter, which is a member of the tripartite ATP-independent periplasmic (TRAP) transporter family, and to determine its role in the virulence of *H. influenzae*. The presentation will also include a general review of this family of binding protein-dependent secondary transporters, which are emerging as a diverse group of organic anion transporters in prokaryotes.

## Colicin translocation and the role of native disorder

Colin Kleanthous

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Colicins and other bacteriocins are species-specific antimicrobial proteins that translocate into bacteria by an as yet undefined pathway(s). One of the remarkable features of colicin import is the ability of these 40-60 kDa proteins to translocate structurally and functionally diverse cytotoxic domains into the periplasm or cytoplasm from where they elicit cell death through depolarisation of the inner membrane, cleavage of cell wall intermediates or degradation of nucleic acid (rRNA, tRNA or DNA). We are investigating how Group A nuclease colicins translocate into *E. coli*; this process begins with high affinity binding of the colicin to the vitamin $B_{12}$ receptor in the outer membrane (OM), with the porin OmpF and periplasmic Tol-complex recruited subsequently. This self-assembled 'translocon' is thought to be the trigger for translocation across the OM. My talk will focus on recent published and unpublished work where we have begun to delineate how colicins recruit binding partners after the initial receptor-recognition event. This has thrown a spotlight on the importance of native disorder to colicin translocation and in particular how such regions 'competitively recruit' binding partners in order to expedite entry to the periplasm.

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## A genomics approach to understanding bacterial transport proteins of the Major Facilitator Superfamily

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*Abstract not received*

## Small Multidrug Resistance (SMR) transporter family

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Small multidrug resistance (SMR) proteins were defined as a family in 1996. These proteins of ~110 amino acids were initially described as cationic dye / quaternary ammonium compound proton-drug antiporters. Bioinformatic investigation recovers 53% of sequenced bacteria species possessing at least one SMR. The most studied member is EmrE from *Escherichia coli*. We have explored the biochemical nature of this protein using fluorescence and CD in various membrane mimetic environments. Furthermore we have investigated the multimeric state of the protein though SEC-HPLC and ultracentrifugation. Additionally ligand binding has been explored using isothermal titration calorimetry and fluorescence. Through the work of other groups and ours, EmrE has provided constantly evolving functional and structural models.

SugE, another *E. coli* SMR protein was initially assigned the phenotype of suppressor of *groEL*. We have investigated this designation, as it was not clear how a transporter homologue could have protein folding chaperone activity. Our studies confirmed that indeed overexpression of *sugE* does indeed provide rescue of a *groEL* mutant. Complementing this study we have used mutagenesis to 'evolve' SugE into EmrE, which demonstrated that SugE can have import activity. This in combination with proteomic work, leads to SugE appearing to import a regulatory molecule.

## A novel exclusion mechanism for carbon resource partitioning in the human oral pathogen *Aggregatibacter actinomycetemcomitans*

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The bacterium *Aggregatibacter actinomycetemcomitans* is a common commensal of the human oral cavity and the causative agent of the disease localized aggressive periodontitis. *A. actinomycetemcomitans* is a slow-growing bacterium that possesses limited metabolic machinery for carbon utilization, which likely impacts its ability to colonize the oral cavity where growth and community composition is mediated by carbon availability. The most noted carbon sources available for growth in the oral cavity are carbohydrates including glucose and fructose; however there is significant competition for these substrates, as they are preferred carbon sources for many bacteria residing in this community. We present evidence that in the presence of the *in vivo* relevant carbon substrates glucose, fructose, and lactate, *A. actinomycetemcomitans* preferentially metabolizes lactate. This preference for lactate exists despite the fact that *A. actinomycetemcomitans* grows faster and obtains higher cell yields during growth with carbohydrates. The preference for lactate is mediated by a novel exclusion mechanism in which metabolism of lactate inhibits uptake of carbohydrates. We also show that *A. actinomycetemcomitans* utilizes lactate produced by the oral bacterium *Streptococcus gordonii* suggesting the potential for cross-feeding in the oral cavity. These results suggest that within the oral cavity, *A. actinomycetemcomitans* has evolved a novel mechanism for resource partitioning mediating preference for a 'non-optimal' metabolic waste product (lactate) produced by other community members.

# Microbial Infection Group / Clinical Microbiology Group joint session

## Mycobacteria in clinical practice

### Bacterial colonization, latency and host adaptation

#### Bacterial colonization: the example of *Neisseria meningitidis*

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Like most extra-cellular bacterial pathogens responsible for invasive infections, *Neisseria meningitidis* is a commensal of human. The natural niche is this nasopharynx and in some circumstances the bacteria invade the bloodstrean, and after crossing the blood brain barrier invade the meninges. An essential step for the dissemination of *N.meningitis* in a population is therefore its ability to colonize the human nasopharynx, and the bacterial attributes which have been selected to allow the bacteria to colonize its niche are also used by *N.meningitidis* to cross the blood brain barrier once in the bloodstream.. Type IV pili azre playing an essential role in the interaction of *N.meningitidis* withh host cells. They are required for this adhesion and to signal to the cells. The molecular basis of this interaction as well as the consequence of the bacterial signaling to the host cells will be discussed.

#### *In vitro* respiratory organ culture models for bacterial colonisation and pathogenesis studies

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*Background In vitro* models allow the reduction, refinement and replacement of experimental animal use. Organ culture models have many advantages over simple cell cultures for studying the pathogenesis of respiratory bacteria: they are more complex; bacteria can only interact with the ciliated epithelial surface; and they have an intact mucociliary clearance system which responds to infection.

*Methods and results* We have developed organ culture systems for canine, equine and porcine respiratory tissues maintained at an air interface, rather than in submerged culture. Uninfected tissues remain viable with normal mucociliary function for 120 hrs. Tissues respond to infection with increased mucus production (<10% surface coverage pre-infection to >80% by 24 hr pi), delayed mucociliary clearance (streptococci) or ciliary stasis (*B. bronchiseptica*) and altered cytokine production. The models exhibit regional colonisation differences and are capable of differentiating mutant bacteria with decreased colonisation potential. The porcine system has been successfully used to screen pools of mutants using STM protocols and the attenuated phenotype of mutants identified confirmed in experiments in pigs.

*Conclusion* Air interface respiratory organ culture models are more physiological than submerged systems and can be successfully used for functional genomics studies.

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#### Pathophysiology of pneumococcal invasive disease

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The pneumococcal surface engineers complex interactions with human cells that result in attachment, invasion, inflammation, and death of either the bacteria or the host cell or both. The architecture of the surface is constructed by the cell wall and its noncovalent adducts of choline binding proteins. Each body site presents a different array of receptors that mediate adherence and invasion. In the nasopharynx, a key player is the interaction of CbpA with the receptor for sIgA. In contrast, platelet activating factor receptor (PAFr) is important during adherence and invasion in the bloodstream.

A new innate immune recognition system is mediated by PAFr and phosphorylcholine-containing bacterial surface molecules. This system applies to pneumococci, *Haemophilus influenzae* and *Neisseria meningitidis*. Interaction between pneumococcal cell wall and PAFr results in two independent events: 1) death of the animal, and 2) uptake of bacterial components into host cells. Intravascular choline-containing cell walls marginate onto endothelial cells and cause rapid lethality in wild type, *Tlr2*$^{-/-}$ and *Nod2*$^{-/-}$ mice, but not in *Pafr*$^{-/-}$ mice. Cell wall exits the vasculature into the heart and brain, accumulating within endothelial cells, cardiomyocytes and neurons in a PAFr-dependent way. Physiological consequences of the cell wall/PAFr interaction are noninflammatory in endothelial cells and neurons but cause rapid loss of cardiomyocyte contractility that contributes to death. Thus, PAFr shepherds phosphorylcholine-containing bacterial components such as cell wall into host cells from where the response ranges from quiescence to severe pathophysiology.

#### Living in a sewer: the progression of bacterial colonization in the tammar wallaby

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The tammar wallaby, *Macropus eugenii*, has a short gestation period of 28-32 days and weighs approximately 0.4g at birth. The neonate has only a rudimentary gastro-intestinal tract, forelimbs to climb from the urogenital tract into the pouch, and a tongue to assist in suckling. For the first three months the pouch young has no mature lymphoid tissue and is presumed to lack immune competence.

In humans, the microflora of the gut changes from facultative anaerobes to obligate anaerobes during the first few postnatal months. We hypothesize that changes also occur with the developing tammar wallaby and that these correlate with the development of the gut associated lymphoid tissues and their functioning. We have therefore analyzed the microflora of the developing pouch young and compared them to maternal samples from the oral cavity, milk, outer pouch skin, the pouch, urogenital tract and the anus using the techniques of T-RFLP and ARDRA to determine the possible sources of bacteria colonizing the pouch young.

#### Mechanisms of microbial competition

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Since mucosal surfaces are often occupied by numerous microbes, competition both within and between these species is likely to be a major challenge to successful colonization. This seminar will examine *in vitro* and *in vivo* mechanisms of intra- and interspecies competition involving *Streptococcus pneumoniae*, a common resident of the human airway. These mechanisms include the organism's production

of antimicrobial products and its stimulation of selective host innate immune responses during co-colonization. The implications for the changing dynamics of pneumococcal and upper airway bacterial colonization due to medical interventions such as vaccination will also be discussed.

## Recognition and response of *Pseudomonas aeruginosa* to soluble compounds released during host stress

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*Pseudomonas aeruginosa* is the most common gram-negative bacterium isolated among cases of nosocomial infection and carries the highest reported case fatality rate (40%) of all hospital acquired infections. The mere presence of *P. aeruginosa* within the intestinal tract of a critically ill patient is associated with a 4 fold increase in mortality, independent of its dissemination to remote organs. While it is generally reasoned that sicker patients colonized by opportunistic pathogens die at higher rates as a simple matter of lowered host resistance, an alternative explanation for this observation is that pathogens themselves sense the liability of a physiologically stressed host and respond with enhanced virulence.

Our laboratory studies how *P. aeruginosa* is cued by changes in the local intestinal microenvironment during critical illness. We are interested in how these cues might transform *P. aeruginosa* from that of indolent colonizer to a lethal pathogen. Among the main risk factors for the development of life-threatening sepsis in critically ill patients are 1.) Ischemia/Reperfusion injury and 2.) Inflammation. We have made novel observation that physiological stress, such as occurs following major surgical intervention or intestinal ischemia/reperfusion injury, leads to the local release of host cell-derived bacterial signaling compounds into the intestinal lumen that directly activate virulence genes in *P. aeruginosa*.

Immune activation is recognized by *P. aeruginosa* through sensing of IFN-γ, a cytokine involved in the regulation of the immune system. IFN-γ is released by Th1 cells and recruits leukocytes to a site of infection, resulting in increased inflammation. *P. aeruginosa* specifically recognizes IFN-γ through its outer membrane protein OprF. Specific binding of IFN-γ to OprF triggers a signaling cascade resulting in the activation of a core regulator block of quorum sensing system, RhlRI and downstream RhlRI regulated virulent determinants, PA-IL lectin/adhesin and pyocyanin [1].

Intestinal ischemia and hypoxia are physiologic disturbances that invariably complicate the course of critically ill patients as blood flow is re-distributed away from the intestinal tract to more vital organs. As a compensatory response, HIF-1α, a global transcriptional regulator is activated resulting in the extracellular accumulation of the cytoprotective compound adenosine that develops as a result of: 1.) upregulation of 5' ectonucleasidase (CD73) which accelerates the conversion of AMP to adenosine, 2.) down-regulation of adenosine deaminase which prevents adenosine metabolism to inosine, and 3.) down-regulation of adenosine kinase preventing conversion of adenosine back to AMP. *P. aeruginosa* recognizes adenosine and metabolizes it to inosine a compound that more potently compare to adenosine induces quorum sensing signaling system (QS) as judged by the expression of QS-regulated PA-IL lectin/adhesin [2,3].

The first compounds appearing at the site of injury, ischemia, and inflammation are opioids interacting with opioid receptors on peripheral sensory nerves leading to analgesia. We have determined that *P. aeruginosa* can recognize various classes of opioid-receptor agonists. Particularly, kappa-opioid dynorphin is incorporated into cytoplasm of *P. aeruginosa* and interacts with the MvfR-regulated branch of the QS signaling system resulting in enhanced production of three known intercellular QS related signals, 2-heptyl-4-hydroxyquinoline N-oxide (HQNO), 4-hydroxy-2-heptylquinoline (HHQ), and *Pseudomonas* quinolone signal (PQS). The action of dynorphin on the MvfR regulated QS system results in activation of several virulence factors that can disrupt epithelial cells membranes such as pyocyanin and the PA-IL lectin/adhesin [4].

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## Thoughts on the complexities of animal models for *Escherichia coli*

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*Escherichia coli* is a broad species possessing many variants, some of which are major pathogens of both humans and animals. Human pathogenic *E. coli* man can be divided into three groups encompassing enteric, urinary and systemic pathogens. Similar classes are found in animals. The merits of models including tissue culture, *in vitro* organ culture, ligated gut loops and oral inoculation models will be discussed in the context of understanding host-pathogen interactions. Whilst classical approaches readily define obvious virulence factors such as host ligands, adhesins and toxins in many *E. coli*–host relationships, others are less well defined. For avian *E. coli* (APEC) models of infection require some predisposing factor whereas the zoonotic *E. coli* O157:H7 (EHEC) is 'commensal' in the farmed animal species causing no disease. The demand for animal models for the development of control methods for these pathogens will be discussed. Approaches used to understand the dynamics of *E. coli* evolution with the specific goal of identifying the next emergent pathotype will be discussed. The discussion will focus on whole genome analyses with specific emphasis upon approaches to define pathogens rapidly which is a key tool for those charged with limiting the entry of pathogens into the food chain.

## Novel intraepithelial time-dependent remodelling of *Salmonella enterica* sv. Typhimurium SL1344 transcriptomic profile

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The last ten years have seen impressive developments in our understanding of the genesis and evolution of the *Salmonella* containing vacuole (SCV) inside mammalian cells; nevertheless, the capacity of *Salmonella* to modulate its gene expression pattern in response to changes occurring inside the host cell remains poorly described. We report here the complete transcriptomic profile of *S.* Typhimurium inside epithelial cells and highlight its similarities and the differences with our previously study in macrophage cells [Eriksson *et al.*, 2003, *Mol Microbiol* **47**, 103–118]. The expression profiles suggest differences in metabolic activity between *Salmonella* isolated from epithelial cells and macrophages.

Surprising expression patterns of virulence genes were observed inside epithelial cells; SPI1 effectors and structural genes were upregulated inside epithelial cells. We discovered that flagella biosynthetic genes

were induced in a time dependent manner inside epithelial cells, but are not expressed inside macrophages. This *de novo* production of flagellin and upregulation of SPI1 expression coincides with intriguing changes in the SCV micro-environment.

## Dynamic immune responses in the lungs during tuberculosis

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T cells are an essential component of the successful immune response against Mycobacterium tuberculosis. Both CD4 and CD8 T cells are present in the granuloma of active and latent tuberculosis infections. The exact functions of these cells during control of tuberculosis is not clear, It is known that IFN-γ production is essential to control of infection, but since both CD4 and CD8 T cells produce this cytokine during infection, it is not clear why both subsets of T cells are necessary. In a mouse model, we have attempted to address the requirement for CD4 T cells in control of *M. tuberculosis*, using adoptive transfer of CD4 T cells into RAG-/- mice. IFN-γ production by CD4 T cells was essential to control of the infection. In the non-human primate model. In this model, depletion of CD4 T cells by antibody during latent infection caused reactivation of *M. tuberculosis* infection, but only at antibody levels that efficiently depleted CD4 T cells within the lungs and hilar lymph nodes. Long term peripheral depletion without depletion in the granuloma had little effect on the infection. However, CD4 T cells can also play a regulatory role. In a non-human primate model, we have identified that regulatory (FoxP3+) cells are present in the blood prior to infection, and then are depleted between 2 and 8 weeks post-infection, suggesting a rapid migration to the lungs. In the murine model, we have identified FoxP3+ cells in the lymph nodes and lungs, and have data to suggest that an increase in these cells may reduce priming of T cell responses and subsequent effector responses in the lungs. Thus, the T cell response in tuberculosis is complex and dynamic. A clearer understanding of both CD4 and CD8 T cell responses is essential to the design and development of an effective tuberculosis vaccine.

## Persistence mechanisms in *Mycobacterium tuberculosis*

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Tuberculosis is notoriously difficult to treat, requiring administration of multiple antibiotics for a period of 6-9 months. Unless closely supervised, many patients are unable or unwilling to complete such a demanding regimen, resulting in high rates of default, treatment failure, and emergent drug resistance. Our laboratory is focused on identifying the cellular and molecular mechanisms that are responsible for persistence of *Mycobacterium tuberculosis* in the face of antimicrobial therapy. We are also seeking to elucidate the strategies that the bacterium deploys to establish and maintain persistent, lifelong infections despite eliciting powerful cell-mediated immune responses from the host. In this context, the mechanisms that we study include metabolic adaptations to the nutritional environment in the tissues of the infected host and counter-immune mechanisms that blunt the impact of the host immune response. Our experimental approaches include mycobacterial genetics, animal and tissue culture models of infection, and time-lapse microscopy of bacteria cultivated in microfluidic chambers. A long-term goal of our studies is to identify and validate candidate targets for anti-mycobacterial drug development, in partnership with our collaborators in the pharmaceutical industry.

## Persistent *Chlamydia* infections

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The UK is facing a growing epidemic of sexually transmitted diseases. The forces driving the epidemic are multifaceted but are undoubtedly linked to young people having an increased number of sexual partners. This is occurring as the safe – sex message fades and the overstretched clinics that treat sexually transmitted infections (STIs) are forced to delay appointments and even turn people away. Genital chlamydial infection is the commonest diagnosed sexually transmitted infection in the UK with 109,832 new cases reported in 2005. The greatest burden of disease is in young people under 25 years of age. In women, persistent untreated infections may lead to pelvic inflammatory disease, infertility or ectopic pregnancy resulting in significant physical and psychological costs to the individual and financial implications for patient care. The role of persistent infections in the spread of genital chlamydial infection is not known.

Chlamydiae are obligate intracellular bacterial pathogens that have a unique developmental cycle. There are two morphologically distinct forms of the microorganism observable during a productive infection of eukaryotic cells. Elementary bodies (EBs) are the infectious, extracellular form of the organism, by contrast the noninfectious, metabolically active reticulate bodies (RBs) are only observed in infected cells. Chlamydial replication occurs within a specialized cytoplasmic structure known as an 'inclusion'.

After entry into the cell, the infectious form, or elementary body (EB), differentiates into a reticulate body (RB) that subsequently undergoes several rounds of binary fission before differentiation back into EBs. The ultrastructural changes that take place during the developmental cycle have been well documented. However, the signals that govern the developmental cycle are not well characterised, principally because there is no means by which to manipulate the chlamydial genome. Several complete genome sequences for *C. trachomatis* have recently become available and this has made it possible to design microarrays and quantitative RT-PCR assays, which have revealed that stage specific genes are expressed at different times during the developmental cycle. However, it is likely that regulation of the developmental cycle is brought about mainly by transcriptional control through a combination of factors including differential use of sigma factors, influence of metabolite depletion (through repressor activity), transcriptional activation and through the higher structure of the chromosomal DNA.

The typical chlamydial developmental cycle can be altered by a number of factors including the use of antibiotics, growth in continuous culture, depletion of essential amino acids, depletion of iron and treatment of host cells with IFNγ. Heat shock, infection of monocytes and bacteriophage infection also have similar effects. Under these various conditions chlamydiae lose infectivity and enter into a 'persistent' infection, whereby RB to EB transition is retarded. IFNÁ treatment depletes the pools of available tryptophan by inducing host indoleamine 2,3-dioxygenase and thus mimics the effects of amino acid deprivation in retarding the developmental cycle. Penicillin treatment of *C. trachomatis* infected cells *in vitro* results in a similar phenotype; the mechanism by which penicillin causes this effect is unknown. Recovery of viable chlamydia is possible when penicillin is removed, as the normal developmental cycle is resumed, although this is both dose and time dependent. Similar effects have been reported *in vitro* with antibiotics used to treat chlamydial infections. Persistent, metabolically active chlamydiae induced by antibiotic treatment might represent a potentially important reservoir for infection. Thus, there is a growing and urgent need to understand the molecular basis of chlamydial persistence. We have studied the effects of penicillin treatments on *C. trachomatis*, it does not prevent EB to RB conversion, nor does it stop the growth of RBs or the

replication of their chromosomal DNA, however, it does block their division and prevents their conversion into EBs. Study of chlamydial persistence *in vitro* may reveal insights into understanding chlamydial persistence *in vivo*. In addition, the development of a high resolution typing protocol for *C. trachomatis* based on the new genome sequence data will allow the differentiation of re-infection from persistent infection.

## Characterization of the strategy used by *Burkholderia cenocepacia* to survive within murine macrophages

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Cystic fibrosis (CF) is a recessive genetic disorder caused by a mutation in the *cftr* gene, which encodes for a chloride channel named cystic fibrosis transmembrane conductance regulator (CFTR). A mutation in *cftr* causes an overproduction of thick and sticky mucus within the airway leading to breathing problems and susceptibility to chronic bacterial lung infections. *Burkholderia cenocepacia* has become an important opportunistic pathogen infecting the lungs of patients with CF. *B. cenocepacia* are multi-drug resistant bacteria that have been associated with the 'cepacia syndrome', characterized by rapid lung deterioration, acute necrotizing pneumonia and septicemia. This syndrome contributes to higher morbidity and mortality in infected CF patients. To date very little is known about the virulence factors involved in the persistence of the bacteria within the airway and how the bacteria interact with the CF lung environment. Our laboratory and others have previously demonstrated that *B. cenocepacia* can survive within macrophages. This study focuses on characterizing the strategy by which *B. cenocepacia* survives within macrophages. Using different fluorescent probes and immunolabelling, we show that *B. cenocepacia* survive within poorly acidified vacuoles that delay the accumulation of the late endosomal/lysosomal marker LAMP-1. We demonstrate that the *B. cenocepacia*-containing vacuoles (BcCV) also delay fusion with lysosomes but maintain an interaction with the incoming endosomal pathway. Our data suggest that BcCVs experience a delay in their progression into phagolysosomes demonstrating that the bacteria can modulate the maturation of their vacuole. In addition, we have demonstrated that the absence of a functional CFTR protein in macrophages prolongs the *B. cenocepacia* delay into phagolysosomes while heat -inactivated bacteria reach the lysosomes within 30 minutes. It is conceivable that such a delay is advantageous for the bacteria, enabling them to activate genes that could confer resistance to the hostile environment of lysosomes and may explain the persistence of the bacteria within the CF lungs.

## Regulatory T cells modulate pathogen-induced immunity, inflammation and disease in *Helicobacter pylori* infection

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Recent studies indicate that $CD4^+CD25^+$ $Foxp3^+$ regulatory T cells (Treg) against bacterial, viral and parasite antigens can be activated and expanded *in vivo* during an infection. Such pathogen-specific Treg can prevent infection-induced immunopathology but may also increase the magnitude of infection and prolong pathogen persistence by suppressing protective immune responses. Findings in both experimentally infected mice and humans with natural infection caused by *Helicobacter pylori* indicate that Treg are important in protecting the *H. pylori*-infected host against excessive gastric inflammation and disease symptoms but at the cost of promoting bacterial colonization at the gastric and duodenum mucosa, which may increase the risk in *H. pylori*-infected individuals to develop duodenal ulcers. In addition to the importance of the balance between Treg and different effector T cells in the gastric intestinal mucosa and/or in the draining lymph nodes for modulating natural infection, this balance could also have bearing on the prospect for vaccine development against *H. pylori*, especially when a vaccine for use in already infected individuals is being considered. These aspects are being studied in both experimental systems and in humans with natural *H. pylori* infection.

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# Clinical Virology Group session

## Blood-borne virus infections

### Significance of HBsAg-negative hepatitis B virus infection

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Since its discovery, hepatitis B surface antigen (HBsAg) has been the diagnostical mainstay for hepatitis B virus. As an essential virus component it does not have the disadvantages of antiviral antibodies which leave a considerable diagnostic window. Inspite of the high sensitivity of contemporary assays in certain conditions, detection of HBsAg may be missed for two reasons: Too low levels of antigenemia or altered antigenicity. Expression of HBsAg is low in the early and late phases of infection or in silent infections with very low replication activity. Mutations in the HBs antigen loop are selected for in cases of occult HBV infection. Both conditions create problems for the screening of blood and organ donors. Furthermore, occult infection may reactivate under immune suppression and cause life-threatening hepatitis. Assay of HBV DNA by very sensitive methods is particularly useful for detection of early phases of HBV infection and for detection of reactivation. Antibody to HBV core protein (anti-HBc) is often but not always present in occult HBV infection and occasionally it also undectable in chronic HBV carriers. Thus reliable diagnosis of HBV should include testing for HBsAg, HBV DNA and anti-HBc.

### Host immune response to HBV

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The hepatotropic hepatitis B virus (HBV) is non-cytopathic; liver disease resulting from this infection is therefore thought to be immune-mediated. In order to develop immunotherapeutic strategies for improving the treatment of HBV, there is a pressing need to dissect out the immune components contributing to viral control versus disease pathogenesis.

Defects in many aspects of the coordinated innate and adaptive immune response have been described in patients failing to control HBV infection, but one of the most profound and critical is depletion of the virus-specific CD8 T cell response. Gene expression profiling has suggested that these CD8 cells are highly susceptible to Bim-mediated apoptosis, partially accounting for their failure to persist in the face of high antigen load.

Data from humans and HBV transgenic mice has pointed to the non-antigen-specific lymphocytes infiltrating the liver in the presence of uncontrolled HBV replication as a major contributor to the ensuing damage. We have identified a pathway whereby NK cells, which account for 30-40% of intrahepatic lymphocytes, can mediate hepatocyte death and have shown that this pathway can be stimulated by cytokines induced during flares of HBV-related liver disease.

### Advances in therapy of chronic hepatitis B virus infection

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Despite an effective prophylactic vaccine, nearly 400 million people worldwide are chronically infected with hepatitis B Virus (HBV). Chronic infections often result in progressive liver disease including cirrhosis, liver failure, and hepatocellular carcinoma, often necessitating liver transplant. Various type I interferon treatments can be effective, but response rates are low and side effects often severe, despite recent improvements. Approved nucleoside analog therapies including lamivudine, adefovir, entecavir and telbivudine. Lamivudine, first approved in 1998, shows adequate response rates and viral load reductions, however, viral resistance occurs at rates exceeding 20% in year 1 to 70% after 4 years. Adefovir, approved in 2002, is active for wild-type and lamivudine resistant HBV and resistance rates low for patients with wild-type virus but higher with lamivudine resistant HBV. Response rates and viral load reductions are also suboptimal with adefovir. Telbivudine, approved in 2006, is more potent given its higher dose than lamivudine, but is inactive against lamivudine resistant HBV and apparently results in a high rate of resistance. Entecavir, approved in 2005, is more potent and active against wild-type, lamivudine, and adefovir resistant viruses. Entecavir resistance is rare (<1%) in patients in treatment naïve patients out to three years, but occurs with increasing frequency in lamivudine refractory patients harboring lamivudine resistant virus. A high barrier to entecavir resistance results from potent suppression of HBV replication and a requirement for at least three amino acid substitutions for resistance. Several other nucleoside analogs are in development but they represent analogs similar to adefovir or lamivudine and telbivudine. Recent results suggest that multiply drug-resistant HBV can result with prolonged therapy. Therefore combinations of agents may be required in the future for effective treatment of the experienced patient.

### The evolutionary origins of HIV

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The AIDS viruses, HIV-1 and HIV-2, each originated through multiple transmissions of non-human primate lentiviruses (SIVs) to humans. Non-invasive sampling of primates in west and central Africa has identified the SIVs most closely related to, and thus the most likely source of, the human viruses. The closest relatives of the epidemic forms of HIV-2 (groups A and B) were found in sooty mangabeys in Cote d'Ivoire. The closest relatives of HIV-1 were found in apes in Cameroon.

The SIV clade including HIV-1 evolved in chimpanzees. The three groups of HIV-1 (M, N and O) reflect three independent ape-to-human transmissions. The closest relatives of HIV-1 groups M (the pandemic form) and N (a very rare group) were found in chimpanzees in southeast and south central Cameroon, respectively. The closest relatives of HIV-1 group O were found in gorillas in Cameroon. Presently, it is unclear whether group O-like viruses were transmitted from chimpanzees to gorillas and humans independently, or whether gorillas transmitted these viruses to humans.

The availability of SIV sequences closely related to HIV-1 allows a search for evolutionary changes involved in adaptation of these viruses to their new (human) host.

### The science and Opractice of CCR-5 inhibition

M. Westby

Pfizer Ltd, Kent

*Abstract not received*

## Recent developments in hepatitis C virus cell culture systems: towards a better understanding of the viral life cycle

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Hepatitis C viruses (HCV) comprise a group of positive-strand RNA viruses belonging to the *Flaviviridae* family and are a major cause of chronic liver disease that currently affects 170 million people worldwide. A hurdle in HCV research has been the difficulty to propagate the virus in cell culture. A first step to overcome this block was the establishment of selectable, subgenomic replicons that replicate autonomously to high levels in the human hepatoma cell line Huh-7. In spite of this remarkable progress, the replicon system is limited because only the intracellular steps of the HCV life cycle can be studied. This limitation has recently been overcome with the molecular cloning of a novel HCV isolate, designated JFH-1, replicating to exceptionally high levels in cell culture and giving rise to the production of infectious virions. Cell culture-grown HCV is also infectious *in vivo* demonstrating that cell culture grown HCV is authentic. Furthermore, chimeric genomes carrying the region from core up to NS2 from various HCV isolates fused to the JFH-1 replicase have been generated. These novel cell culture systems will help to understand the early and late steps of the HCV life cycle.

## Pathogenesis of HCV infection – the host

Paul Klenerman

University of Oxford

HCV persists in the majority of those infected. This occurs despite initial host antiviral T cell and B cell responses. The mechanisms by which such responses are attenuated include viral variation leading to immune escape, T cell exhaustion, and T cell regulation. There is evidence for all 3 effects, and these will be discussed. To what extent they can be circumvented in development of immunotherapy or vaccines will also be discussed.

## Advances in treatment of chronic HCV infection

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Pegylated interferon alfa in combination with the nucleoside analogue ribavirin has been established as standard therapy for chronic hepatitis C virus (HCV) infection with sustained virologic response rates of 54-63%. Treatment duration depends on the HCV genotype with currently 24, 48 or 72 weeks for genotype 1 and 16 to 24 weeks for genotype 2 and 3 infected patients. In genotype 1 infected patients without a 2-log decline of HCV-RNA concentration after 12 and detectable HCV RNA after 24 weeks of therapy treatment can be discontinued due to non-response. In genotype 1 patients with a negative HCV RNA (<50 IU/ml) after 4 weeks of therapy (rapid virologic response) treatment can be shortened to 24 weeks while patients with negative HCV RNA after 12 weeks of therapy should be treated for 72 weeks. Efficient treatment of side effects of interferon-based therapy is essential in order to improve compliance, prevent dose reduction or early discontinuation and therefore enhance the probability of sustained response. Future developments of interferon-based therapy mainly aim at further individualisation of treatment duration. Furthermore, direct antiviral drugs, which are currently investigated in phase I/II clinical trials, will fundamentally expand the treatment options of HCV infection.

## The biology and epidemiology of transmitted HIV drug resistance

Deenan Pillay

University College London

The emergence of HIV variants with reduced drug susceptibilities in antiretroviral drug experienced individuals has been long recognised. Not unsurprisingly, this has led to transmission of such viruses, generating concern that the major benefits conferred by ART may be compromised. In this talk, I will outline the epidemiology of transmitted drug resistance, which appears to be reducing in incidence. This is probably due to recent improvements in maintaining suppression of viraemia in treated individuals. Nevertheless, following transmission, these viruses often persist in the absence of treatment. This is in contrast to the case of resistance emerging during treatment, which rapidly disappears on removal of the drug selective pressure due to the relative fitness deficits of resistant compared to wild type viruses. We have developed in vitro models of the transmission bottleneck, which demonstrate the complex and subtle reversion mechanisms operating following transmission. Finally, the clinical outcome of transmitted drug resistance will be discussed in relation to large European and US-based patient cohort data.

# Education & Training Group / Clinical Microbiology Group / Clinical Virology Group joint session

## Training of medical and dental students – making microbiology more relevant

### An update on PBL debates

Peter H. Dangerfield

University of Liverpool

During 2005, debate on the role of PBL was held at microbiology meetings in the context of medical and in particular clinical microbiology education. The debate was balanced between those who felt a strong role for Problem Based Learning (PBL) could be justified in medical undergraduate education and those who felt that traditional teaching was the best method for learning the complexities of the subject.

The evidence of success in PBL can be demonstrated by its wide adoption throughout the world and the success that graduates of the system can demonstrate in clinical practice. Research reports indicate that the graduate doctor of a PBL system is better adapted to learner centred and time managed professional development and is more adaptive to changing environments. Traditional learners tend to be strategic learners who can have difficulty in developing their knowledge without didactic teaching support.

PBL can also offer solutions to curricular development, particularly in subjects where the prominence of the subject in medical schools has diminished with reduced staffing levels. It can offer specialities such as microbiology a real opportunity to develop their influence and profile within the whole area of educational development. However, it is essential that there is a full engagement of protagonists with content debate to ensure learners in the future fully appreciate the importance of the subject.

The presenter will provide some new insight into the benefits of use of PBL and will present some research evidence which supports application of PBL and its near relative Enquiry Based Learning in the environment of health care education.

### PBL and microbiology in medical training: a difficult combination

Mathew Upton

Medical Microbiology, University of Manchester School of Medicine

Fourteen years ago, the University of Manchester introduced Problem Based Learning (PBL) methods for education of medical students. The general feeling is that graduates from PBL based courses have a deeper understanding of subjects related to clinical practice than those studying more conventional curricula.

However, anecdotal evidence from examination results, from conversations with local clinical microbiologists and from interaction with local junior doctors, supports the suggestion that some of the foundation information in subjects like microbiology is not covered in sufficient detail. These subjects are becoming marginalized and are not recognized by students in Manchester as areas that require even basic understanding.

In Manchester, we have formed a group of academic and clinical microbiologists keen to see relevant subject material emphasized throughout the course and to have greater weight placed on assessment of practical and theoretical aspects of microbiology. Students need to be attracted to the subject and made aware of the importance of infection in medicine. We are looking at the ways in which this will be achieved most efficiently in the framework of a PBL environment. It may be useful to apply the approaches that are adopted to learning support in other subjects allied to medicine.

### Postgraduate training in medical microbiology: a trainee's perspective

Fiona J. Cooke

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With the introduction of Modernising Medical Careers, postgraduate medical training in the UK is being drastically re-structured. Concerns voiced by current trainees in the infection specialties will be presented, with particular emphasis on Academic Medical Microbiology. It is unclear how a 3-4 year formal period of research for a higher degree will fit in with run-through training.

General postgraduate training in medical microbiology varies considerably between different regions in the UK, as demonstrated by a recent questionnaire survey by the Association of Medical Microbiologists. Particular differences were found in terms of qualifications on recruitment, service vs. elective activity, time spent 'signing out' and overall satisfaction with the program. Results of a questionnaire-based survey to assess infection training overseas, involving 33 countries, will be presented.

To disseminate information about training opportunities, I helped compile a 'training in infection' manual and website, with Dr Alison Holmes of Imperial College, London. This resource (www.trainingininfection.org.uk/) is 'by trainees for trainees', and provides a platform to disseminate information about courses, conferences, meetings, grants, journals, textbooks etc. It now operates in collaboration with the National Electronic Library of Infection and the British Infection Society. There is considerable potential for expansion in the UK and internationally.

### Champion students!

**G. Phillips**, J. Ker & E. Burnett

NHS Tayside, Dundee

Teaching infection control to medical students can be challenging. In the University of Dundee we have used the Cleanliness Champions resource pack on 2 consecutive groups of final year medical students. For both years we have obtained feedback from the students and used an audience response exercise with them. The presentation will outline the programme content and some aspects of the delivery, student and mentor feedback, the results of the audience response exercises and future direction.

### Specific issues facing virologists

Judith Breuer

Barts and The London School of Medicine and Dentistry

Trainees in clinical virology increasingly need a broad background in clinical infection as well as working knowledge of molecular methods for viral detection and monitoring. In common with clinical microbiology in general, medical virology is moving towards a more clinically based service and in many cases, joint training with infectious disease. At the same time, the increasing complexity of antiviral, especially antiretroviral, therapy and resistance monitoring, requires that highly specialised practitioners with expertise in these areas continue to be trained. Teh demands of clinical and diagnotic laboratory training and delivery of the service, have squeezed out

time learning for research and development skills. However, with the discovery of a new viral pathogen roughly every 2-3 years, it is important that clinical virologists receive training in this area. The traditionally academic bent of clinical virology has also suffered in recent years, but the establishment of clinical academic fellows may go someway to redress this balance

## Interaction between the practitioner and the diagnostic microbiology laboratory

Rob Allaker

Institute of Dentistry, Queen Mary, University of London

There is an increasing need to encourage appropriate microbiology testing and to avoid the overuse of antimicrobial agents. The diagnostic microbiology laboratory has a key role in monitoring the emergence of antibiotic resistance in infections and to decrease the spread of resistant strains. As more than 80% of antibiotics are prescribed by general practitioners, the control of antibiotic resistance depends largely on rational prescribing behaviour in this group of professionals.

With particular reference to dental practitioners and the specialised oral microbiology laboratory, the predictive value of either positive or negative test results for selected species of bacteria in common infections, including adult periodontitis, is not high enough for routine use. Likewise, in most cases of severe orofacial infections no change in therapeutic management is made as a result of laboratory findings.

To optimize both diagnosis and treatment, a key to the future will be improved communication between the practitioner and microbiology laboratory, with an increasing emphasis on training in microbiology. Diagnostic laboratories also need to improve the communication of their potential role in addressing the increasing burden of antibiotic resistance and encourage the appropriate use of their services.

## Lessons on the patient's views of superbugs

K.B. Bamford

Imperial College London

*Abstract not received*

## Emerging diseases

J. Oxford

Retroscreen Virology, London

*Abstract not received*

## Infectious agents that disobey all the rules; what prions teach us about cleaning and decontamination

J. Mark Sutton

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The emergence of bovine spongiform encephalopathy (BSE) during the 1980s and its human form, variant Creutzfeldt–Jakob disease (vCJD), in the 1990s have posed a number of problems. These protein-only infectious agents challenge many assumptions as to the basis of infectious disease, not least of which is the effectiveness of cleaning and decontamination procedures. Whilst the decontamination of surgical instruments will never be a glamorous profession, the emergence of prions should mean that an understanding of the key issues is a minimum requirement for all medical training.

Routine sterilisation methods, developed to decontaminate bacteria and viruses, fail to inactivate these novel types of infectious agents. This has driven a significant re-evaluation of the standards of cleaning and decontamination procedures in hospitals with the introduction of a traffic light system. This institutional approach to improving standards is harder to assess in general practice or dentistry, although recent reports have suggested that there is significant room for improvement.

The paper will describe our current understanding of the risks of iatrogenic prion transmission through surgery, dentistry and other medical interventions, based on our ongoing research in this area. This will focus not only on the relative hazards of different tissues and procedures but also on the ability of current approaches to control these risks. Remaining uncertainties that may have an impact on future healthcare provision will be discussed.

## Training in antimicrobial prescribing – the need for guidelines in the curriculum

C.H. Webb

Royal Hospitals Belfast

Antimicrobial prescribing and stewardship as a specific competence has been largely overlooked in undergraduate curricula. Elements are to be found in therapeutics, microbiology and clinical skills courses, each giving an emphasis particular to the speciality perspective. None may holistically address the unique characteristic of antimicrobial prescribing, which is the conflict between fidelity to the individual patient and conservation of a finite resource for the wider public health.

'Appropriate' antimicrobial prescribing is difficult to define and often rigorous clinical assessment of the patient and a knowledge of the natural history of common infections is overlooked in the preoccupation to choose the 'correct' antibiotic.

Effective education in prescribing can address key GMC learning objectives by using defined learning outcomes which cover skills, knowledge, professional behaviour, communication and development. These can be built around common clinical infection problems which may include difficult issues with carers and the public. The most effective setting for learning may be the ward/clinic, with the programme integrated into the clinical teaching and delivered by clinical trainers who may not be specialists in microbiology or therapeutics.

Experience with such a model will be described.

## 'Involve me and I'll understand' – the future of infection control teaching?

A. Smith

University of Glasgow

*Abstract not received*

# Environmental Microbiology Group session with the British Society for Soil Science and the British Mycological Society

## Soil microbiology: revisiting the past and developing the future

### Traditional methodologies – values and applications

#### How can the experience of the past help us in the questions and methods of the future?

E.A. Paul

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Science progresses through the application of new theory (questions) and technology (methods). Applied sciences require adherence to the above with the added requirement that results must provide economically viable approaches. The beginning of the last century was organism based with the hope that understanding organism type and function would lead to control of soil processes and inoculation with beneficial organisms. It has only been recently understood that complex, ecological –organism interactions govern the possible management of soil biota. The last century brought knowledge of nutrient dynamics, tracers, computers and molecular techniques. These should provide major breakthroughs in theory and knowledge concerning biodiversity, food webs and closed system nutrient cycling. Present challenges involve responses to global change and the utilization of our knowledge of combined tracer -molecular - constituent measurements and modeling to improve soil organic matter dynamics in improved nutrient utilization and carbon sequestration. Sustainable biomass, fuel production, at a time of food deficiencies, without damaging the environment will provide both opportunities and challenges. There is a great need for understanding the role of microbial populations and dynamics relative to autotrophic and heterotrophic respiration in terrestrial-atmospheric-interaction-global models required in global change predictions.

#### The processing of soil carbon by soil micro-organisms

D.W. Hopkins

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Among the earliest questions to engage soil microbiologists was how do soil micro-organisms lead to or contribute to the decomposition of organic residues in soils? Over the centuries the impetus for such research changed from needing to understand the mechanisms of residue breakdown as the counterpoint to photosynthesis, nutrient release for plants and the contribution stabilized organic matter makes to soil properties, to include the role of soils as repositories of sequestered carbon and their roles supporting the diversity of terrestrial organisms. Quantifying the size of the microbial biomass and the rate at which carbon flows through it as it is processed by its organisms is central to being able to understand and model the processing of soil carbon by soil micro-organisms. Unfortunately, some would say, this operational necessity almost achieved the status of being a research objective in itself. The purpose of this presentation is to explain the concept of the soil microbial biomass from a functional perspective and how it can be used as a measure of soil processes.

#### Using scaling and soil function to address key soil ecological issues

K. Killham

University of Aberdeen

*Abstract not received*

#### Temporal and spatial variations of microbial activity in Histic Andosols

**R. Guicharnaud**, O. Arnalds & G.I. Paton

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Iceland has unique geological and climatic characteristics creating distinctive soils. Despite the high latitudes, the Andolsols and Histic Andolsols have great maturity and high levels of fertility due to their basaltic parent material. Although attention has been paid to the chemical and agronomical aspects of the soils, little has been given to soil biology. In this study a transect of Histic Andolsols (under similar landuse) from across Iceland (n=7) was sampled and measurements of biomass, microbial and enzymatic activity and nutrient cycling made. For comparison one of these sites was intensely sampled (n=36). There was a greater variability within the single site than across all of the Icelandic samples taken. Furthermore, the Histic Andosols were significantly different from other soils groups (Histosols, Andosols, Leptosols). A traditional pedological soil classification system was respectful of soil biological parameters. A study of the impact of temperature on soil processes was made using the before mentioned techniques. The Histic Andosols were found to yield similar results to those reported for permafrost impacted soils of the high Arctic in that there was significant microbial activity even at sub-zero temperatures. These findings have significant impacts for our consideration of high latitude soils both as a resource and as a player in global carbon cycling.

### Linking the traditional and the new technologies

#### Who does what in soil – what can nucleic acids tell us?

J.I. Prosser

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Molecular techniques have revolutionised our view of the diversity and composition of microbial communities in the soil. Analysis of 16S rRNA gene sequences amplified from soil nucleic acids have replaced cultivation based approaches as the method of choice for community analysis and 16S rRNA and functional genes are increasingly being used to assess microbial activity and function. Theoretical analysis of molecular data has led to estimates of species abundance as high as 1 million distinct genomes per gram of soil and quantitative PCR amplification has increased estimates of bacterial abundances by more than one order of magnitude. In addition, metagenomic techniques have identified roles for previously uncultivated groups in major biogeochemical processes. These advances have been achieved with acknowledgement of the practical limitations of nucleic acid-based techniques and of the need for use of additional approaches to address the important questions in soil microbiology. Conceptual and theoretical limitations are considered less frequently but are increasingly important as we move from qualitative descriptions to quantitative understanding and prediction of the role of microbial communities in soil ecosystem processes.

### Altering the mineral composition of soil causes a shift in microbial community structure

**Jennifer Davis**[1], Deirdre Gleeson[2], Deirdre Rooney[2], Nicholas Clipson[2] & Lynette Abbott[1]

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This study tests the hypothesis that mineralogy (via mineral addition) influences microbial community structure in a nutrient deficient soil. Microcosms were established by adding mica (M), basalt (B) and rock phosphate (P) to soil separately, and in combination (MBP), and by planting with *Lolium rigidum*, *Trifolium subterraneum* or by leaving unplanted. Microcosms were destructively sampled after 78 days and the effect of mineral and plant treatments on microbial community structure assessed using automated ribosomal intergenic spacer analysis (ARISA). Bacterial community structure was significantly affected by both mineral (global $R = 0.73$ and $p < 0.001$) and plant (global $R = 0.71$ and $p < 0.001$) treatments, as was fungal community structure: mineral (global $R = 0.65$ and $p < 0.001$) and plant (global $R = 0.65$ and $p < 0.001$) treatments. All of the pairwise comparisons of bacterial and fungal communities between different mineral treatments and between different plant treatments were significantly different ($p < 0.05$). PERMANOVA analysis for both bacterial and fungal community structure also showed a significant interaction between mineral and plant treatments ($p = 0.001$). This study has shown that the addition of minerals to soil microcosms resulted in substantial changes in both bacterial and fungal community structure, dependent on type of mineral added and the plant species present. These results suggest that soil mineralogy may be an important factor influencing microbial community structure in soil.

### Use of molecular methods to study how agricultural fertilization regime affects the community structure of desulfonating bacteria in wheat rhizosphere

**Achim Schmalenberger** & Michael Kertesz

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Sulfonates are a key component of the sulfur present in agricultural soils, and their mobilization by bacteria involves the oxidoreductase AsfA. The effect of fertilization regime on bacterial *asfA* distribution in wheat rhizospheres was examined using the longterm Broadbalk field experiment (Rothamsted).

Desulfonating bacteria isolated from wheat rhizosphere included *Variovorax*, *Polaromonas* and *Rhodococcus* strains, all of which contained the *asfA* gene. Rhizosphere DNA was isolated from wheat plants in plots fertilized with different levels of nitrogen and sulfate, or with manure, and community analysis was done by genetic profiling (T-RFLP) and with *asfA* clone libraries generated from this DNA. About 40% of the molecular isolates from the clone libraries of *asfA* were affiliated to the genus *Variovorax*. Analysis of *asfA*-based T-RFLP fingerprints by Principal component analysis showed significant differences between sulphate-free treatments and those where sulphate was applied. The results suggest the occurrence of desulfonating bacterial communities that are specific to the fertilization regime chosen, and that arylsulfonates play a major role in rhizobacteria sulfur nutrition.

### Integrating new approaches to understanding the rhizosphere

G.A. Kowalchuk

Netherlands Institute of Ecology, Heteren, The Netherlands

*Abstract not received*

### Application of microbiomics to soil microbiology

J. Jansson

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*Abstract not received*

### Molecular applications in the study of community structure

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Molecular tools are increasingly applied to estimate the community structure and population size of microbial communities in soil and elsewhere. There are, of course, benefits and disadvantages associated with these techniques. Essentially they allow the measurement of the size and structure of a community independently of issues associated with culturability. As with other fields there is a vast range of methods available, targeted toward both DNA and RNA, which are suitable in a range of applications. In particular there is a trade off in the relationship between throughput and focus. Some methods, such as T-RFLP, allow the analysis of large numbers of samples but at a low relative resolution whilst others, such as DGGE or cloning and sequencing, allow the fine scale assessment of a limited range of samples. Obviously questions relating to the requirements of any given hypothesis have to be addressed before the correct tool can be selected. As with any other suite of tools the power of molecular techniques is greatly increased when used in combination with other methods that allow results measures to be assessed in combination with, for example, physiological or biochemical measures.

## Future developments and needs

### Integrating techniques to understand molecular and functional aspects in soil microbial ecology

Mark J. Bailey

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The development of high throughput molecular analyses of environmental samples and in particular the application of genomics has underpinned advances in microbial ecology over the last decade. Knowledge of gene function and distribution have facilitated the detection and monitoring of individual species in complex environments and advanced understanding of the contribution microorganisms provide to ecosystem services. Microbes are central to biogeochemical cycling and play an essential role in maintaining soil processes and hence the regulation of terrestrial ecosystems. However, we skill know little of their ecology and evolution and need to determine the link between diversity and function. In short who does what where and when? Knowledge of microbial plasticity informs on the extent of redundancy and the resilience of ecosystems to perturbation and manipulation. The challenge is to develop approaches that provide sensitivity and specificity to allow *in situ*, quantitative assessment of the relative abundance and functional relationship that exist between populations present in mixed communities. To illustrate this I will draw on examples that define links between above and below ground diversity that identify not only the key players in relation to carbon flow but also the drivers of microbial succession and activity in communities.

## Modern soil ecology – how useful is resistance-resilience as a concept for assessing soil health using microbiological indicators?

Colin D. Campbell

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Recent progress in quantifying and understanding soil microbial diversity and its importance in underpinning the functioning of soil has led to a proliferation of microbial assays and indicators to measure soil health. The huge diversity of methods is starting to match the diversity of organisms and there is a great need to develop theories and concepts that are useful both for developing the fundamental science and for applying our ecological knowledge to help sustain and protect soils. The concept of resistance-resilience is one approach that has gained acceptance in diverse scientific disciplines and is also being used to examine microbial structure-function relationships in soil. It is interesting to evaluate this approach in relation to what we understand about how microbial cells, populations and communities respond to different stresses. This paper will review the concept and the evidence from case studies on how useful it might be in modern soil ecology studies and attempt to link our basic understanding through to useful approaches for measuring soil health.

## Development of quantitative tools to better understand fungal growth and function in soils

Fordyce A. Davidson

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Recent advances in systems biology have driven many aspects of biological research in a direction heavily weighted towards computational, quantitative and predictive analysis, based on, or assisted by mathematical modeling. In particular, mathematical modeling has played a significant role in the development of our understanding of the growth and function of the fungal mycelium. One of the main problems that faces modelers in this context is the choice of scale. In the study of fungal mycelia, the question of scale is expressed in an extreme manner: their indeterminate growth habit ensures that the investigation of growth and function has to consider scales ranging from the (sub) micron to the kilometre. Further complexity is added when modeling the effects of the physical and nutritional heterogeneity of soils. We will first discuss the general process by which a mathematical model for fungal growth can be developed before studying in detail how some recent models have assisted our understanding of fungal mycelia at a range of scales. Emphasis will be on current models for growth in soils.

# Fermentation & Bioprocessing Group session

## Protein expression strategies: a comparative view

### Mammalian expression systems: cell factories for harvesting therapeutic proteins

A.J. Dickson

University of Manchester

*Abstract not received*

### Baculovirus expression: history and current practice

Ian Jones

School of Animal & Microbial Sciences, University of Reading

The expression of proteins using recombinant baculoviruses is now a mature technology that has found widespread use from basic research to vaccine development and from bench to industrial scale. In this review I will outline the core components of the system that have allowed such diverse use and introduce the more recent developments that have focused on methods that facilitate high throughput expression. The focus will be advances in viral genome engineering, vector design and cell culture but the development of recombinant baculoviruses for the assembly of multi-subunit complexes and gene transfer into mammalian cells will be discussed.

### A yeast expression system for the enhanced production of therapeutic proteins

**C. Finnis**, D. Sleep & D. Mead

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The Baker's yeast, *Saccharomyces cerevisiae,* has a long history of safe use and has GRAS status from the FDA. Novozymes Delta Ltd has undertaken extensive development to offer *Saccharomyces cerevisiae* as an alternative system for the commercial production of a wide range of proteins. In order to produce recombinant proteins in high yields and of optimum quality *Saccharomyces cerevisiae* strains have been subjected to a series of genetic manipulations. Combined with the development of whole 2µm-based expression vectors, the expression hosts contain only yeast DNA and the gene(s) of interest. These vectors have been improved to allow production of multiple subunit proteins, or a single heterologous protein plus a chaperone to aid folding. Overall, the system confers the advantages of stable, high-level protein expression using animal component free materials, delivering a highly competitive cost of goods, typical of a microbial fermentation process. The suitability of the system has been proved through the large-scale production of Recombumin® – the only commercially available recombinant human albumin authorised for use in human therapeutics.

### The *Bacillus* protein secretion factory: snip, snip, your nicked!

**C.R. Harwood**[1], A. Bloor[2], M. Leckenby[2], J. Hulme[2], D.S. Radford[1] & R. Cranenburgh[2]

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The use of secretion systems for the production of heterologous proteins has a number of advantages, including reduced downstream processing costs. *Bacillus subtilis* is an attractive secretion host since it has the potential to secrete proteins at yields in excess of 20 g/l. However, such concentrations are rarely obtained during the production of heterologous proteins.

In *B. subtilis,* secreted proteins are transported *via* the Sec-dependent translocase in an essentially unfolded form. Consequently, they must fold in an environment that is dominated by a high density of immobilised negative charge. Recent progress has identified post-translocational folding in the extracytoplasmic environment as the main bottleneck to achieving high yields of heterologous proteins. While native *Bacillus* proteins have intrinsic characteristics that facilitate their rapid folding (assisted by a variety of extrinsic folding factors, including chaperone-like enzymes, peptides and metal ions), heterologous proteins do not. Heterologous proteins are therefore sensitive to membrane- and cell wall-associated proteases that act to clear misfolded or otherwise aberrant proteins from the translocase and/or the cell wall. We discuss strains that are designed to by-pass the undesirable effects of these proteases.

### Towards high-throughput process development for recombinant proteins – possibilities, tools and challenges

Peter Neubauer

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The expression of proteins in recombinant hosts is a multifactorial process. Optimisation for functional proteins often is long lasting and based on personnel experiences as well as on trial and error. We see the developmental process from the decision for a target protein, over cloning and transformation into the desired vectors and host systems, optimisation of expression, high cell density cultivation, scale-up, and protein purification as a holistic process, which can be shortened by parallelisation and better monitoring.

New tools developed in our laboratory aim to decrease the process development time significantly, including computational tools for gene and process optimisation, robot-based parallelisation of experiments, and improved monitoring tools.

These tools include a new scalable biocatalytic method for controlled high cell density cultivation of microorganisms in microtiter plates (EBaD). The SENBIT® wireless modular on-line monitoring and control system for shake flask cultivations[1] has been very useful for the evaluation of various cell cultures. Further, a sandwich hybridisation platform has been implemented which allows the quantitative automated detection of RNAs and proteins from cell extracts as a useful monitoring tool for the expression of target genes, cofactors, and the cellular environment[2]. Finally, a scale-down bioreactor[3] is integrated into the system, which allows the simulation of large-scale bioreactor process conditions in a laboratory environment.

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## Omics meets old-fashioned cell physiology – tools to understand protein producing *Escherichia coli* cells

Ursula Rinas

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Protein production can be stressful to the host organism. Through classical and nowadays available 'omic tools a wealth of information can be generated and used in a 'Systems Biotechnology Approach' for a better understanding of the adaptation strategy of the bacterial cell to the challenges resulting from the forced production of a foreign protein. Although it may not always be required to strive for stress avoidance *per se*, one should be able to recognize and understand the stress-related cellular reaction to be able to avoid the reaction when it obstructs the desired quality and quantity of the recombinant product.

Protein production related cell responses during the production of a recombinant human growth factor in batch and industrial relevant fed-batch cultures will be evaluated using proteome, transcriptome, metabolome and fluxome profiling techniques. It will be shown that commonly observed responses towards induced recombinant protein production such as growth rate reduction, changes in cell morphology, induction of stress responses and corresponding alterations in gene expression profiles, as well as alterations of central catabolic and anabolic activities strongly depend on, and thus can be effectively manipulated by process and induction conditions to achieve the goal of product formation as desired.

## Automated approaches for the expression of recombinant proteins using *Escherichia coli*

**R.M. Hall**[1], A.M. Bridges[1], J.A. Bullock[2], J.A. Cole[3], A.P. Fosberry[1], P. Homes[1], I.R. Hudson[2], O. Jenkins[1], J.J. Jones[1], P.K. Singh[1] &. R. Wales[2]

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Major advantages of using *Escherichia coli* as a host for recombinant protein generation include cost effectiveness, high yields (up to 50% of total cell protein) and speed of production. Rapid turnaround is especially important to the pharmaceutical industry where time lines are constantly under pressure. Rapid, parallel approaches supporting the identification of suitable clones are clearly needed to improve protein production efficiency.

Piccolo® is a fully automated, high throughput system, specifically designed to support the rapid optimisation of protein production from both insect and microbial cells. The system offers an unparalleled capability to evaluate a large number of different conditions (up to a maximum of 1152) in a single experiment. Cell growth is monitored robotically and can be linked to inducer addition. Following expression, cells are centrifuged, lysed and the target protein is purified using affinity chromatography. Selected conditions can then be scaled up into fermenters. Further efficiencies can be gained if a GFP fusion protein is used. An on-line sensor to measure GFP-tagged protein expression in fermenters has been developed at GSK. Combining these automated approaches is going some way towards our goal of reducing time lines and improving productivity.

## Influence of glucose feed rate on solubility and proteolysis of recombinant proteins in *Escherichia coli*

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The rapid production of small soluble quantities of target proteins calls for a trial and error production methodology. This implies a large number of production methods which should be chosen from techniques which are proven to lead to soluble production and a high quality full length protein. However, if many parameters are to be rapidly tested a multiparallel but unsupervised reactor system is called for. This disqualifies several of the techniques used for production of protein products since they are too elaborate and thus too time consuming and use equipment which cannot be translated to the small ml-scale.

We have shown that the feed rate of glucose controls the specific production rate of a selected number of recombinant proteins. In this paper we will further discuss how the feed rate can be used to control the proteolysis and inclusion body formation. The feed rate can further be used to control the cell retention of recombinant protein secreted to the periplasm thereby increasing the final productivity. In order to control the feed rate in small scale reactor volumes a set of genetically engineered strains were designed with allow the accumulation of increased cell densities without acetic acid production and without the use of traditional fedbatch control.

# Food & Beverages Group session

## Mechanisms of survival of Gram-positive pathogens during food processing

### *Bacillus cereus* survival strategies

Tjakko Abee

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*Bacillus cereus* is a common cause of food-borne disease that thrives in many different ecological niches. For the control of this pathogen, it is especially relevant to know which mechanisms it can utilize to sustain growth in the many environments that it can inhabit. We aimed to assess global regulation in *B. cereus* highlighting the role of a range of sigma factors, including the general stress sigma factor $\sigma^B$ in the performance of *B. cereus* under various growth and stress conditions, relevant in the processing and preservation of foods.

Using *B. cereus* ATCC 14579 and targeted sigma factor deletion mutants, the impact of these regulators and their regulons on *B. cereus* growth performance, stress response, sporulation efficiency and surface behaviour, including biofilm formation, were assessed. In addition, proteomics and gene profiling, employing *B. cereus* whole genome ORF-based micro-arrays, are used to further identify key elements in *B. cereus* eco-physiology and virulence that may affect its performance and survival in industrial settings. This approach may supply tools for enhanced control of this versatile food-borne human pathogen.

### *Listeria monocytogenes*: acid stress responses in foods and beyond

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The ability of the intracellular foodborne pathogen *Listeria monocytogenes* to survive and grow under low pH conditions is fundamental to the infectious cycle. Resistance to acid stress is necessary for growth in low pH foods, for passage through the gastric barrier and for subsequent growth within the macrophage phagosome. We have characterized two key systems that play a critical role in pH homeostasis in this organism, namely; the glutamate decarboxylase (GAD) and arginine deiminase (ADI) systems. Through the use of a functional genetics approach we have created a series of deletion mutants lacking specific components of both pathways and have analysed the contribution of individual genes to pH homeostasis in acidic environments, including foods and the mammalian host. Our work has demonstrated that a specific glutamate decarboxylase enzyme (GadD2) is absolutely essential for survival of lethal acid conditions (pH2.5) whilst a second enzyme (GadD1) promotes growth of the pathogen under mildly acidic conditions (pH5.5). Intriguingly, the *gadD1* locus is part of a 5-gene genetic islet (*Stress Survival Islet-1*) that is present in *Listeria* serotypes that are common in foods but is absent in epidemic strains. Creation of a mutant lacking the entire islet and analysis of naturally islet-negative strains clearly demonstrated a role for this region in strain-specific stress resistance in *L. monocytogenes*.

### The regulation of toxin formation by *Clostridium perfringens* and *Clostridium botulinum*

Michel R. Popoff

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*Clostridium perfringens* and *Clostridium botulinum* produce potent toxins which are responsible for food-borne intoxications in humans. *C. perfringensi* enterotoxin (CPE) causes a food poisoning diarrhea, whereas botulinum neurotoxin (BoNT) induces a severe flaccid paralysis. Toxin synthesis in clostridia is a highly regulated process. CPE formation is strictly dependent of sporulation. Transcription of *cpe* gene is under the control of sporulation specific RNA polymerase sigma factors. *C. perfringens* sporulation and the concomitant production of CPE do not occur in regular culture mediums or food, but take place in intestinal content. Inorganic phosphate is an important environmental signal inducing sporulation and CPE synthesis. In contrast, BoNT is formed and secreted in food during the vegetative growth phase of *C. botulinum*. Synthesis of BoNT and associated non-toxic proteins forming the botulinum complexes are regulated at the end of the exponential growth phase and early stationary phase by a specific alternative sigma factor (BotR). Environmental signals triggering BoNT synthesis are still unknown.

### Survival of *Mycobacterium avium* subsp. *paratuberculosis* during dairy processing

Irene R. Grant[1] & Michael T. Rowe[2]

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Interest in the impact of dairy processing on *Mycobacterium avium* subsp. *paratuberculosis* (MAP) has arisen in recent years because cows' milk may be a potential vehicle by which MAP is transmitted from cattle with Johne's disease to humans. In susceptible individuals MAP may have some involvement in the inflammatory bowel condition Crohn's disease, although this association remains controversial. Research has shown that high temperature, short time (HTST) pasteurisation of milk may not completely inactivate all MAP present. Low numbers of surviving MAP were isolated from 2% commercially pasteurised cows' milk in a UK survey, and similar findings have now been reported for other milk surveys worldwide. Potential explanations for the observed heat resistance of MAP include the occurrence of cells in clumps and heat activation of dormant cells. Laboratory studies suggest that dairy processes that physically remove MAP from milk, such as centrifugation and microfiltration, may be useful adjuncts to HTST pasteurisation. More recent studies on UV inactivation of MAP in milk indicate that UV treatment achieves only 0.5-1 $\log_{10}$ reduction in viable MAP before adverse organoleptic changes arise in milk, so this novel preservation method is not a viable alternative non-thermal process to HTST pasteurisation for inactivation of MAP.

### Staphylococcal stress responses in food

H. Ingmer

Royal Veterinary & Agricultural University, Frederiksberg, Denmark

*Staphylococcus aureus* is a bacterial pathogen that for many decades has been known for its robustness against environmental stress and thus, in recent years research has focused on understanding the underlying molecular mechanisms. With offset in the response to heat, we have studied a group of proteases, the Clp proteases and have found that they are particularly important for growth and survival

both during environmental adverse conditions and during infection. The Clp proteases are composed of the proteolytic compartment (ClpP) as well as of a substrate binding unit formed by the one of several possible Clp ATPases that among other activities also determines substrate specificity of the protease. Particularly, we have investigated the role of four Clp ATPases and have shown that ClpX is required for expression of major virulence factors and for virulence of *S. aureus*, but not for survival during heat shock whereas ClpC and ClpB and to a minor extent ClpL are required for growth at high temperature and in the case of ClpB also for induction of thermotolerance. Presently, we are investigating the role of proteolysis in modulation of the general stress response as well as in further characterizing the role of Clp proteins in stress tolerance and virulence of *S. aureus*.

## Listerial survival upon surfaces

**T.G. Aldsworth**[1], R. Nova[1], C.E.R. Dodd[1], S. Perni[2], G. Shama[2] & the LMTOOCHE partnership

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Attachment of listerial species to fomites poses a major problem for the food industry as its ability to survive in food factory environments results in the organism becoming endemic in processing areas, and attachment to surfaces is believed to contribute to this. Hence removal of listerial cells from surfaces is essential to the manufacture of safe foods. The aim of this work was to study survival of listerial strains on surfaces under a variety of conditions. Listeria cells were attached to stainless steel surfaces and the strength of attachment assayed under flow conditions using a radial flow chamber (Fowler Cell). Significant strain-dependent variation in attachment strength was seen, suggesting differential expression of cell surface components possibly influencing establishment of strains. The influence of nutrients on attachment of Listeria has also been investigated and shown to play a role. These findings have important implications for sanitisation regimen as strain and growth conditions will influence their effectiveness.

## Lag time in *Clostridium botulinum*

S.C. Stringer, M.D. Webb & **M.W. Peck**

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Sales of minimally heat processed chilled foods are currently increasing in Europe by about 10% per year. The principal microbiological hazard in these foods is *Clostridium botulinum*. Foodborne botulism is a severe and often deadly disease, with as little as 30ng of neurotoxin potentially fatal. The continued safe development of these foods depends on an improved understanding and prediction of lag time and its variability, since any growth is likely to initiate from a low number of spores. This requires knowledge of the distribution of lag times from individual spores. A semi-automated phase-contrast microscopy/image analysis system has been developed to quantify distinct stages of lag for individual spores. Lag time from individual spores was highly variable, as a result of the variability for each distinct stage of lag (germination, emergence, cell maturation and doubling). The duration of each stage of lag was independent for individual spores. Consequently, it was not possible to predict the total duration of lag phase from information on just one of the stages of lag (e.g. from germination time alone), and the first spore to germinate was not the first to give actively dividing cells.

## Characterisation of a putative *agr* system in *Clostridium botulinum* and *Clostridium sporogenes*

**C.M. Cooksley**[1], I.J. Davis[1], W.C. Chan[1], K. Winzer[1], A. Cockayne[1], M.W. Peck[2], & N.P. Minton[1]

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One of the most notorious members of the genus *Clostridium* is *C. botulinum*, the causative agent of botulism. Aside from wound botulism in intravenous drug users, the major clinical concerns relate to intestinal toxaemia (common in the USA) and foodborne botulism – the major UK concern. To help prevent botulism, it would be useful to understand those environmental factors that affect the ability of the organism to grow and/or elaborate toxin. Insight into the regulatory mechanisms that control both spore formation and the germination process would also considerably benefit control measures. Such adaptive responses require co-ordinate control of gene expression.

The genome sequence of the *C. botulinum* Group I strain ATCC 3502 has recently been determined. *In silico* analysis has revealed the presence of two distinct loci capable of encoding proteins with homology to AgrB and AgrD of the *Staphylococcus aureus agr* quorum sensing system. We have begun the functional characterisation of these genes in order to determine whether they play a role in quorum sensing.

The equivalent regions were shown to be present in *C. sporogenes*, and to be highly conserved. Transcriptional linkage assays have shown some of the genes of the *C. sporogenes agr* regions to be co-expressed, and to have higher expression during early exponential growth. Modulation of the expression of the identified *agr* genes is a prerequisite to determining their function. We have used antisense RNA expression for this purpose, and have shown that down regulation of the *agrB* gene affects sporulation. We are now in the process of producing knockout mutants of some of the *agr* genes using the recently-developed ClosTron system.

## *Enterococcus* oxidative stress responses

Jean-Christophe Giard

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The genus *Enterococcus* belongs to the lactic acid bacteria (LAB) group of micro-organism. It is naturally present inside the intestinal tract of humans, sometimes used in dairy food industry and even as a probiotic. However, it is probably the most unusual LAB since enterococcci, and particularly *E. faecalis*, have also been associated with a number of human infections. During the process of infections, one of the stresses mainly encountered by the bacteria is the oxidative stress. Many studies using Gram positive and negative pathogens established clear relationships between oxidative stress responses and virulence. If the pathogenic power of certain bacteria directly results from a synthesis of toxic molecules, their adaptation to stresses imposed by the host (i.e. ROS) remains an important criterion to develop their pathogenicity. Then, enzymes that allow *E. faecalis* to cope with oxidative stress may be considered as 'colonization factors'. In addition to virulence traits, such factors should undermine the commensal relationship and may explain why *E. faecalis* is an opportunist pathogenic bacterium. This presentation describes the oxidative stress responses in *E. faecalis* and their relationships with virulence.

# Physiology, Biochemistry & Molecular Genetics Group session

## Systems biology of regulatory networks

### Use of Chip-chip to look at the role of global regulators in *Escherichia coli*

D. Grainger

University of Birmingham

Chromation Immunoprecipitation (ChIP) is a tool that can be used to monitor interactions between DNA binding proteins and their targets. Proteins are cross-linked to DNA *in vivo* (typically by treatment with formaldehyde) then, after cell disruption, the cross-linked nucleoprotein is sheared randomly by sonication. Protein-DNA complexes are selectively immunoprecipiated with a suitable antibody, the cross-linking is reversed, and the precipitated DNA is purified. PCR can then be used with specific primers to test whether candidate target sequences are enriched in the precipitated DNA population. In ChIP-chip, the precipitated DNA is labelled and hybridised to a microarray to generate a chromosome-wide map of DNA binding. The ChIP-chip approach has several advantages compared to other methods that are used to define regulons of global transcription factors and these will be discussed.

### Novel insights into the evolution of negative self-regulating transcription factors

D.J. Stekel

University of Birmingham

Many prokaryotic transcription factors, typically associated with functionally associated operons, repress their own transcription. It is often asserted that such regulation enables a cell to homeostatically maintain protein abundance. We use a variety of stochastic modelling techniques to explore the role of negative self regulation in controlling protein variability. We demonstrate that mathematical analysis using standard approximations gives the surprising result that protein variance relative to its mean should be independent of repressor strength in physiological ranges. However, computer simulations show that this result does not apply to strong repressors: as repressor strength increases, so too does protein variability. Instead, we propose that strong negative self-regulation is evolutionarily favoured because it can allow a protein to be strongly expressed using minimum mRNA levels.

We use in silico evolution to back this claim and demonstrate that negative self repressors can produce protein with a 70% improvement in mRNA usage relative to an equivalent unrepressed system.

### Regulatory networks in yeast

N. Luscombe

European Bioinformatics Institute, Cambridge

*Abstract not received*

### Studying the response of *Escherichia coli* cells to temperature shift: a network inference approach

Shabnam Moobed[3], Katrine Semeith[1], Fernando Ortega[1], Thomas Carzaniga[1], Gianni Dehò[2], David Wild[3] & **Francesco Falciani**[1]

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Adaptation of *E. coli* cells to thermal stress involves simultaneous and global adaptations of the cell's most essential machinery. The heat shock response essentially depends on two master regulators; transduction of the 'heat signal' will, in turn, modulate their abundance and activity via complex but fairly well understood mechanisms. The control of bacterial response to cold shock appears to be different. A temperature downshift causes a transient inhibition of transcription and translation of most genes whereas a few dozen 'cold shock genes' are strongly induced before a new steady state is established a few hours later. Surprisingly, adaptation mechanisms of bacteria shifting from cold to 'warm' (optimum) growth conditions have been much less explored. Nevertheless, physiological rearrangements as drastic as those observed during cold adaptation must occur upon a 'warm shock' and specific regulatory mechanisms are expected to be implicated in 'warm adaptation'. Moreover, transition from cold to 37 °C, the mammalian host temperature, may represent an initial signal that triggers invasion and pathogenicity response. Therefore, understanding 'warm response' may also provide insights into the early stages of bacterial infection of a warm-blooded host. We have characterized *E. coli* response during the shift from 10 °C to 37 °C using expression profiling. In order to formulate hypothesis on the mechanisms underlying this response we have used network inference methodologies to deduce the structure of gene regulatory networks. In this communication we describe the result of our analysis and discuss the biological relevance of our results.

### Regulatory insights from EnviCom, a compendium of *Salmonella* gene expression profiles

**Mark Alston**, Sacha Lucchini, Arthur Thompson & Jay Hinton

Molecular Microbiology, The Institute of Food Research, Norwich NR4 7UA

*Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is a major pathogen of animals and man causing more human deaths than any other food-borne pathogen.

To successfully infect its host, *Salmonella* must survive a number of host defences. DNA microarrays have been used to define the *Salmonella* transcriptome in response to several infection-relevant environmental stresses and these data assembled into the *Salmonella* Environmental Compendium (EnviCom). EnviCom has allowed similar, or opposing, patterns of gene expression to be identified and linked to gene regulation, and has helped us to highlight possible interactions between infection-relevant regulatory networks. For example, using the *Salmonella* Pathogenicity Island (SPI1) invasion genes as a focus we have discovered that the *nap* operon appears to be co-regulated with SPI1. The environmental compendium has utility for the identification of new regulatory networks and will be queried to investigate the role played by particular regulons in the response to environmental stresses. Furthermore, it lends itself as an operon discovery tool. We are in the process of expanding EnviCom and developing equivalent compendia for regulons and for gene expression during infection of different host niches.

### Mathematical modelling of genetic regulation in *Escherichia coli*

I. Goryanin

University of Edinburgh

*Abstract not received*

## Use of *lux* and *gfp* fusions to examine the induction dynamics of the acid stress response genes of *Escherichia coli*

**Neil Burton**, Barbara Russell, Sundeap Moore & Peter A. Lund

School of Biosciences, University of Birmingham, Birmingham B15 2TT

A system that provides effective protection for *Escherichia coli* at extremely low pH (2-2.5) is glutamate dependent acid resistance. In order for this system to be functional at low pH, the structural components GadA and GadBC, must be pre-induced. The regulation of this induction at the molecular level is remarkably complex and involves numerous transcriptional regulator proteins, including the essential activator GadE. Modelling of such a system can only be done with the availability of high density time series data. We have utilised promoter fusions to a *lux* reporter operon or to *gfp* on low copy number plasmids to gather such data on the activities of key promoters of the Gad network in *E. coli* MG1655 in response to a mild acid shock (pH5.6). Our data suggest that the *gadE* promoter is induced first, followed by the *gadBC* promoter and, after a further delay, the *gadA* promoter. The *gad* structural genes show significant differences in post-induction kinetics. In addition, we have used these promoter fusions to investigate gene expression in strains of *E. coli* which have been selected to show enhanced resistance to low pH in exponential phase. Preliminary results show both the *gadE* and *gadBC* promoters are strongly up-regulated compared to their expression in the wild type strain, even in the absence of pre-inducing conditions, suggesting a possible molecular basis for the evolved acid resistant phenotype.

## Growth rate-dependent flipping of an artificial genetic switch in *Escherichia coli*

**S. Lucas Black**[1], Sarah Spragg[1,2], Gail P. Ferguson[2] & **Rosalind J. Allen**[1]

[1]School of Physics, Edinburgh University; [2]School of Biological Sciences and CSEC, Edinburgh University

Genetic switching is of great interest in the fields of molecular biology, biotechnology and bio-computing as well as being relevant to pathogenesis and antibiotic resistance. In this project we explore how the rate of flipping of an artificial genetic switch varies as a function of growth rate in *E. coli*. To do this, we measure GFP fluorescence in a 96-well plate reader. This poster describes our results so far.

## Modelling of metabolic regulation in prokaryotes

Lorenz Wernisch

Birkbeck College, University of London

Surprisingly little is known about regulatory processes in prokaryotes outside a small group of model species such as Escherichia coli. Probabilistic models allow one to combine the comparatively sparse direct experimental evidence for regulation in less well mapped organisms such as *Mycobacterium tuberculosis*. Control of metabolism in prokaryotes is tightly linked to gene expression by just-in-time expression of enzymes required for the activation of a particular pathway. That means gene expression data can be used in conjunction with bioinformatics and genomics data to map genes to enzymes and to track the activity profiles of metabolic pathways. I will discuss the challenges of such a project, suitable statistical concepts and tools, as well as applications to the lipid metabolic pathways in *M. tuberculosis*.

## Modelling of gene networks (theoretical, and in yeast)

M. Stumpf

Imperial College London

*Abstract not received*

## Bistable differentiation in *Bacillus subtilis* colonies

**Leendert Hamoen**, Eric Stewart, Thomas Berngruber, François Taddei, Wiep Klaas Smits, Oscar Kuipers & Jan-Willem Veening

University of Newcastle

The soil bacterium *Bacillus subtilis* is well known for its capacity to develop different cell types such as motile cells, genetic competent cells and sporulating cells. These differentiation processes are a response to starvation. Surprisingly, not all cells within a culture follow the same differentiation pathway, and several of these adaptive responses turn out to be bistable. We have recently shown that the positive feedback architecture of the regulation pathways involved is responsible for the bistable expression of the competence and sporulation transcription factors. The choice to differentiate may be noise based; however, cell age or growth rate of the cell could play a decisive factor as well. We have used special time-lapse microscopy to follow the outgrowth of a single cell into a sporulating micro-colony. This enabled us to show that *B. subtilis* does age, but that aging does not determine cell fate. However, we found that the physiological state of the cell's ancestor, which can be up to six generations removed, affects cell fate through a process of epigenetic inheritance. This inter-generational 'memory' may be important for the development of multi-cellular structures such as fruiting bodies and biofilms.

## Modelling of chemotaxis network in *Escherichia coli*

M. Kollman

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*Abstract not received*

## Mapping promoter input functions of *Escherichia coli*

**Shai Kaplan**, Anat Bren & Uri Alon

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A major goal of biology is to understand the computations done by transcription networks in cells. In transcription networks, transcription factors bind promoters of genes to control the rate at which they are transcribed. The edges or arrows in the network, which define which transcription factor regulates which gene, are becoming well characterized in model organisms. However, the connectivity of the network is not sufficient to understand its behavior. One also needs to know the way that inputs are integrated at each promoter (input functions), and the relative strength of each interaction ('numbers on the arrows'). These functions are currently mostly unknown. We present a novel robotic assay that employs a library of fluorescent reporter strains covering the vast majority of promoters in *E. coli* that measure the response of promoter activities to input signals at high time resolution and accuracy. Our preliminary results of input functions of various sugar systems in *E. coli* show that in addition to functions that resemble AND gates or OR gates, input functions can be intricately designed, and display diverse shapes. This systematic characterization of input functions provides new insights into the design principles of the transcription network.

## Redefining the regulatory network that controls flagellar assembly in *Salmonella*

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The bacterial flagellum is structurally comprised of a basal body, a hook and a long filament that acts as the propeller. Assembly is temporally regulated by a checkpoint dictated by the length of the intermediate hook-basal body structure. Three promoter classes organised into a transcriptional hierarchy control flagellar gene expression coupled to flagellar assembly.

A substrate specificity switch of the flagellar secretion apparatus defines the assembly checkpoint and is coupled to the activation of post-switch subunit gene expression by the flagellar specific transcription factor $\sigma^{28}$. Proteins destined for post-switch secretion by the secretion apparatus require secretion chaperones. The secretion chaperones are molecular timing devices: having a second role as regulators of gene expression. We have begun to define the complete regulatory network that couples flagellar gene expression to assembly in *Salmonella enterica* serovar Typhimurium. Using null mutations in genes coding T3S-chaperones and their substrates we have shown: 1) the T3S-chaperone FliT regulates two flagellar promoter classes antagonistically 2) the FliT substrate, FliD, acts as an anti-regulator of FliT 3) there are two further regulatory circuits responsive to basal body assembly and filament polymerisation and 4) Secretion substrate competition influences $\sigma^{28}$-dependent post-switch gene expression via changes in secretion of its anti-$\sigma^{28}$ factor, FlgM.

## Regulation of flagellar and chemotaxis genes in *Rhizobium leguminosarum*

Dinah D. Tambalo[1], Kate L. Del Bel[1], Denise E. Bustard[1], Paige R. Greenwood[1], Audrey E. Steedman[1], Christopher K. Yost[2] & **Michael F. Hynes**[1]

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The genome of the root nodule bacterium *Rhizobium leguminosarum* contains 27 chemoreceptor (*mcp*) genes, 7 flagellin genes (*fla*), two chemotaxis (*che*) operons, and other genes (*flg*, *fli* and *mot*) involved in motility. To investigate the regulation of the motility system in *R. leguminosarum*, we cloned three potential regulatory genes (*visN*, *visR*, and *rem*) located within the *fla/che* gene cluster. Mutants in *visN*, *visR*, and *rem* were used to determine their role in regulation of *gusA* fusions to promoters of *mcp*, *fla*, *mot* and *che* genes. Expression of the *visN* and *rem* genes in free-living and symbiotic conditions was also monitored. The results showed a regulatory hierarchy whereby VisN and VisR regulate expression of *rem*, and Rem regulates the principal *che* operon, *flaA*, *flaC*, *flaD*, and *mpcD*. The second *che* operon, *flaE* and *mcpC* are not part of this regulatory cascade. Expression of *visN* and *rem* was reduced in pea nodules, as was expression of chemotaxis and flagellar genes.

## Mathematical modeling of gene regulatory networks: overview of problems, predictions and utility

Michael C. Mackey

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This talk will focus on recent mathematical modeling efforts to understand dynamic behaviour in the lac and tryp operons as well as the phage lambda switch. All three examples highlight the techniques and problems facing modelers, as well as the advantages to both experimentalists and modelers that can ensue from joint efforts.

## Surviving heat shock: control strategies for robustness and performance

M. Khammash

University of California, USA

The heat shock response in bacteria is an important mechanism for combating the stress associated with an increase in temperature in the cellular environment. The resulting increased heat causes the unfolding or misfolding of cellular proteins and leads to a state of cellular stress. The cell responds to the accumulation of nonfunctional proteins by the heat induced upregulation of the heat shock proteins (HSPs), including both chaperones and proteases. The production of HSPs is regulated directly by alterations in the level, activity, and stability of the sigma factor sigma-32. The logic of the heat shock response is implemented through a hierarchy of feedback and feedforward controls that regulate both the amount of sigma-32 and its functionality. In this talk we present a mathematical model that captures known aspects of the heat shock system. With the aid of this model, we discuss the logic of the heat shock response from a control theory perspective, drawing comparisons to synthetic engineering control systems.

Robustness, performance, and optimality of the control architecture will be addressed and related to the complexity of the heat-shock response system.

# Systematics & Evolution Group session

## *Alphaproteobacteria*: unifying a diverse class

### Genomes, phylogenies and evolution

J.P.W. Young

University of York

*Abstract not received*

### Genomic diversity: exploring the bacterial genomic species concept

Denis Costechareyre[1], Vincent Daubin[2], Christine Oger-Desfeux[3], Franck Bertolla[1], & **Xavier Nesme**[1]

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The bacterial species is presently defined on the basis of the genomic coherence of its members determined by DNA/DNA hybridization studies. This operational definition is still lacking biological investigations that will allow to make us the bacterial species a concept comparable to the biological species concept (BSC) accepted for Eukarya. In addition to AFLP and MLSA studies performed to propose easiest molecular alternatives to hybridizations for the determination of species, we are exploring candidate forces that lead or maintain the genomic coherence of species. Two forces were considered: i) the sexual isolation characterised by a drop in homologous recombinations between couples of strains; and ii) the ecological specificity of genomic species. Using closely related but highly diverse genomic species of *Agrobacterium spp*., we first experimentally compared sexual isolation to genetic and genomic distances to test whether a drop do readily occur from within to between species matings. Secondly, we set up a comparative genomic approach to determine the species specific set of genes of a given species that is likely involved in its adaptation to a specific ecological niche. Results showed that even very closely related species have specific genes, but are not significantly sexually isolated, suggesting that sexual isolation is a consequence rather a cause of the speciation in bacteria.

### Thousands of gene trees to reconstruct the tree of life

Sophie Abby, Manolo Gouy & **Vincent Daubin**

Université Lyon 1; Laboratoire BBE, F-69622 cedex, France

Despite the increasing amount of genomic data and the development of new phylogenomics methods, the tree of life is still largely unresolved thirty years after Woese's classification based on ribosomal RNA. Indeed, gene histories typically intermingle duplications, horizontal gene transfers (HGT) and gene losses and are therefore difficult to convert into information on the history of species.

Here we propose a new approach which allows to use numerous gene families usually not included in phylogenetic analysis by testing for orthology hypothesis using maximum-likelihood (ML). Orthologous genes can be practically defined as a set of genes that produce a phylogeny congruent with the species tree.

Our studies focused on eight bacterial and one archaeal phyla and showed that a reference tree maximizing the support of numerous genes can be constructed at the phylum scale. Moreover, the results demonstrate the possibility of extracting orthologous sequences from families containing duplications. Future improvements may allow the use of this approach at the inter-phylum scale and could provide sets of sequences to reconstruct the tree of life.

### Mobile genetic elements

Dariusz Bartosik

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Sequencing projects have revealed that bacterial genomes are not monolithic structures. They can contain various mobile elements such as plasmids and integrated transposable elements (TE), integrons, bacteriophages as well as genomic islands and islets, acquired by different lateral transfer events. Identification of these 'foreign' components of the genome mosaic provides evidence supporting genome plasticity and enables prediction of the direction and frequency of lateral gene transfer in the environment. In our studies we analyzed plasmids and TE harbored by *Paracoccus* spp. (*Alphaproteobacteria*), which are among the most metabolically versatile bacteria. As a result of these studies over 40 plasmids were identified, ranging in size from 2.7 to over 600 kb. Several plasmid genomes were sequenced, which allowed identification of functional modules coding for replication, stability and transfer functions. Some of the modules were used to construct entrapment vectors useful in the identification of TE of *Paracoccus* spp. This allowed identification of (i) many insertion sequences (IS), (ii) transposons, (iii) a transposable genomic island, as well as (iv) transposable modules – a novel type of TE generated by an insertion sequence. These elements were shown to play a significant role in shaping the structure of plasmids naturally occurring in *Paracoccus* spp.

### Solute binding protein dependent transporters in the rhizobia

Philip Poole

School of Biological Sciences, University of Reading, Whiteknights, Reading

There has been a large increase in the number of solute binding protein dependent transport systems in the rhizobia. These systems are comprised of the ATP binding cassette-transporter (ABC) and tripartite ATP-independent periplasmic transporter (TRAP) families. ABC systems consist of four domains: two hydrophobic integral membrane domains (IMP) and two ATP-binding cassettes (ABC). In addition bacterial ABC uptake systems contain an SBP. TRAP systems possess one small and one large integral membrane protein as well as an SBP. Unlike ABC-Ts, which use ATP hydrolysis to energise uptake, TRAP-Ts use the proton motive force.

There are 200 ABC genes in *Sinorhizobium meliloti*, 216 in *Mesorhizobium loti* 269 in *Rhizobium leguminosarum* and 240 in *Bradyrhizobium japonicum*, compared to 67 in *Escherichia coli* and 124 in *Pseudomonas aeruginosa*. The ABC genes of *S. meliloti* are organised into 146 uptake systems and 18 export systems. Whereas, the TRAP systems number 15 in *S. meliloti*, 22 in *B. japonicum* and curiously only two in *M. loti* compared to one in *E. coli* and six in *P. aeruginosa*. The increase in the number of systems may be due to the high affinity of these transporters enabling the acquisition of a broad range of nutrients from the oligotrophic and nutrient-limited soil and rhizosphere habitats of free-living rhizobia.

## Energy conservation in the respiratory chain

Peter Brzezinski

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The energy metabolism of biological systems involves electron and proton-transfer reactions across biological membranes. One component of this machinery is an integral membrane protein, cytochrome *c* oxidases, which catalyses the four-electron reduction of dioxygen ($O_2$) to water and oxidation of different types of cytochromes *c* (cyt.c): $4cyt.c^{2+} + O_2 + 4H^+ \rightarrow 4cyt.c^{3+} + 2H_2O$

The oxygen-reducing site of cytochrome *c* oxidases consists of a haem-copper center, buried within the protein. The protons used for $O_2$ reduction to water (substrate protons) are taken up from the more negative (*N*-) side of the membrane while cyt. *c* reacts on the opposite, more positive (*P*-) side of the membrane. Thus, the chemical reactions catalysed by cytochrome *c* oxidase are arranged topographically in such a way that they result in a charge separation corresponding to a net transfer of one positive charge from the *N*-side to the *P*-side per electron transferred to $O_2$. In addition, for most oxidases characterized to date, part of the free energy released in this catalytic reaction is also used to pump (translocate) protons from the *N*-side to the *P*-side side of the membrane, with an average stoichiometry of one proton per electron. Hence, on average two charges are transferred across the membrane per electron transferred to oxygen. This transmembrane proton and voltage gradient, generated in part by the oxidases is used, for example, for synthesis of ATP by the ATP synthase.

The proton-pumping function of the oxidases is reflected in specific structural elements of the molecular machine. Some of these components have been identified, however, the molecular mechanism by which cytochrome *c* oxidase pumps protons is not understood. The general view is that during the process the enzyme must provide an alternating access of protons to the two sides of the membrane and have this alteration strictly coupled to specific transitions of the catalytic cycle. Such changes in the accessibility of protons to the two sides of the membrane are likely to be achieved by breaking and formation of hydrogen bonds in internal proton-transfer pathways, for example through local rearrangements of amino-acid residue side chains.

In my talk I will discuss results from experimental studies aimed at uncovering the molecular mechanism by which cytochrome *c* oxidase pumps protons. In addition, I will discuss fundamental mechanistic principles that are utilized to couple the exergonic electron and proton transfer from cyt. *c* to $O_2$, to proton pumping.

## The chemical diversity of the *Alphaproteobacteria*: charting the forgotten depths

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Over the past 5 decades a significant amount of data on the chemical composition of prokaryotes has become available. Respiratory lipoquinones, polar lipids and the nature of their hydrophilic side chains have, during the course of evolution provided us with a myriad of different compounds, all of which are synthesised by the cell. The biochemical pathways leading to their biosynthesis are encoded on the genome. Within the *Alphaproteobacteria* the full diversity of polar lipids remains to be documented, but in addition to phospholipids one finds amino acid based lipids, glycolipids, and polar hopanoids. The distribution of these lipids is not random, but appears to be linked to evolutionary and physiological changes, which affect the infrastructure and functioning of the (ubiquitously present) highly organised cell membrane(s). However, a functioning membrane does not consist of lipids alone and there are complex, and not fully understood interactions with proteins, which are part of one of the most complex and essential components of the cell. Direct studies on membranes are hampered by their hydrophobicity and their inherent dynamic nature. One of the first steps is to document that diversity and locate the enzymes and genes responsible for their biosynthesis.

## Evolution of methylotrophic metabolism

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Growth on single-carbon ($C_1$) compounds, or methylotrophy, is a unique metabolic capacity requiring dozens of genes, and is present in multiple microbial phyla, including several groups of *Alphaproteobacteria*. The genes encoding $C_1$ metabolism are generally highly conserved between these groups, but display phylogenetic relationships discordant with other genes, including 16S rDNA due to the key role of horizontal gene transfer (HGT) in the evolution of this metabolic mode. Additionally, characterized strains from methylotrophic phyla are almost exclusively carbon-source specialists, even though their sister phyla are typically generalists. What physiological changes accompany the incorporation of new metabolic modules? Or the loss of metabolic capacities during prolonged specialization? In order to directly address these questions in the context of the evolution of methylotrophic metabolism, we have evolved replicate experimental populations of the alphaproteobacterium *Methylobacterium extorquens* AM1. These populations have allowed us to follow through time adaptation subsequent to HGT and the tradeoffs associated between $C_1$ and multi-C growth. Furthermore, we have begun to uncover the physiological bases for adaptation in the laboratory setting and relate them to systems-level physiology through computational models of the $C_1$ metabolic subsystem.

## Type IV Secretion systems

Christoph Dehio

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Among the repertoire of protein secretion systems evolved in Gram-negative bacteria (type I to type V), the type IV secretion systems (T4SS) are particularly prevalent in the *Alphaproteobacteria*. Type IV secretion systems have evolved as DNA-transfer systems in the context of bacterial conjugation and many alphaproteobacterial plasmids encode such bacterial conjugations systems. Several alphaproteobacterial species establishing pathogenic or mutualistic interactions with eukaryotic host cells have independently adopted bacterial conjugation systems for the direct delivery of macromolecular substrates (such as protein or DNA) into the host cell cytoplasm. The prototypic T4SS is the VirB/VirD4 of *Agrobacterium tumefaciens*, which mediates conjugation-like transfer of the tumor-inducing DNA and the associated proteins into infected plant cells. The T4SS involved in mediating host cell interaction by other *Alphaproteobacteria* are considered to deliver effector proteins that subvert host cellular functions. We are using the mammalian pathogen *Bartonella* as a model alphaproteobacterium for studying the evolution of T4SS and the thereby translocated effector proteins. Further to the role of T4SS in establishing long-term host cell interaction I will discuss a novel role for T4SS in mediating adaptation to the host – a major factor driving the evolution of host-restricted bacteria.

## The chemosensory pathways of *Rhodobacter sphaeroides*

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Analysis of the genome of *R. sphaeroides* reveals that it has operons loci encoding homologues that could form 3 complete chemosensory pathways. Expression studies have shown that their trasncription is independently regulated. Deletions studies show that 2 of these are essential for normal chemotactic behaviour. Why does *R. sphaeroides* have 2 pathways when *E. coli* and *B. subtilis* manage with only one?

*In vitro* analysis of the phosphotransfer pattern of the purified proteins showed a complex behaviour with the histidine protein kinase CheA2 able to phosphotransfer to all the response regulators, while CheA3 could only phosphotransfer to two. Despite being able to phosphorylate all the response regulators, CheA2 did not complement a CheA3 deletion. *in vivo* analysis of the cellular location of each chemosensory protein shows that all of the proteins of one operon localise to the cell poles with the membrane spanning chemoreceptors while the others form a tight cluster in the middle of the cell with receptors lacking the membrane spanning domain. The two pathways are therefore physically separate in the cell. Molecular analysis of localisation reveals that the requirements to localise to different positions is different, with the cytoplasmic cluster needing a specific chemoreceptor and a protein homologue of the ParA plasmid partitioning factor.

Possible reasons why *R. sphaeroides* has two chemosensory pathways will be discussed.

## The mechanism of flagellar filament assembly in *Caulobacter crescentus*

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The *Salmonella* flagellar system is the most intensely studied and is thus regarded as the paradigm of flagellar assembly. In the *Salmonella* paradigm, two junction proteins, one flagellin subunit and a filament capping protein are required for efficient filament assembly. In contrast in the alphaproteobacterium *Caulobacter crescentus* six flagellins are incorporated into the flagellar filament while no homologue of the *Salmonella* filament cap is found in the genome.

We have created combinations of in-frame deletions of the six flagellin genes to determine their role in filament formation in the absence of a filament cap. Individual and combination deletions of flagellins range from non-motile, partially motile to motile. When filaments were produced they were physically distinct from each other and from wild type. Characterisation of mutant phenotypes showed that *C. crescentus* releases monomeric flagellins suggesting filament polymerisation is inefficient compared to *Salmonella*. MALDI-TOF analysis of the monomeric secreted flagellins by wild type identified four out of six *C. crescentus* flagellins. Therefore we conclude that the absence of a filament cap to direct filament assembly and the presence of six flagellins suggests an alternative mechanism of filament assembly in this member of the *Alphaproteobacteria*.

## Quorum sensing and biofilm formation among the *Alphaproteobacteria*

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The multicellular interactions of prokaryotes have gained great attention over recent years as it has become clear that microbes utilize a range of different mechanisms to communicate or monitor their own activity. Likewise, the physical manifestations of these interactions, biofilms and other multicellular assemblies, have also garnered intense interest. Structured microbial assemblies are often the site for microbial signaling mechanisms, hence linking these phenomena. *Alphaproteobacteria* have served as important model systems for the study of these processes in free-living and host-associated bacteria. Additionally, some of the most compelling and complex examples of these multicellular phenotypes are found in *Alphaproteobacteria* and they continue to yield surprising new discoveries. Acylated homoserine lactones (AHLs) serve as a common signaling language among *Alphaproteobacteria*, regulating a diverse range of microbial activity, and often influencing parasitic and commensal interactions with hosts. In plant-associated bacteria such as species of *Agrobacterium* and *Rhizobium*, AHLs control important functions including virulence, horizontal gene transfer and symbiosis. In several cases, there are multiple AHL signaling systems, with overlapping or hierarchal organizations, and global regulatory functions. Biofilm formation also plays an important role in host interactions for these microbes, and is often integrated with signaling processes.

# Virus Group session

## Molecular aspects of virus replication from entry through to exit

### Influenza A viruses

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Influenza A viruses are important human pathogens that are responsible for seasonal epidemics and for periodic high mortality pandemics. Here I focus on the viral NS1 protein, a multi-functional dimeric protein that participates in both protein-RNA and protein-protein interactions. Its N-terminal RNA-binding domain binds double-stranded RNA (dsRNA) with low affinity. The primary role of this dsRNA-binding activity is inhibition of the (IFN)-induced oligo (A) synthetase/RNase L pathway. This activity has no detectable role in inhibiting the production of IFN-β mRNA or inhibiting activation of PKR. The rest of the NS1A protein, denoted as the effector domain, has binding sites for several cellular proteins, including CPSF30, a cellular factor required for the processing of cellular pre-mRNAs. As a result, the production of IFN-β mRNA and other cellular mRNAs is inhibited, and a large amount of IFN-β pre-mRNA accumulates. The X-ray crystal structure of the NS1 protein complexed to a fragment of CPSF30 identifies the CPSF30 binding site on the NS1 protein. Changing a single amino acid in this NS1 binding site restores the production of IFN-β mRNA and attenuates the virus, verifying the crucial role of the CPSF30-binding site of the NS1 protein in influenza A virus replication.

### Interactions of HSV with the inner nuclear membrane and nuclear pore

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During entry, after fusion of the virus and host membranes, herpesvirus capsids traffic to the nuclear pore where they dock prior to de-encapsidation and transport of the genome across the pore for transcription, replication and packaging. After assembly in the nucleus, capsids exit via a pathway which is not thought to involve the nuclear pore, but instead involves navigation across the nuclear lamina and then a budding process through the inner and outer nuclear membranes (INM and ONM respectively). We have presently little detailed understanding of the mechanism controlling the pathways of infection at the nuclear membrane or of the host components involved. We are currently exploring virus–host interactions at the INM, including the lamina components and membrane components such as Emerin and LBR, which we believe are likely to represent key steps in exit and assembly pathway. Accumulated evidence also indicates that the large tegument protein VP1-2 encoded by the UL36 gene plays a key role in both inward transport and outward transport and assembly. We will present current studies on the impact of infection on INM structure and the role of VP1-2 in multiple aspects on inward and outward virus trafficking.

### Persistence of bovine viral diarrhea virus requires restriction of viral replication by a host factor

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More than 1% of the cattle population is persistently infected by the pestivirus bovine viral diarrhea virus (BVDV). Lifelong persistence depends on a sophisticated interplay between virus and host. Due to intrauterine infection adaptive immune response is disabled by acquired immunotolerance while innate immune response is antagonized by virus-encoded proteins. A third essential prerequisite for viral persistence is the non-cytopathogenic (noncp) phenotype of the infecting BVDV strain.

The noncp phenotype depends on the restriction of viral replication by a host factor. This restriction is based on the regulation of a vital autoprotease residing in non-structural protein 2 (NS2). The latter enzyme catalyzes the release of NS3, an essential component of the viral replicase. Surprisingly, the NS2 autoprotease is only active in the first hours after infection. As a consequence, the NS3 amount declines at later time points, leading to a strong decrease in viral RNA replication. For its activity the NS2 autoprotease depends on a cellular cofactor, termed Jiv (J-domain protein interacting with viral protein). Overexpression of Jiv interfered with temporal downregulation of NS2-protease activity and strongly enhanced viral RNA replication resulting in a switch of the viral biotype from noncp to cp.

According to our data, the limiting amount of a cellular protein is crucial for maintaining the noncp phenotype of BVDV and thereby for its ability to establish persistent infections.

### Studies of restriction factor-retrovirus interactions

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Evidence obtained from the study of endogenous retroviruses implies that retroviruses, in modern form, and their vertebrate hosts have coexisted for tens of millions of years. It is therefore unsurprising that a number of host genes regulating retrovirus replication have evolved and that a degree od co-adaptation has followed. Two such genes are the primate Trim5 (alpha isoform) and murine Fv1 genes. Both genes interact with polymerised viral capsid protein present on incoming virions in newly infected cells and act to block virus replication prior to integration; Trim5 inhibiting reverse transcription and Fv1, in some manner, preventing integration. Trim5 can act on a range of lentiviruses, including HIV, as well as murine leukaemia virus (MLV) whereas Fv1 acts only on MLV. Genetic, cell biological and biochemical studies, from a number of labs, designed to investigate the mode of action of these factors, and their effects on retroviral replication, will be presented.

### A reverse genetic approach to understanding dengue virus replication and pathogenesis

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The Dengue viruses (DENVs) are mosquito borne flaviviruses infecting 50-100 million individuals annually worldwide. Dengue disease ranges from a mild fever to the potentially fatal dengue haemorrhagic fever/shock syndromes. The DENV particle is comprised

of an RNA genome and three structural proteins (capsid (C), membrane and envelope). Translation of the ~11 kb positive stranded RNA genome results in a single polyprotein which is processed by cellular and a viral encoded protease to the three structural and seven nonstructural (NS) proteins; NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Recent evidence suggests that aside from their key role in genome replication, specific DENV nonstructural proteins are involved in perturbing the host innate immune response. The multifunctional NS5 protein is central to viral replication, possessing enzymatic activities required for both viral RNA capping and synthesis. Although flavivirus replication is believed to occur in the cytoplasm, the NS5 protein can also be detected in the nucleus, potentially affecting host cell processes. Using reverse genetics, in combination with recent protein structural data, we aim to identify and characterise specific determinants in the DENV nonstructural proteins, with an emphasis on the NS5 protein, that regulate the viral lifecycle.

## Genetic interaction between *cis*-acting packaging elements in the segmented genome of influenza A virus

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Influenza A virus utilises *cis*-acting packaging signals to package its segmented RNA genome. These signals extend into coding regions, where distinguishing between selection for RNA structure and for conservation of the encoded protein frustrates mapping projects. By considering codon conservation, we identified putative *cis*-acting elements within the coding regions, including likely packaging signals. An 8-plasmid reverse genetics system was used to introduce synonymous mutations into conserved codons in segment 7 (M1/M2). Mutants with changes in conserved regions replicated to significantly lower titres than wild-type virus, whereas viruses with alterations to less-conserved codons replicated normally. Three debilitated mutants were serially passaged to select fitter variants and all regained high titre growth. In all cases, sequencing showed that the original mutations remained, suggesting compensatory second-site mutations. Identification (currently underway) of these mutations will help delineate interactions between disparate sites in influenza's segmented genome during the process of viral assembly.

## Mutational analysis of herpes simplex virus DNA packaging protein UL33

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The products of the UL15, UL28 and UL33 genes of herpes simplex virus type 1 are thought to form a viral terminase complex which cleaves concatemeric viral DNA into monomeric genomes, and packages them into preformed capsids. Random insertional mutagenesis of the UL33 gene produced 16 distinct mutants encoding polypeptides with 5aa insertions located throughout the protein. The mutants were tested for their ability to complement the growth of mutant viruses containing null and temperature-sensitive lesions in UL33. This allowed identification of several regions of UL33 required for virus growth. Each of these regions was also vital for the packaging of replicated amplicon DNA in a transient DNA packaging assay. Twelve of the mutants retained the ability to interact with UL28, as demonstrated in an immunofluorescence assay. Surprisingly, two of the mutants that failed to interact with UL28 were capable of supporting viral growth and DNA packaging. These data suggest that interaction between the UL33 and UL28 proteins may not be essential for efficient cleavage and packaging of viral DNA.

## Secretion of hepatitis C virus particles is dependent on glycoprotein stability conferred by the ion channel function of p7

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Hepatitis C virus (HCV) infects over 170 million people world-wide causing serious liver disease. Interferon / ribavirin therapy has limited efficacy due to a high level of innate viral resistance, making the search for virus-specific drug targets a priority.

The development of an HCV culture system based on a genotype 2a isolate, JFH-1, provides the first opportunity to study HCV assembly. We identified the function of HCV p7 as an amantadine-sensitive ion channel and demonstrated its ability to protect influenza HA proteins from low pH during export, suggesting an equivalent role for p7 in the assembly of HCV virions. Here, we have investigated the effects of p7 null-mutants and p7 inhibitors in the JFH-1 background. Blocking p7 function prevented secretion of particles and p7 function could be *trans*-complemented; partially restoring infectivity. These producer cells were shown to contain a similar level of E2-p7 precursor to wild type virus, yet showed a pronounced decrease in the levels of mature E2 glycoprotein and p7 itself was undetectable; suggestive of a lack of protein stability following cleavage of the precursor. We propose that p7 functions to preserve HCV glycoproteins in a correctly folded state on virus particles, thereby facilitating their secretion.

## Activation of PI3-Kinase by the NS1 protein of influenza A virus

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Influenza A virus NS1 protein is a multifunctional virulence factor, and contributes significantly to disease pathogenesis by modulating virus replication, cell death, and host immunity. Previous work has identified a novel role for NS1 during infection: the direct binding and activation of phosphatidylinositol-3-kinase (PI3-Kinase), a cellular enzyme involved in the control of gene transcription, protein synthesis, cell survival and cytokine production. Specifically, NS1 binds the p85β regulatory subunit of PI3-Kinase, a function that requires Y89 of NS1. Y89 is totally conserved among all influenza A virus strains sequenced to date, including those of human and avian origin. Substitution of Y89 for phenylalanine (Y89F) prevents NS1 from binding p85β or activating PI3-Kinase signalling. Thus, a recombinant influenza A virus (Udorn strain) expressing NS1-Y89F exhibits a small-plaque phenotype, and grows more slowly in MDCK cells than wild-type virus. Here, we discuss recent work characterising the mechanism of PI3-Kinase activation by NS1. *In vitro* binding assays, domain mapping, and kinase activity experiments suggest a potential mode of action by which NS1 activates PI3-Kinase signalling.

## Unusual features of the Epstein–Barr virus lytic replication inducer – *BZLF1*

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A viral gene, *BZLF1*, is required to initiate Epstein-Barr virus (EBV) replication. The product of *BZLF1*, Zta, is a transcription factor for viral and host genes and a replication factor for viral DNA. Zta is an atypical member of the bZIP protein family, both with respect to its dimerisation domain and its DNA-binding specificity. The dimerisation domain contains the expected coiled coil motif, adjacent to a unique region. We and others have shown that the unique region folds to position itself in intimate contact with the coiled coil. This contributes to the dimerisation, transactivation and replication functions of Zta. The second atypical feature of Zta is its preference for binding to

DNA-binding sites (ZREs) when they are methylated at CpG motifs. As the EBV genome is heavily methylated *in vivo*, this feature may be essential to allow transactivation of viral genes and replication of the genome. Analysis of the DNA-contact region of Zta identified a critical amino acid residue that allows differentiation between a methylated and a non-methylated ZRE. Furthermore, mutation of this residue prevents transcriptional activation of the endogenous viral gene and dramatically reduces viral replication.

## The viral polymerase complex confers replication-competence of Borna disease virus in a new host species

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Borna disease virus (BDV) can persistently infect the central nervous system of a broad range of mammalian species. Mice resist infections with primary BDV isolates but certain laboratory strains can be adapted to replicate in mice. We determined the molecular basis of adaptation by studying mutations that a cDNA-derived BDV strain had acquired during one passage in rats and three passages in mice. The adapted virus propagated efficiently in mice and induced neurological disease. Its genome contained seven point mutations of which three caused amino acid changes, namely two changes (L1116R and N1398D) in the L polymerase and one change (R66K) in the polymerase cofactor P. Recombinant BDV carrying these mutations either alone or in combination all showed enhanced multiplication speed in Vero cells, indicating a general improvement of viral polymerase activity. Mutations R66K and L1116R, but not N1398D, conferred replication-competence of recombinant BDV in mice. Combination of both L mutations enhanced virus propagation, but infection remained largely non-pathogenic. Inclusion of all mutations drastically enhanced BDV virulence and resulted in early disease induction and lethality in mice. The virulence-enhancing effect of the R66K mutation in P was due to reduced negative regulation of polymerase activity by X, a small non-structural viral protein. Our data demonstrate that replication-competence of BDV in a new species is mediated by changes in components of the polymerase complex rather than the viral envelope. They further suggest a role of virus-encoded negative regulator X in this process.

## In HIV-2, a dimeric genome is required for infectivity and particle maturation

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The packaging signal of Human Immunodeficiency Virus type-2 (HIV-2) is located upstream of the splice donor and hence is present on both spliced and unspliced RNA. However, only the latter is incorporated into virions by a co-translational mechanism. Interestingly, deletion of the packaging signal in this virus renders the RNA monomeric. Since the genomes of all retroviruses studied to date are dimeric in virion particles, we investigated whether a dimeric genome was a prerequisite for encapsidation in HIV-2. Here, we show that HIV-2 genomic RNA is dimeric in the virion as well as in the cytoplasm of infected cells, suggesting that dimerisation occurs at an early stage of viral replication. Furthermore, viruses with a disrupted dimer phenotype were packaged with much reduced efficiency and fail to establish an infection in T cells as compare to wild-type virus. Infectivity of dimerisation-deficient virus was also reduced and electron microscopy of these virus cores revealed a larger proportion of immature cores, suggesting that dimerisation may be important for particle maturation.

## Coronavirus replicase function: beyond RNA synthesis

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The coronavirus replicase gene encodes non-structural, replicative proteins that are expressed as fusion proteins by translation from the genomic RNA. Proteolytic processing results in the release of 16 processing endproducts termed nsp 1-16. Here we report coronavirus replicase gene-encoded functions that impact on virus pathogenesis. We targeted, by reverse genetics, the ADRP domain encoded in nsp3 and nsp1 function. Mouse hepatitis virus (MHV) ADRP mutants replicated to wild-type levels in the spleen, but were attenuated in the liver. The deletion in the C-terminal third of nsp1 resulted in a strong attenuation in mice which was, to a large extend, related to innate immune responses. Remarkably, the mutant virus did still grow in conventional DCs and induced IFN-· in plasmacytoid DCs. In mice, the mutant virus was rapidly cleared but it (i) replicated for 2 days in the spleen, (ii) induced a potent cytotoxic T cell response and (iii) protected against homologous and heterologous virus challenge. Our results provide a paradigm for the rational design of live attenuated coronavirus vaccines encoding altered replicase nsps.

## KSHV ORF57 mimics splicing by mediating the recruitment of hTREX to intronless viral mRNAs

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Recent studies have shown that mRNA nuclear export in humans is regulated not by the exon junction complex, but rather by a distinct, highly conserved set of proteins known as the transcription export complex (hTREX). Recruitment of hTREX is dependent upon both an intact 5' cap and splicing. In contrast to most human genes, analysis of herpesvirus genomes has highlighted that the majority of lytically expressed genes lack introns and as such do not undergo splicing. As herpesviruses replicate in the nucleus, this leads to an intriguing problem for the virus, namely how do the herpesvirus intronless mRNAs which do not undergo splicing get efficiently exported from the nucleus. The KSHV ORF57 gene product achieves this function by binding viral RNA, shuttling between the nucleus and cytoplasm, thereby promoting nuclear export of viral mRNAs. Here we show that ORF57 interacts with hTREX, leading to the assembly of the hTREX complex, not EJC components, on intronless viral mRNA. This process effectively mimics splicing allowing efficient export of viral intronless mRNAs.

## Arterivirus non-structural protein 1: the key to regulating a complex life cycle?

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The Arteriviruses, a family of plus-stranded, enveloped RNA viruses, are united with Coronaviruses and Roniviruses in the order Nidovirales. Their genomes are polycistronic and share a common organization, with the 5' two-thirds of the viral genome encoding the replicase polyprotein, which is processed into non-structural proteins by virus-encoded (auto)proteases. The viral structural genes are encoded in the 3'-end of the genome and are expressed via a unique mechanism of discontinuous RNA synthesis. For arteri- and coronaviruses this process results in the production of a nested set of subgenomic (sg) mRNAs that are 5'- and 3'-coterminal with the viral genome.

Non-structural protein 1 (nsp1) of Equine Arteritis Virus (EAV) – the prototype arterivirus – is a key factor for sg RNA synthesis but not essential for genome replication per se. Prominent features of this 29-kDa protein are two papainlike autoprotease domains (PCPa and PCPb), the latter one possessing autoproteolytic activity, and a predicted zinc finger domain close to its N-terminus. Amino acid substitutions of putative zinc-coordinating residues produced a complex pattern of phenotypes. Certain replacements had a similar effect to deleting the whole protein from the viral genome – sg RNA synthesis was completely abolished. Other substitutions, however, did not have a significant effect on sg RNA production, but resulted in a decrease of virus infectivity, or seemed to affect the ratio between amounts of genomic and sgRNAs accumulated in transfected cells. Analysis of second site revertants mapped suppressor mutations to the zinc finger and PCPa domains of nsp1, providing genetic support for an interaction between these subdomains. Detailed characterization of nsp1 mutants in terms of viral RNA and protein synthesis levels, as well as nsp1 oligomerization, was performed.

## A role for transportin in nuclear import of adenovirus core proteins and DNA

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Efficient delivery of the viral genome into the cell nucleus is a feature of many viral infections including adenovirus. Adenoviruses target their dsDNA genome to the interphase nucleus, entering through the nuclear pore complex. We have used digitonin permeabilised cell import assays to study the cellular import receptors involved in entry of the virus DNA and associated core proteins, protein V and protein VII, into the nucleus. We show that inhibition of transportin results in aberrant localisation of protein V and inhibition of protein VII and DNA import whereas disruption of the classical importin alpha-importin beta mediated pathway has little effect. Import can be restored in import reactions to which transportin has been added. In addition we demonstrate that transportin is necessary for protein V to localise to the nucleolus. Our results point towards transportin being an important import receptor for the adenovirus core.

## Exceptional strain of influenza A virus with high virulence in mice carrying a functional *Mx1* resistance gene

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It is still poorly understood how influenza A viruses (FLUAV) can become highly pathogenic in immunocompetent hosts. The interferon (IFN)-induced resistance factor Mx1 is a main component of innate immunity against FLUAV in mice. Animals carrying a wild-type *Mx1* gene ($Mx1^{+/+}$) differ from regular laboratory mice ($Mx1^{-/-}$) in being highly resistant to infection with standard FLUAV strains. We identified an extraordinary variant of the FLUAV strain PR8 (H1N1) (designated hvPR8) which is unusually virulent in $Mx1^{+/+}$ mice. hvPR8 was well controlled in $Mx1^{+/+}$ but not $Mx1^{-/-}$ mice if the animals were treated with IFN before infection, indicating that hvPR8 exhibits normal sensitivity to growth restriction by Mx1. hvPR8 multiplied much faster than standard PR8 due to highly efficient viral gene expression in infected cells at early times post infection. Studies with reassortant viruses containing defined genome segments of hvPR8 and standard PR8 demonstrated that the HA, NA, PB1 and PB2 genes of hvPR8 all contributed to virulence, indicating that efficient host cell entry and early gene expression renders hvPR8 highly pathogenic. These results reveal a surprising simple concept of viral virulence gain which is based on high multiplication speed that may prevent a timely antiviral response in the infected host.

## Interaction between hepatitis C virus core protein, cellular DDX3 and splicing factor SF2/ASF

**Richard Adair**, David Dalrymple, Allan Angus & Arvind H. Patel

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Hepatitis C virus (HCV) core protein binds to many host cell proteins, including the RNA helicase DDX3, which it redistributes in normal Huh-7 cells, cells harbouring full-length viral genomic replicons and cells infected with HCV strain JFH-1. We have identified a set of fully conserved 6 core residues which are critical for binding and sequestering DDX3. Here a detailed analysis of the mutants at the level of amino acid sequence, virus particle assembly, viral RNA replication, and infection will be presented. We also report that HCV core interacts with, and redistributes, the essential cell splicing regulator protein, Splicing Factor 2/Alternative Splicing Factor (SF2/ASF). Importantly, SF2/ASF has additional roles in stimulating translation and RNA stability since it shuttles continuously between the nucleus and cytoplasm. We show by immunoprecipitation that SF2/ASF interacts with DDX3 and this interaction is enhanced in HCV infected cells. Using immunofluorescence we show that HCV core co-localises with and sequesters SF2/ASF in infected cells and describe studies to investigate the influence of HCV infection on cellular splicing.

## Replication of rabies virus, structure of its nucleocapsid

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Negative strand RNA viruses such as rabies virus have their RNA totally covered by the viral nucleoprotein. The viral RNA-dependent RNA polymerase binds to these N-RNA complexes through the viral phosphoprotein and uses the protein-RNA complex as the matrix for transcription and replication. The N-RNA complexes are helical and very flexible making it impossible to study them by structural biology techniques. When the nucleoprotein is expressed alone in insect cells, it binds to cellular RNAs and forms long complexes (like viral N-RNA) when bound to long RNAs or closed circular complexes when bound to short RNAs. Depending on the length of the residing RNA there are between 8 and 15 N-protomers per ring. We were able to prepare pure fractions of these rings containing only one ring size and could crystallize the pure complexes. The structure of the complex shows that N binds to the phosphate sugar backbone and totally covers the RNA, allowing no access of the polymerase to the RNA in this conformation. Presently we are studying whether the binding of the phosphoprotein to the N-RNA can induce a (partial) conformational change in the nucleoprotein, releasing the RNA.

## Adenovirus late gene expression: a controlling role for L4 proteins

Keith N. Leppard

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The basic structure of the adenovirus gene expression temporal cascade was established many years ago in studies of two closely related human adenoviruses, serotypes 2 & 5. Initially upon entry to the cell nucleus, only the E1A gene is expressed. This provides proteins that activate the remaining viral early genes, as well as a number of host genes, to provide an intracellular environment in which viral DNA replication can occur. During this early phase of gene expression, the 28 kbp viral major late transcription unit (MLTU) is weakly active, encoding just one protein from its L1 region, but

after the onset of DNA replication the promoter is upregulated and the pattern of RNA processing changes progressively, ultimately achieving regulated expression of around 15 proteins from MLTU regions L1 – L5. The MLTU L4 region is expressed very early in this activation process and recent work has shown that L4 proteins play a critical role in achieving full MLTU expression. The L4 33K protein serves as a viral splicing factor that is required for the production of several late mRNAs while the multifunctional L4 100K protein regulates translation among other activities. The L4 22K protein, only recently shown to exist, plays a role in DNA packaging but may also act to regulate MLTU expression. Evidence for these L4 functions will be discussed.

## Early steps in the life cycle of hepatitis B virus

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Our knowledge of the viral life-cycle is still limited. In the past, the lack of a feasible *in vitro* infection system for HBV has hampered elucidation of HBV structures involved in viral binding and entry. Despite the high species specificity of HBV, primary hepatocyte cultures from Asian tree shrews, *Tupaia belangeri* could be efficiently infected with this virus, providing an invaluable model for HBV infection studies. Using a system that allows analysis of interaction of the viral envelope with cell surface structures, we were able to determine cell surface heparan sulfate proteoglycans serving as low affinity receptors for HBV. Mapping of neutralising epitopes and competing viral lipopeptides revealed a stretch of 10 aminoacids of the large HBV surface protein (LHBs) to be essential for viral entry. We suggest that HBV is trapped within the liver in the space of Dissé by heparan sulfate proteoglycans that serve as low affinity binding sites. Thereafter, HBV binds via its preS1 attachment site and the N-terminal myristic acid located within the LHBs to a yet unknown high affinity receptor. The S-domain, although non-essential for high affinity attachment, is important for further steps e.g. for fusion of the viral envelope in a yet undefined compartment.

## Papillomaviruses; molecular aspects of virus replication from entry through to exit

John Doorbar

Division of Virology, MRC National Institute for Medical Research, London

Papillomaviruses infect skin and mucosal epithelium. Typically they cause harmless lesions such as warts and verrucas, but certain types cause only inapparent infections, while others can be associated with the development of cancers. High-risk human papillomavirus (HPV) types cause most of the half million or so cases of cervical cancer that occur each year, while the low-risk HPV types cause genital warts.

To replicate at epithelial surfaces such as the skin, the papillomavirus genome must become resident in dividing basal cells in the lowest epithelial layers. Only as these cells leave the basal layer and are pushed towards the epithelial surface are events in the virus productive cycle triggered. How these events are controlled is still only poorly understood, but it is starting to become clear how the viral proteins can work together to subvert the normal differentiation programme of the skin. Different HPV types have subtly different strategies for completing their life cycle at the sites that they infect. The ability of some HPV types to undergo abortive rather than productive infection appears to underlie the development of many HPV-associated cancers. Such basic research is now beginning to have an impact on the treatment and management of HPV-associated disease.

## Picornaviruses: Uncapped viral mRNA stability, translation and replication

Brian J. Kempf, Benjamin P. Steil, Hannah Townsend, Jian-Qiu Han & **David J. Barton**

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Poliovirus (PV), the prototypic group C enterovirus in the *Picornaviridae* family, possesses a single-stranded RNA genome. Like all positive-strand RNA viruses, PV RNA is translated and replicated in the cytoplasm of host cells. My laboratory exploits cell-free reactions containing cytoplasmic extracts from uninfected HeLa cells. When programmed with PV RNA these reactions faithfully support all of the intracellular metabolic steps of replication, culminating in the assembly of infectious virus (Molla *et al.*, 1991; Barton *et al.*, 1993). These reactions are advantageous because they support the synchronous and sequential translation and replication of PV RNA allowing for detailed kinetic analyses of particular steps of replication. Using these reactions we recently examined de novo polysome formation and discovered mechanisms by which naturally uncapped PV mRNA evades degradation by cellular 5' exonuclease (Xrn1). We also elucidated mechanisms by which the viral protein of the genome (VPg) and uridylylated forms of VPg ($VPgpUpU_{OH}$) prime the initiation of viral negative- and positive-strand RNA replication. Our investigations reveal concerted strategies by which viral RNA and proteins redirect host cell components from cellular purposes to those of viral gene expression and replication.

# Prize Lectures



## Fleming Prize Lecture

*The Fleming Lecture is awarded for outstanding research by a microbiologist in the early stages of their career.*

### Mining microbial genomes for new natural products and biosynthetic pathways

Gregory L. Challis

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Bioinformatics analyses have identified gene clusters encoding cryptic or orphan secondary metabolite biosynthetic pathways, not associated with the production of known metabolites, in numerous microbial genome sequences. An overview of different approaches for the discovery of the products of such gene clusters will be presented. The development of sequence analysis tools that predict the substrates of enzymes encoded by cryptic gene clusters will be described. An example of how such tools can be applied to predict the physicochemical properties and biological function of putative metabolic products of cryptic gene clusters will be discussed. A gene knockout / comparative metabolic profiling approach has been developed to identify the products of cryptic gene clusters. Application of this approach to the discovery of the products of cryptic gene clusters identified in *Streptomyces* genomes will be described. The biosynthetic pathways and biological functions of several metabolites discovered by this approach will be discussed. The lecture will conclude with a discussion of the potential for genome mining to usher in a second 'golden age' of novel bioactive microbial natural product discovery.

Greg Challis obtained a BSc in Chemistry from Imperial College London and a DPhil in Organic Chemistry at the University of Oxford. In 1998 he was awarded a Wellcome Trust International Prize Travelling Research Fellowship to undertake postdoctoral research in the Department of Chemistry at Johns Hopkins University in Baltimore, USA. He returned to England in 2000 to carry out postdoctoral research on the same fellowship in the Streptomyces group at the John Innes Centre in Colney, Norwich. The following year he was appointed as Lecturer in Chemical Biology in the Department of Chemistry at the University of Warwick and began his independent research programme at the interface of Chemistry and Microbiology. In 2003 he was promoted to Senior Lecturer and in 2006 he was promoted to his current position of Professor of Chemical Biology. Challis was the recipient of the 2002 Meldola Medal and Prize of the Royal Society of Chemistry.

## Colworth Prize Lecture

*The Colworth Prize Lecture is awarded for an outstanding contribution of importance in applied microbiology.*

### Look who's talking: communication and co-operation in the bacterial world

Professor Paul Williams

University of Nottingham

Paul is currently Professor of Molecular Microbiology in the School of Molecular Medical Sciences and Director of the Institute of Infection, Immunity & Inflammation at the University of Nottingham. He graduated in Pharmacy at Nottingham in 1979 and did a PhD in microbiology with Mike Brown at the University of Aston in Birmingham. After a short postdoc at Aston, he moved back to Nottingham in 1985 as a lecturer in the department of Pharmaceutical Sciences and was promoted to Professor in 1995. Paul's research interests primarily focused on the molecular basis of bacterial pathogenicity but a chance observation in the early 1990s that carbapenem antibiotic biosynthesis in the plant pathogen *Erwinia* re-directed his focus to the study cell-to-cell communication (quorum sensing) in diverse bacteria of biotechnological, agricultural and medical importance. This work in collaboration with colleagues in the UK and overseas has resulted in the discovery of new signalling molecules and communication strategies not just between bacteria but between bacteria and higher organisms. Consequently this has created new opportunities for developing novel therapeutics and in particular antibacterial and immune modulatory agents.

# Posters

## Cells & Cell Surfaces Group

### CCS 01 The *Escherichia coli* β-propeller protein NanM (YjhT) is a novel monosaccharide mutarotase involved in sialic acid utilisation

**Emmanuele Severi**[1], Axel Müller[1], Jennifer Potts[1], Andrew Leech[3], Keith S. Wilson[2] & Gavin H. Thomas[1]

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Bacteria use sialic acid for a number of purposes, including nutrition and LPS modification, all of which depend on high-affinity uptake systems. In *Haemophilus influenzae* the sialic acid transporter genes *siaPQM* cluster with the genes required for sialic acid catabolism. Directly downstream of *siaPQM* is an uncharacterised gene, *HI0148* that encodes a periplasmic protein that has been suggested to be involved in sialic acid utilisation, and that has orthologues in many other sialic acid-utilising bacteria.

In this work we report the functional and structural characterisation of the NanM (YjhT) protein from *E. coli*, an orthologue of the *HI0148* gene product. We provide evidence that NanM, a 6-bladed β-propeller protein that forms dimers in solution, is a novel monosaccharide mutarotase that accelerates the equilibration rate between the α and β anomers of sialic acid. We also discuss the possible roles that enzyme-catalysed mutarotation might have in the physiology of sialic acid-utilising bacteria.

### CCS 02 Characterisation of a novel galactose transporter from *Escherichia coli*

**R.S.P. Horler**, A. Muller, J.R. Potts & G.H. Thomas

Dept of Biology, University of York, York YO10 5YW

There are two known galactose transporters in *E. coli* a MFS Symporter and an ABC transporter. Using tryptophan fluorescence spectroscopy and mass spectrometry we show the existence of an additional ABC transport system for galactose. Characterizing the extracytosolic solute receptor (ESR) of this system using NMR and X-ray crystallography revealed that the ligand is the rarer furanose form of galactose, present at 7% in solution. The system has homologues in free living soil bacteria and in several human pathogens, indicating a potentially wide range of organisms that can utilize galactofuranose during growth.

### CCS 03 Structural and functional studies of the novel TRAP family of transport proteins

**Christopher Mulligan**[1], Peter Henderson[2] & Gavin H. Thomas[1]

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Tripartite ATP-independent periplasmic (TRAP) transporters are widespread in prokaryotes and consist of an extracytoplasmic solute receptor (ESR) and two unequally sized membrane proteins, their novelty being a method of substrate transport that uses an ESR in conjunction with the membrane potential.

Only a few of these transporters have been studied, including the $C_4$-dicarboxylate transporter from *Rhodobacter capsulatus* and a sialic acid transporter from the human pathogen *Haemophilus influenzae*. The ESR has been subjected to the most research, leaving the membrane components relatively unstudied.

The membrane components of the 2,3-diketo-L-gulonate TRAP transporter from *Escherichia coli* and the sialic acid TRAP transporter from *H. influenzae* have being overexpressed and purified for biochemical and biophysical studies, with the aim of elucidating the protein-protein interactions between the membrane components and also their interactions with the ESR.

### CCS 04 How the expression phase of *Escherichia coli* outer membrane protein Ag43 is determined and inherited?

**Renata Kaminska** & Marjan van der Woude

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Ag43 is an autotransporting, self-recognising adhesin involved in cell aggregation and biofilm formation. It is expressed in a phase variable manner that depends on methylation pattern of Ag43 promoter region. The methylation reaction is conducted by Dam methylase that acts as an activator of Ag43 expression. Dam recognition sequences overlap with a binding site of global regulator OxyR. When OxyR binds first it prevents methylation and represses transcription of Ag43 encoding gene *agn43*. This regulation is epigenetic as it is reversible but heritable and does not involve changing in DNA sequence.

It remains unknown how DNA methylation state is inherited. We have previously shown that Dam can methylate and OxyR can bind DNA that became hemi-methylated as a result of replication event. They both modulate affinity of sequestration protein SeqA to Ag43 regulatory region in vitro. Here we use both *in vitro* and *in vivo* approaches to analyse the sequence of events that lead to maintenance and switch of Ag43 expression phase after DNA replication.

### CCS 05 The BacA protein and lipid A are important for the chronic persistence of *Sinorhizobium meliloti*

**Victoria L. Marlow**[1], Mustapha Aouida[2], Sebastian Beck[1], Euan James[3], Dindial Ramotar[2], Graham C. Walker[4] & Gail P. Ferguson[1]

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*Sinorhizobium meliloti,* a legume symbiont requires the *bacA* gene which encodes a putative inner membrane protein for persistence. Although the precise function of BacA is unknown, *S. meliloti bacA* mutants display an array of phenotypes including a low level resistance to the glycopeptide bleomycin and increased sensitivity to detergents. The latter phenotype led us to discover that BacA affects an unusual fatty acid lipid A modification. Subsequent analysis revealed that this unusual lipid A is important, but not crucial for host persistence. This suggested that loss of BacA may result in further cell envelope alterations and additional host-induced lipid A changes may be occurring. We are currently characterizing candidate genes which may be involved in possible host-induced lipid A changes. We recently discovered that the bleomycin

resistance phenotype of the *bacA* mutant is independent of the unusual lipid A. Using a *recA* mutant and transport studies we have shown bleomycin is able to enter *S. meliloti*, even in the absence of BacA, suggesting a BacA-independent mechanism of uptake. We have shown polyamines and glutathione protect against bleomycin damage and this protection is independent of BacA. Thus, we present a model for the potential role of BacA in the persistence of *S. meliloti*.

## CCS 06 The piezophilic and psychrophilic bacterium, *Photobacterium profundum* SS9, adapts its lipopolysaccharide in response to temperature and pressure

**D. Allcock**[1], **T. Tryfona**[1], F. Lauro[2], D. Bartlett[2], R. Carlson[3] & G.P. Ferguson[1]

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*Photobacterium profundum* is a marine bacterium capable of growing over a range of temperatures (2-20°C) and pressures (0.1-70MPa) and therefore can be used as a model organism for high pressure and low temperature adaptation. Our results indicate that *P. profundum* can adapt its lipopolysaccharides (LPS) in response to changing environmental conditions. In this study we are investigating the composition of *P?. profundum* LPS and identifying fatty acid and carbohydrate alterations. *P. profundum* putative LPS mutants were found to be sensitive to cold temperatures and had altered growth under elevated pressures. SDS-PAGE analysis of the above mutants indicated altered LPS phenotypes when compared to the parent strain. Studying the specific alterations in the LPS composition of the putative mutants will lead to a better understanding of the molecular basis of cold-temperature and high-pressure adapted growth of *P. profundum* SS9.

## CCS 07 Investigating a putative *hcp* mutant of the deep-sea bacterium *Photobacterium profundum* SS9

**Marc Gillingwater**[1], Federico Lauro[2], Douglas Bartlett[2] & Gail P. Ferguson[1]

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Over 70 % of the Earth's surface consists of oceans at an average pressure of 38 MPa and temperature of 1-3°C, yet very little is known about deep-sea microorganisms. *Photobacterium profundum* SS9 is a pressure- and cold-loving gammaproteobacterium that grows optimally at 15°C (28 MPa). It is capable of growth at 2–20°C and 0.1–70 MPa, making it an idea model organism for the study of pressure- and cold-adapted growth. A *P. profundum* SS9 mutant strain with a mini-Tn*5* insertion in a putative haemolysin-coregulated protein (*hcp*) gene was isolated from a screen of transposon mutants deficient in growth at 4°C. Analysis of the putative *hcp* mutant revealed a cold sensitivity in both liquid and on solid media. Additionally, microscopy suggested this strain exhibited greater motility than the parent strain and this was confirmed by analysis of swimming motility on low percentage agar. Preliminary experiments also suggest the putative *hcp* mutant exhibits an altered biofilm and this is currently being investigated further.

## CCS 08 Characterising the role of exopolysaccharides in *Sinorhizobium meliloti* biofilms

**Gary Dorken**[1], **Sarah Spragg**[1], Graham C. Walker[2], Wilson Poon1 & Gail Ferguson[1]

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Biofilms are clusters of surface-attached bacteria that are encased in a matrix of exopolysaccharides (EPS). To further understand the role of EPS in biofilm formation and structure we are studying *Sinorhizobium meliloti*, an agriculturally important legume symbiont that fixes nitrogen into ammonia for its plant host. *S. meliloti* forms three distinct EPS molecules (succinoglycan, EPSII and K antigen), which have well-characterised biosynthesis pathways and structures. We are investigating biofilms using the classic crystal violet staining assay and confocal microscopy of GFP-expressing stains. A mutant that is overproducing succinoglycan has been shown to have altered crystal violet staining and confocal microscopy suggests this mutant forms a more compact biofilm that attaches to surfaces more readily. We are currently studying other EPS mutants of *S. meliloti*. Finally, analysis of biofilms is now being further extended to investigate the susceptibility of biofilms to antibiotics, beginning with studies on *Escherichia coli*.

## CCS 09 Iron acquisition by *Rhodococcus equi*

**Raúl Miranda-CasoLuengo** & Wim G. Meijer

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Despite iron being an essential requirement for life, $Fe^{3+}$ is highly insoluble under physiological conditions ($10^{-18}$ M), making iron scarce in all environments. Intracellular pathogens such as *Rhodococcus equi* face a greater challenge as they must acquire iron from their hosts. Thus, bacteria have evolved iron acquisition systems relying on secretion and uptake of siderophores, small molecules with high affinities for $Fe^{3+}$.

We previously showed that the expression of the IupABC transporter system was iron-regulated and involved in iron acquisition during the saprophytic life of *R. equi*. Here we show that mutation of *iupA* imposes a permanent condition of iron starvation leading to the secretion and accumulation of a catecholate-siderophore even under iron-replete conditions. UV/Vis and mass spectroscopy were used to characterize this siderophore. Genes thought to be involved in siderophore biosynthesis were targeted using the suicide plasmid pAP1. A mutant, SID3, which did not produce the siderophore, showed high sensitivity to iron limitation and its growth was restored by cross-feeding with the supernatant of wild-type culture grown under low-iron conditions.

## CCS 10 Transport of hydroxamate siderophores in *Sinorhizobium meliloti*

**Damien Keogh**, Paraic Ó Cuiv, Peter Collins, Paul Clarke & Michael O'Connell

School of Biotechnology, Dublin City University, Dublin 9, Ireland

Iron is a critical element for the growth and metabolism of rhizobia in both the free living state and in symbiosis. Iron acquisition mediated by hydroxamate siderophores has been detected in species of the rhizobia. The FhuCDB system of *Escherichia coli* has served as the model ABC transport system for the utilisation of hydroxamate siderophores in gram negative bacteria. However, *Sinorhizobium meliloti* is distinguishable in that it does not encode a FhuCDB system and must utilise hydroxamate siderophores by a novel process.

The *hmuPSTUV* region of the *S. meliloti* genome has been shown to be involved in haem uptake. By mutagenesis we have demonstrated that HmuU, a permease and HmuV, an ATPase, also function in hydroxamate siderophore utilisation. We have identified *smc01659* as encoding the periplasmic binding protein that forms an ABC transport system with HmuU and HmuV for hydroxamate siderophores. Heterologous expression of *smc01659*, *hmuU* and *hmuV* in an *E. coli fhuC* mutant restored the ability to utilise hydroxamate siderophores. Thus, utilisation of hydroxamate siderophores and haem compounds is effected by a split-ABC transport system.

## CCS 11 The AmtB-GlnK complex of *Escherichia coli*

Anne Durand[1], **Mike Merrick**[1], Matthew Conroy[2], Per Bullough[2], Domenico Lupo[3], Xiao-Dan Li[3] & Fritz Winkler[3]

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Amt proteins are found in all domains of life and encode high-affinity ammonia channels that facilitate the influx of ammonia into cells under conditions of ammonium limitation. In eubacteria and archaea they are almost invariably co-expressed with GlnK which is a member of the PII signal transduction protein family. PII proteins act as sensors of intracellular nitrogen status and regulate the activities of a wide range of proteins by protein-protein interaction. We have previously demonstrated that in *E. coli* GlnK is sequestered to the inner membrane by AmtB in response to an excess of extracellular ammonium; that this process is rapid and reversible; and that GlnK apparently inhibits ammonia flux through AmtB.

We now report the purification of the AmtB-GlnK complex from *E. coli*, the analysis of the role of effectors in controlling association and dissociation of the complex in vitro, and the solution of the X-ray crystal structure of the complex to a resolution of 2.5 Å.

## CCS 12 The novel ammonium transport protein of *Leptospira interrogans*

**Elizabeth Scanlon**, Lucy Foulston & Mike Merrick

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Amt proteins are a ubiquitous group of membrane-localised channels for the conduction of ammonia, found in organisms ranging from bacteria to humans. The recently sequenced genome of the pathogenic spirochaete *Leptospira interrogans* contains two potential Amt homologues, one of which appears to encode a hybrid protein between Amt and the intracellular domain of a chemoreceptor. This protein could therefore have the novel capacity both to conduct ammonia and to communicate its availability directly to the chemotaxis machinery. The protein has been expressed as a His-tagged fusion in *Escherichia coli,* where it is localised to the membrane, and in *Saccharomyces cerevisiae.* $^{14}$C-methylammonium uptake assays in *E. coli* and growth complementation assays in *S. cerevisiae* have been used to examine the protein's functionality as an ammonia channel.

## CCS 13 Functional conservation in *Streptococcus pneumoniae* of selected ABC and PTS transporters previously identified in *Streptococcus mutans*

Harpreet Kalsi, Alexander J. Webb & **Arthur H.F. Hosie**

Microbiology, King's College London Dental Institute, London SE1 9RT

Analysis of the *Streptococcus pneumoniae* genome sequences has indicated that this species is able to transport and metabolize a wide range of sugars. It has been proposed that this confers a fitness advantage, contributing to effective colonization of the nasopharynx and growth. However, many of the gene annotations are not supported by experimental evidence. A number of the predicted carbohydrate transporters in *S. pneumoniae* belong to the ABC superfamily, including a member of the carbohydrate uptake (CUT2) subfamily, which is annotated as a galactose transporter. However, this permease has identity to a ribonucleoside ABC transporter we have characterized in *Streptococcus mutans*, so this annotation may be misleading. Similarly, *S. pneumoniae* has a gene annotated as *ptsG*, a glucose specific PTS EII component. However, our data on the characterization of a putative orthologue in *S. mutans* indicates that this *S. pneumoniae* PtsG is most probably not a glucose specific PTS. We present the phenotype of *S. pneumoniae* strains mutated in the CUT2 ABC and putative *ptsG* genes and compare it with our previous findings for the orthologues in *S. mutans.*

## CCS 14 Carbohydrate uptake by ABC transporters in *Streptococcus pneumoniae*

**Kevin Crawford**, Alexander J. Webb, Karen A. Homer & Arthur H.F. Hosie

Microbiology, King's College London Dental Institute, London SE1 9RT

Analysis of the *Streptococcus pneumoniae* genome sequences have indicated that this species may transport and metabolise a wide range of sugars. It has been proposed that this confers a fitness advantage, contributing to effective colonisation of the nasopharynx and growth. A number of the predicted carbohydrate transporters in *S. pneumoniae* belong to the ABC superfamily. We have mutated the *S. pneumoniae* genes encoding components of the carbohydrate uptake (CUT1) subfamily of ABC transporters. Phenotypic analysis of the resulting mutants, including growth and fermentation assays with a range of carbohydrates, indicate that a single ATPase interacts with multiple membrane complexes with specificity for different carbohydrates. The ATPase is essential for the uptake of raffinose, stachyose and maltotetraose, which are transported by two separate ABC membrane complexes, MalXCD (maltotetraose uptake) and RafEFG (raffinose and stachyose uptake). The specificities of the other ABC transporters of the CUT1 family are being investigated. It is typical for the component subunits of ABC transporters to interact exclusively in distinct transport complexes so it is unusual that the single CUT1 ATPase in *S. pneumoniae* associates with multiple complexes to form active transporters.

## CCS 15 Carbohydrate uptake by ABC transporters in *Streptococcus mutans*

**Alexander J. Webb**, Karen A. Homer & Arthur H.F. Hosie

Microbiology, King's College London Dental Institute, London SE1 9RT

*Streptococcus mutans* is one of the dominant etiological agents of dental caries. Carbohydrate uptake and metabolism are central to its pathogenesis, as the lactic acid produced as an end product of fermentation contributes to caries. However, many aspects of *S. mutans* physiology and carbohydrate metabolism are incompletely understood. *Streptococcus mutans* has a large number of transporters of the ATP-binding cassette (ABC) superfamily, including two members of the carbohydrate uptake 1 (CUT1) subfamily. One of these, Msm, which is encoded by the multiple sugar metabolism operon, has been previously characterised. However, although it is annotated as a maltodextrin (Mal) transporter due to its similarity to the maltose permease of *E. coli*, the function of the second CUT1 transporter in *S. mutans* is unknown. We report the phenotypic analysis of mutants with the genes encoding either the Msm or Mal ABC transporters inactivated. These data indicate that Msm and Mal transport distinct carbohydrates. However, the data also indicate that some components of these two related ABC transporters are interchangeable.

## CCS 16 Capsular polysaccharide export in pathogenic *Escherichia coli* – the role of the KpsE and protein

**James Thompson**, Tom Hudson & Ian S. Roberts

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The biosynthesis and export of capsular polysaccharides in *Escherichia coli* provides a fascinating biological problem. How is a large negatively charged macromolecule synthesised and then exported across two membranes and the intervening peptidoglycan. Recently we have shown that the export of group 2 capsular polysaccharides involves a biosynthetic/export complex at the poles of the cell. The KpsE protein, essential for the movement of polysaccharide across the inner-membrane and periplasm, is anchored to the inner-membrane by an N-terminal trans-membrane domain. Site-directed mutagenesis of this region has shown important conserved amino acids and that the first 26 amino acids located in the cytoplasm appear to be dispensable. In addition we have been able to show that KpsE interacts with peptidoglycan and that this may be important in the export of group 2 capsular polysaccharides.

## CCS 17 Potential accessory factors for autotransporter secretion in *Pseudomonas aeruginosa*

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Autotransporters are the largest group of secreted proteins in Gram-negative bacteria, and many possess important roles in promoting virulence. Autotransporter proteins are assembled with a characteristic three domain structure; (i) the N-terminal Sec signal peptide for inner membrane translocation, (ii) the central passenger domain carrying the active component of the protein, and (iii) the C-terminal domain which forms a β barrel in the outer membrane necessary for secretion. Autotransporters were originally thought to contain the entire machinery for outer membrane translocation within themselves. However, an additional factor has been recently found which facilitates the secretion of these molecules, namely Omp85.

Prompted by the isolation of six independent mutants deficient in autotransporter secretion in *Escherichia coli*, we hypothesize that other accessory secretion factors exist. Genes with similar genomic contexts and high sequence homology to those inactivated in these mutants have been identified in *Pseudomonas aeruginosa*, an important human pathogen. In frame deletion of these genes was undertaken, and the impact on secretion assessed, to provide an insight into this important mechanism of secretion.

## CCS 18 The characterisation of an arsenic efflux pump in *Escherichia coli*

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Arsenic poisoning is a major problem worldwide with a possible 330 million people at risk of drinking arsenic-contaminated water and consequently developing an associated disease. The elucidation of the mechanism of bacterial resistance to arsenicals will provide a greater understanding of the control of intracellular arsenic levels, not only in bacteria but also in higher organisms, and this may lead to strategies to prevent the contamination of drinking water. Bacterial resistance to arsenicals is generally conferred by *ars* operons. In this study the *arsRDABC* operon which encodes an oxyanion-translocating efflux pump found on plasmid R773 in *Escherichia coli* was investigated with particular focus on the *arsDA* genes. *arsD* encodes a repressor protein and *arsA* encodes the ATPase subunit of the efflux pump. It has been shown that ArsD has an additional role as a metallochaperone, transferring the arsenical to the ArsA component of the pump. From recent experiments, we have evidence which suggests that these proteins may also have a role in plasmid segregation.

## CCS 19 Evaluation of single tat deletions on Tat complex assembly in *Streptomyces lividans*

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*Streptomyces lividans* contains homologues of *E. coli* TatA, TatB and TatC. Systematic analysis of these Tat components showed that TatA and TatB, but not TatC are individually dispensable for Tat-dependent secretion in *S. lividans*. In addition, the structural organisation of the membrane-embedded Tat proteins in *S. lividans* is described. Next to a 600 kDa complex in which all Tat subunits participate, a TatAB complex with varying size between 200 and 400 kDa and homo-oligomeric TatA complexes ranging from below 100 kDa to over 600 kDa could be isolated.

Here, we studied the role of each Tat component in the assembly and stability of *S. lividans* Tat complexes in the membrane. To this purpose, proteins present in membrane fractions from *S. lividans* wild type and single *tat* mutants were subjected to TatA- or TatBC-mediated ion exchange and subsequent gel filtration chromatography.

In the absence of TatA, TatB and TatC are still present in a high molecular weight complex of which the observed size is similar to that of the wild-type core TatABC complex. Similarly, TatA and TatC co-elute in a complex with a molecular mass of about 600 kDa in the absence of TatB. However, we observed some degradation of TatC suggesting that TatC stability is dependent on the presence of TatB. Finally, our data showed that that in a strain lacking the TatC component, TatA and TatB do not co-elute in one single peak corresponding to a molecular mass of about 600 kDa. Instead TatAB complexes have been found to elute over a wide range of fractions with corresponding molecular masses of over 600 kDa to less than 100 kDa

This study showed that in *S. lividans* TatB is necessary for stabilization of TatC whereas a key role in driving Tat complex assembly is suggested for TatC.

# Posters

## Clinical Microbiology Group

### CM 01 Antimicrobial susceptibilities of anaerobic bacteria isolated from the sputum of cystic fibrosis patients

**T.R. Field**[1], J.S. Elborn[2] & M.M. Tunney[1]

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Pulmonary infection is the leading cause of morbidity and mortality in patients with Cystic Fibrosis (CF). We have shown that anaerobic bacteria are present in significant numbers in ~60% of the sputum samples that we have examined. The susceptibility of anaerobes to the antibiotics ceftazidime, meropenem, tobramycin, piperacillin/tazobactam, metronidazole and clindamycin was examined by the agar dilution method according to NCCLS guidelines. Where possible the MIC of matching *P. aeruginosa* isolates was also determined. The concentration of tobramycin required to inhibit the growth of some anaerobic isolates was higher than that achievable clinically following IV administration. Surprisingly, the majority of anaerobes tested demonstrated resistance to metronidazole but were sensitive to clindamycin and piperacillin/tazobactam. Additionally, some strains demonstrated high levels of resistance to multiple antibiotics. There was no correlation between antibiotic resistance observed between matched anaerobe and *P. aeruginosa* strains. These results suggest that alternative antibiotic treatment regimens may be necessary to treat CF pulmonary infection if anaerobes are present.

### CM 02 Biofilm formation by anaerobic bacteria isolated from the sputum of patients with cystic fibrosis

**T.R. Field**[1], J.S. Elborn[2] & M.M. Tunney[1]

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Pulmonary infection, primarily with *Pseudomonas aeruginosa,* is a leading cause of morbidity and mortality in Cystic Fibrosis (CF) patients. We have isolated anaerobic bacteria from ~60% of the sputum samples that we have examined. Bacterial biofilms are surface attached communities encased in an exopolymeric matrix and are increasingly being recognised as a significant contributor to chronic and recalcitrant infections. The ability to form biofilms can therefore, be considered an important virulence factor for CF pulmonary infection. The ability of anaerobic bacteria, isolated from the sputum of CF patients, to form biofilms was assessed using crystal violet in a micro-titre tray assay. Isolates from the genus's *Prevotella, Veillonella, Propionibacterium* and *Actinomyces* were assessed. The majority of the isolates tested demonstrated the ability to form biofilms after 4 hours of attachment, with several strains forming substantial biofilms after as little as 24 hours. These results indicate that anaerobes present within the CF lung are capable of biofilm formation *in vitro.*

### CM 03 Anaerobic bacteria in acute exacerbations of cystic fibrosis pulmonary infection

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Respiratory disease is the main cause of mortality and morbidity in patients with Cystic Fibrosis (CF) with more than 95% of deaths due to respiratory failure. We have previously shown that anaerobes are present in the sputum of stable CF patients. The aim of this study was to quantify and identify obligate anaerobes in the sputum of CF patients experiencing acute exacerbation of pulmonary symptoms.

Samples were obtained from 27 CF patients on admission to Belfast City Hospital prior to intravenous antibiotic treatment and again at discharge. Samples were processed using strict anaerobic bacteriological culture techniques and identified by sequencing of the 16S rRNA gene. Anaerobes were detected in all sputum samples on admission with a mean of $7.1 \times 10^6$ CFU/g of sputum (range $6.0 \times 10^4$ to $2.3 \times 10^7$). On discharge, anaerobes were also detected in all sputum samples with a mean of $1.6 \times 10^7$ CFU/g of sputum (range $1.0 \times 10^4$ to $1.0 \times 10^8$). The anaerobes identified have included *Prevotella* species such as *denticola, nigrescens* and *melaninogenica, Veillonella* species such as *dispar* and *parvula* and other anaerobes such as *Propionibacterium acnes and Fusobacterium nucleatum.*

These results suggest that anaerobic bacteria are found in CF sputum in high numbers at both admission and discharge from hospital. Comparison of specific anaerobes in sputum at the onset of exacerbation and at discharge may indicate if anaerobes play a role in exacerbations of pulmonary symptoms of CF patients.

### CM 04 Rapid detection of MRSA in a routine diagnostic laboratory using a real-time PCR assay

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Rapid, specific and sensitive screening methods play a vital role in detecting MRSA in hospitals, with a number of commercial assays available for routine use. This study is the first reported in the United Kingdom to compare the use of one of these assays, IDI-MRSA™, a rapid real-time PCR based assay, with isolation and culture on MRSA-ID chromogenic agar for detection of MRSA in nasal and cutaneous axilla/groin swabs. Swabs were collected from 690 patients admitted to two wards in Antrim Area Hospital. In nasal swabs, 22/690 (3.2%) and 31/690 (4.5%) were positive by culture and IDI-MRSA™ assay, respectively. In axilla/groin swabs, 14/690 (2.0%) and 22/690 (3.2%) were positive by culture and IDI-MRSA™ assay, respectively. Sensitivities of 88.8% and 87.5% and specificities of 98.6% and 98.8% for nasal and axilla/groin swabs, respectively, were obtained. Including the axilla/groin swab in the routine screening protocol identified 7 patients as MRSA positive by PCR, (4 of whom were subsequently confirmed as positive by culture), in whom MRSA was not detected by culture or PCR of the nasal swabs. These results suggest that within a clinical setting, the IDI-MRSA™ assay is an invaluable tool in accurately identifying MRSA negative patients thus avoiding the unnecessary and costly implementation of infection control precautions; however, isolation by culture is still required for definitive identification of MRSA positive patients.

## CM 05 Characterisation of meticillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients pre-and post decolonisation

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Meticillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial infections. Colonisation, often the precursor to infection, can prove difficult to eradicate with many patients remaining MRSA positive despite repeated decolonisation attempts. This study aimed to characterise the MRSA isolated from successfully and unsuccessfully decolonised patients prior to and post decolonisation, in terms of strain types and changes in antibiotic susceptibilities. Restriction enzyme digestion followed by pulsed field gel electrophoresis (PFGE) was used to determine differences between isolates at a molecular level. To date, 22 isolates from 4 patients have been characterised by PFGE: no difference in the MRSA strain isolated pre and post decolonisation was observed in 3 of these patients. Further work examining differences in susceptibilities of these isolates to antibiotics commonly used in treatment of MRSA infection is ongoing.

## CM 06 *In vitro* comparison of tea tree oil and terpinen-4-ol against clinical isolates of meticillin-resistant *Staphylococcus aureus*

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Tea tree oil (TTO) contains more than 100 bioactive components. Of these components, terpinen-4-ol is thought to be the main antimicrobial. The aim of this study was to compare the antibacterial activity of terpinen-4-ol and TTO against clinical MRSA isolates. The antibacterial activity of terpinen-4-ol and TTO was analyzed by determination of $MIC_{50/90}$ and $MBC_{50/90}$ values for 30 MRSA isolates. Additionally, time kill assays were performed on 10 MRSA isolates grown planktonically, using both 1% v/v TTO and 1% v/v terpinen-4-ol. The $MIC_{50/90}$ was 0.125/0.25 % v/v for terpinen-4-ol and 0.25/0.5 % v/v for TTO, whilst the $MBC_{50/90}$ was 0.5/0.5 % v/v for terpinen-4-ol and 2.0/8.0 % v/v for TTO. Time kill assays showed that terpinen-4-ol produced total kill by two hours for all isolates analyzed (a seven $\log_{10}$ reduction in viable counts) compared to TTO which produced an average of a two $\log_{10}$ reduction in viable count after three hours. The results of this study have shown that terpinen-4-ol, a component of TTO, has greater bactericidal activity than TTO at equivalent concentrations.

## CM 07 A rapid colorimetric assay for antimicrobial susceptibility testing of staphylococcal spp. and extended-spectrum beta lactamases (ESBLs)

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The application of a colorimetric assay based on reduction of the tetrazolium salt XTT for rapid determination of susceptibility of *Pseudomonas aeruginosa* isolates to bactericidal antibiotics has previously been established. Since the development of XTT, additional tetrazolium salts, such as WST-8, have now been developed which are reported to have improved sensitivity. The aim of this study was to compare XTT and WST-8 in a rapid colorimetric based assay. A total of 32 *Staphylococcus* spp. and 30 ESBL-producing isolates were tested for susceptibilities to various antimicrobial agents using conventional and rapid colorimetric (XTT and WST-8) methods. Results with *Staphylococcus* spp. and XTT showed 100% essential agreement with conventional MIC values according to the BSAC guidelines, compared to 89% using WST-8. XTT displayed a smaller number (11 vs. 14) of very major errors than WST-8. Results for ESBL-producing isolates and XTT showed varying amounts of essential agreement, ranging from 100% (Ofloxacin) to 77% (Ceftazadime). These results suggest XTT is more accurate than WST-8 in rapid determination of MIC values. The high level of essential agreement between XTT and conventional MIC values suggests that XTT has potential for rapidly determining antimicrobial susceptibility in routine clinical practice.

## CM 08 Susceptibility of *Staphylococcus aureus* isolated from a retrieved cochlear implant

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Although cochlear implantation has become a safe and effective method for auditory rehabilitation of sensorineural hearing loss, infection of the implant may occur. The aim of this study was to investigate the susceptibility of an MSSA isolate cultured from an infected cochlear implant to commonly used antibiotics and to Tea-Tree Oil (TTO), and its principal antimicrobial component terpinen-4-ol, both of which have been previously shown to be effective against meticillin-sensitive Staphylococcus aureus (MSSA). The Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC) were determined according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines. The isolate was susceptible to all antibiotics tested with MIC values ranging from ≤0.5-1 µg/mL while MBC values ranged from 0.5-2 µg/mL. The isolate was also susceptible to TTO and terpinen-4-ol, with MIC values of 0.5% and 0.25% respectively. In addition, when the isolate was grown in biofilm for 24 and 72 hours, treatment with TTO or terpinen-4-ol resulted in complete biofilm eradication after 1 hour exposure. Results of this study suggest that in addition to conventional antibiotics, TTO and terpinen-4-ol display significant activity against an MSSA isolate in biofilm responsible for cochlear implant rejection.

## CM 09 Habituation to sub-lethal concentrations of tea tree oil (*Melaleuca alternifolia*) is associated with reduced susceptibility to antibiotics in human pathogens

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*Objectives* To investigate the effect of sub-lethal challenge with tea tree oil (TTO) on the antibiotic susceptibility profiles of significant human pathogens and commensals.

*Methods* The study compared the antibiotic susceptibility (Etest) patterns of *E. coli*, *S. aureus*, methicillin resistant *S. aureus* (MRSA) and *Salmonella* spp. after broth culture for 72 h in the presence or absence of sub-lethal concentrations of tea tree oil (TTO; 0.25%, 0.25%, 0.1%).

*Results* All habituated cultures (exposed to sub-lethal concentrations of TTO) displayed reduced susceptibility to a range of clinically relevant antibiotics compared to non habituated (control) cultures.

*Conclusions* Although tea tree oil may be an effective antimicrobial agent when appropriately used at bacteriocidal concentrations, its application at sub-lethal concentrations may contribute to the development of antibiotic resistance in human pathogens.

## CM 10 Characterisation of bacteraemic *Escherichia coli* isolates from St James's Hospital

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*Escherichia coli* is the leading cause of bacteraemia worldwide. Extra-intestinal pathogenic *E. coli* (ExPEC) has an enhanced ability to cause multitudes of extraintestinal infections including bacteraemia, neonatal meningitis and pneumonia. ExPEC displays a wide range of putative virulence factors and belongs predominantly in phylogenetic group B2. Currently, the specific characteristics of *E. coli* bacteraemia are incompletely defined, with limited data linking resistance and virulence. Previous studies show multidrug-resistant (MDR) *E. coli* are associated with lower virulence potential, with shifts away from phylogenetic group B2 towards groups A, B1 and/or D. Susceptibility of 222 isolates to a panel of antibiotics was determined using agar microdilutions following CLSI guidelines. Phylogenetic grouping and virulence factor analysis was established using triplex and multiplex PCRs respectively. High levels of resistance to penicillins and fluoroquinolones were detected. Intriguingly, the majority of MDR *E. coli* belonged to phylogenetic group B2. This study highlights accelerated spread of virulence genes within MDR *E. coli*. Development of pathogens with increased virulence potential adds further to already limited treatment options.

## CM 11 Elucidation of antibiotic resistance levels in *Pseudomonas aeruginosa*

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*Pseudomonas aeruginosa* is one of the most prevalent nosocomial pathogens found in hospitals. Its innate resistance to a wide variety of antibiotics represents a growing challenge in the treatment of infection. Data concerning the prevalence of resistance amongst *P. aeruginosa* strains in hospitals has been well reported in many European countries. *P. aeruginosa* is a major pathogen amongst cystic fibrosis (CF) patients. Ireland has the highest rate of CF per capita in the world. Therefore, data concerning the levels of resistance in *P. aeruginosa* is important.

This study was undertaken to determine the prevalence of resistance to Fluoroquinolones, Aminoglycosides, Cephalosporins and Polymyxin E (Colistin). MICs were determined by agar dilution according to CLSI and BSAC guidelines. Resistance was found to all antibiotics tested and significant levels of resistance were also found to the less commonly used antibiotic, colistin.

| | % Non-Susceptible (NS) | % NS EARSS 2006 quarter 1/2 |
|---|---|---|
| Ceftazidime | 6.12 | 9.7 |
| Ciprofloxacin | 10.20 | 14.1 |
| Gentamicin | 12.24 | 12.7 |
| Amikacin | 48.90 | |
| Levofloxacin | 8.60 | |
| Colistin | 92.00 | |

## CM 12 Implementation of an in-house typing method for multi-drug resistant *Acinetobacter baumanii*

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Following an outbreak of MDR A. baumanii in the intensive care unit at our hospital, we aimed to establish a rapid in-house MDR *A. baumanii* typing method for infection control purposes. Thirty-six strains isolated from different patients were investigated using random amplified polymorphic DNA (RAPD), repetitive extragenic palindromic sequence-based PCR (REP-PCR) and sequence-based typing of the adeB gene, and compared to Pulsed Field Gel Electrophoresis (PFGE). PFGE analysis showed that the SE Clone accounted for 50% of the isolates, 30% were the RFR Clone and 17% were the Oxa 23 Clone. RAPD showed the same pattern for SE and RFR clones and typing patterns were not reproducible for isolates of the Oxa-23 clone. No point mutations were detected in the sequences of the adeB gene, although it was not possible to sequence the entire gene. We are currently redesigning the primers to improve this assay. REP-PCR was highly discriminatory and has potential for rapid strain typing of MDR A. baumanii for infection control purposes.

## CM 13 Acquisition of ciprofloxacin resistance *in vitro* incurs no cost for *Burkholderia cepacia*

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It is often assumed that antibiotic resistant mutants are disadvantaged as compared to the wild type in the absence of the antibiotic. This study quantifies the cost of fluoroquinolone resistance mutations in *B. cepacia*. First and second step resistant mutants were selected *in vitro* and characterised by sequencing of the quinolone resistance determining regions of *gyrA*, *gyrB*, *parC* and *parE* and by MIC measurement. Fitness of these mutants was assessed using quantification of biofilm growth, planktonic growth, paired competition assays, survival in water and resistance to drying. Single mutations in gyrase A (Asp87Asn and Thr83ILe), conferring moderate increases in MIC (16 and 80 times, respectively) did not incur a measurable fitness cost. High level resistance (>256µg/ml), caused by a second step mutation in *parC* (Ser80Leu) resulted a decrease in relative growth rate (0.85 and 0.83 respectively) compared to parent but did not affect biofilm formation or survive in the environmental survival. As mutants with decreased susceptibility to fluoroquinolones do not necessarily exhibit a large fitness cost then this may allow populations of resistant bacteria to proliferate via clonal expansion in the absence of an antibiotic selective pressure.

## CM 14 Fitness of clinical isolates of *Burkholderia cepacia* complex

**C.F. Pope**[1], J. Pratten[2], S.H. Gillespie[1] & T.D. McHugh[1]

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Most *Burkholderia cepacia* complex (Bcc) infections in CF patients occur with *B. cenocepacia* (genomavar III) and *B. multivorans* (genomavar II). All Bcc bacteria form biofilms *in vitro* but *B. cenocepacia* and *B. multivorans* form the most extensive biofilms.

The aim of this study was to investigate whether clinical Bcc strains of *B. cenocepacia* and *B. multivorans* had differences in biological fitness. The predominance of these genomavars reflects enhanced adaptation to the environment and to the cystic fibrosis lung Models of the *in vivo* growth and survival conditions for *B. cepacia* were selected; quantification of biofilm growth, planktonic growth curve, survival in water and resistance to drying. Biofilm formation: A 96 well plate assay was adopted, quantification of biofilm growth was facilitated by spectrophotometric measurement of crystal violet binding. Planktonic growth: the method of Youmans and Youmans was adapted for use in the Bactec blood culture monitoring system.

Survival assays: Survival in sterile tap water and survival during dessication of the strains were assessed as indicators of the ability to survive in the environment.

Significant differences in fitness, measured as generation time and resistance to dessication, have been found between clinical Bcc isolates isolated from sputum from cystic fibrosis patients. Quantification of the propensity of the strains to form biofilms and to survive in water is ongoing.

## CM 15 Phage display library construction from metagenomic DNA

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The human oral cavity serves as a nutritionally rich environment for many different bacteria. Although approx. 60% of the species in this habitat are cultivable in the laboratory today, little is known about the adherence mechanisms used by these organisms to colonise the different ecological niches of the mouth.

The aim of this project was to identify novel bacterial adhesins present in human saliva using a culture-independent approach of phage display.

A phagemid library was constructed from salivary DNA cloned into the phagemid vector pG8H6, which was screened against immunoglobulin A (IgA) and bovine serum albumin (BSA) in a process called biopanning.

The peptide expressed by the IgA binding clone was found to be homologous to a conserved domain for a chloride channel protein from *Fusobacterium nucleatum*.

A further peptide sequence from a BSA control clone could be identified as a protein homologous to an immunoreactive antigen from *Porphyromonas gingivalis*.

Results indicate that membrane and surface proteins serve a range of purposes.

Additional work will be required to further investigate the role of these proteins in cell adhesion mechanisms.

## CM 16 Typing of methicillin-resistant *Staphylococcus aureus* from Belgium and Hungary, using spa repeat determination

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Methicillin-resistant *Staphylococcus aureus* (MRSA) was first isolated in European hospitals in 1960, soon after methicillin became available clinically. The prevalence of MRSA progressively increased thereafter. Now nosocomial infections caused by MRSA represent an increasing problem in hospital, while MRSA has also been emerging as a community pathogen. There are relatively few epidemic clones spreading globally that can be identified by molecular typing techniques. Such techniques could provide a sensitive and specific method for the detection and tracking of MRSA outbreaks, as well as benefiting therapy.

In this study, 25 isolates of MRSA collected in Belgium and Hungary were typed by *spa* gene repeat determination. Amongst the 25 isolates, 24 were typed into 12 groups showing 6 novel spa types; whereas 1, AUR40 (7004), was untypeable. In addition, 12 new repeat sequences have been identified in the 6 novel spa types. It was also realised that t002 was the most common *spa* type in the isolates collected in Belgium, whereas t045 and t030 were dominant in the isolates brought from Hungary.

## CM 17 Aggregate architecture and bacterial cell dormancy

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Dormancy, a reversible state of low metabolic activity, is an important factor in latency of *Mycobacterium tuberculosis*, the causative agent of tuberculosis. The RPF protein family is believed to be crucial in the resuscitation process of Mycobacteria from dormancy. To study the effects of micro-colony architecture and size on RPF accumulation and cell resuscitation in Mycobacterial aggregates, artificial micro-colonies of dormant *Mycobacterium smegmatis*, the fast growing relative of *Mycobacterium tuberculosis*, were made. Dormancy was induced in bacterial cell suspensions and methods for their resuscitation were established. As large differences exist in the dielectric properties of active, dormant and dead cells, their separation was achieved using a newly developed pulsed-field DEP-FFF separation system. Following this, the formation of aggregates of dormant cells was carried out by attracting them with positive dielectrophoresis to high field regions in an interdigitated-castellated electrode array, and subsequently immobilizing them using flocculants. Using a long-term cell feeding/resuscitation system, it was shown that resuscitation was quicker in larger aggregates than in smaller aggregates.

## CM 18 Investigating *Staphylococcus aureus* virulence-associated genes using microarray technology

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*Staphylococcus aureus* is a classical example of an emerging public health challenge. It causes a wide variety of infections, including several that are life threatening, and is one of the leading causes of nosocomial infection. Its transition from benign coloniser to pathogen is thought to be related to the presence of some, but not all, of the virulence genes maintained by the species.

A virulence-associated gene microarray for investigating *S. aureus* has been developed. The complete array comprises 750 oligonucleotide probes for genes that have a potential virulence association. Strains whose genomes have been sequenced were used to test the array. The results were verified through *in-silico* analysis.

Testing of a group of 85 clinical strains showed a high level of variation in the presence or absence of a subset of 100 genes. These results indicate that the microarray would be a highly discriminatory typing tool.

The microarray described here provides insight into the pathogenicity, epidemicity and evolution of *S. aureus* strains by providing profiles on individual gene content and repertoire of virulence-associated genes.

## CM 19 Characterisation of *Escherichia coli* interactions with plantain fibre: implications for Crohn's disease

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Crohn's disease is increasingly prevalent in westernised societies. *E. coli* has been implicated in triggering disease as these bacteria are more numerous in the mucosa of Crohn's disease patients than in

healthy subjects. These *E. coli* are able to adhere to colon cells in the laboratory, and this is inhibitable by soluble plantain fibre. Here we have shown that Crohn's disease associated *E. coli* are able to metabolise plantain fibre to the same degree as glucose, whereas all other major intestinal bacterial groups tested were unable to do so. Analysis of the composition of plantain fibre before and after bacterial growth will enable us to elucidate the mechanisms of bacterial degradation. We have also used confocal microscopy to show that these *E. coli* are able to adhere to plantain fibre. These results give further support to the hypothesis that bacterial interaction with soluble plantain fibre could prevent adherence of *E. coli* to the gut mucosa therefore preventing relapse of Crohn's disease.

## CM 20 Identification of the pandemic O3:K6 clone in cases of *Vibrio parahaemolyticus* related illness in the UK

Sariqa Wagley

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Cases of food poisoning attributed to *V. parahaemolyticus* are commonly reported in countries with both a high ambient temperature and where seafood is consumed raw. In Europe, *V. parahaemolyticus* infections are rare and sporadic, usually travel related, and often attributed to inadequate hygiene standards leading to the recontamination of cooked foodstuffs. However, recently several outbreaks have been reported in Europe associated with indigenously produced seafood. In 2004, 76 cases of *V. parahaemolyticus*-associated gastroenteritis were reported in Spain. Molecular and serological characterisation of isolates collected from patients during these outbreaks demonstrated the presence of the clinically significant O3:K6 pandemic clone.

In 2004, 3 cases of *V. parahaemolyticus*-associated gastroenteritis were identified in the U.K. Characterisation of these strains using pulsed field gel electrophoresis showed highly significant homology with the pandemic O3:K6 clone linked to Spanish outbreaks. This article demonstrates the presence of *V. parahaemolyticus* O3:K6 in the UK and supports the hypotheses that the recently emergent O3:K6 pandemic clone in continental Europe has also been responsible for isolated *V. parahaemolyticus* cases in the UK.

## CM 21 Humoral responses to the MTB12 antigen of *Mycobacterium tuberculosis* in pulmonary tuberculosis

**Se-Jin An**[1], Ji-Sook Lee[3], Chul-Su Yang[1], Sung-Soo Jung[2], Ji Woong Son[4], Jae-Hee Oh[1], Hwa-Jung Kim[1], Jeong-Kyu Park[1], Tae-Hyun Paik[3], Eun-Kyeong Jo[1] & Chang-Hwa Song[1]

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The *Mycobacterium tuberculosis* MTB12 protein plays an essential role in proinflammatory responses during the early stages of human pulmonary tuberculosis (TB), even though the T-cell immunoreactivity of MTB12 is weaker than that of the 30-kDa antigen. In this study, we evaluated the humoral immune responses and the potential diagnostic utility of the native MTB12 protein of *M. tuberculosis*.

*Methods* The anti-MTB12 IgG and IgM responses were determined in the sera of TB patients and healthy controls using an enzyme-linked immunosorbent assay (ELISA).

The assay sensitivity for MTB12 was 40.4% and the specificity was 94.8%. The assay sensitivity of the 30-kDa antigen was slightly higher than that for MTB12. However, the sensitivity increased to 52.6% when the combination of MTB12 and 38-kDa protein was assayed. Using ELISA, the mean IgG levels against MTB12 alone or MTB12 plus the 38-kDa antigen, but not for MTB12 plus the 30-kDa antigen, were significantly increased in the TB group two months after treatment for TB ($P < 0.05$).

Collectively, these data suggest that MTB12 alone, or in combination with the 38-kDa antigen, can be used to increase the accuracy of pulmonary TB diagnosis.

## CM 22 Molecular epidemiology and trends in antibiotic susceptibility pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates from major hospitals in Riyadh, Saudi Arabia

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Few studies have been done to report MRSA in Saudi Arabia and the level of its endemicity is still not documented for most facilities in the Kingdom.

*Objectives:* For 512 MRSA from 7major hospitals in Riyadh; 1- track the presence of MRSA strains in major hospitals in Riyadh, Saudi Arabia. 2- compare Matushek, standardized Canadian and standardized European techniques for pulsed field gel electrophoresis (PFGE) of MRSA. 3- perform comparative chromosomal DNA analysis of MRSA strains for epidemiological investigation using pulsed-field gel electrophoresis. 4- detect decreased susceptibility to vancomycin among the isolates. 5- correlate the PFGE types generated to microbiological and clinical data of the isolates. 6- perform antibiotic susceptibility and MICs of the isolates tovarious antibiotics and correlate them with PFGE types and trends over time.

*Results:* The prevalence of MRSA in the study hospitals ranged from 12% to 49.4%. Mean patient age was 44 years with males constituting 64.4% and females 35.6%. Approximately 41.5% of the isolates came from patients in the extreme age groups. Matushek technique has been found to give clearer results than the two other studied techniques. A dendogram has been generated using PFGE macrorestriction fragments and 6 types have been identified M1-M6 with M1 being predominant and widespread. A clear link between PFGE types versus some clinical and microbiological data available for the strains, for example M1 was statistically associated with male patients while the unique types were associated with female patients, M2 was associated with isolates from wounds and age group <5 years and M4 was associated with isolates from patients admitted to ICUs. M5 was highly correlated with low sensitivity to linezolid. No vancomycin resistant isolates have been detected. MIC for vancomycin was in the susceptible range for all isolates ranging from 0.25 to 3 ug/ml. The overall susceptibility of MRSA to the various antibiotics tested was: fusidic acid 4.3%, sulfamethoxazole/trimethoprim 33.8%, gentamicin 39.6%, mupirocin 77.0%, gatifloxacin 78.9%, chloramphenicl 80.7%, linezolid 95.1%, quinupristin/dalfopristin 100%. Some differences were noted in the resistance of isolates among the participating hospitals reflecting antibiotic usage. On the whole, inpatient isolates (accounting for 77.5% of the isolates) were more resistant than outpatient isolates (22.5%) except for linezolid. Quinupristin-dalfopristin and linezolid are the most effective antibiotics tested against inpatient isolates while quinupristin-dalfopristin and gatifloxacin seem to be the most effective against outpatient isolates. Approximately one forth of the isolates are no longer susceptible to mupirocin used for eradication of the carrier state reflecting resistance developing after widespread use. Trends over time show a tendency towards decreased susceptibility to gatifloxacin and linezolid with increasing susceptibility to gentamicin and sulfamethoxazole/trimethoprim.

## CM 23 *Helicobacter pylori* related precancerous changes diagnosed by rapid touch imprint cytology of gastric mucosal biopsies: survey of 5000 cases

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Dept of Pathology, Shiraz Medical School, Shiraz, Iran

*Background and objectives* Gastric cancer is the most common malignancy among Iranian population. This study is designed to evaluate role of Helicobacter pylori in gastric mucosal dysplasia by examination of gastric biopsies in rapid touch imprint cytological smears.

*Methods* After through clinical examination of the patients referred due to dyspepsia an upper GI endoscopy done and mucosal biopsy of suspected areas performed. A touch imprint cytology from biopsies done and stained with wright- gimsa method. There was 100 dysplastic cases out of 5000 examined patients all of them showed extensive H.pylori infection. The dysplastic changes confirmed by histopathology and for the confirmation of H.pylori ELISA as well as bacterial culture performed.

*Results:* The data showed that touch imprint cytological examination of gastric biopsies had the sensitivity, specificity and accuracy of 98%, 95% and 97% respectively for the diagnosis of dysplasia caused by helicobacter pylori.

*Conclusion* Our data suggested that rapid cytological examination of gastric mucosal smears from endoscopic biopsies was a helpful accurate and cost effective method for the detection of H.pylori and its related precancerous changes of gastric mucosa.

# Posters

## Environmental Microbiology Group

### EM 01 Expression of 2,4-D degradation genes in biofilm and planktonic cells

**Helena Rintala**, Mojtaba Mohseni, Sara Burton & Hilary Lappin-Scott

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Chloroaromatic compounds, such as the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) are persistent pollutants in soil. Microorganisms capable of degradation of these compounds have been identified and used for bioremediation. However, introduced strains are not as resistant to environmental stress as indigenous populations. Therefore, gene bioaugmentation, *e.g.* cleaning up contaminated sites by introducing degradative genes on conjugative plasmids to the soil, has received increasing interest. Horizontal transfer of biodegradation genes via conjugation and subsequent biodegradation has been shown to occur in biofilms. However, detailed characterization and impact of environmental factors on this gene expression remains unexplored.

Objective of this research is to determine the expression of 2,4-D degradation genes after transconjugation in biofilms and planktonic cells.

A flow cell system for biofilm formation and monitoring of transconjugation with gfp has been established (Mohseni *et al.*, unpublished). Transconjugation between *Burkhoderia hospita* carrying the degradation genes in a conjugative plasmid and *Pseudomonas putida* as a recipient will be performed using this system. Effluent samples and biofilm biomass will be collected and gene expression will be determined using RT-QPCR.

### EM 02 Microbial degradation of a persistent monoaromatic fraction isolated from crude oil

**M. Frenzel**[1,2], S.J. Rowland[2], A. Scarlett[3], A.M. Booth[2], T. Galloway[3], A. Lewis[4] S.K. Burton[1] & H.M. Lappin-Scott[1]

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Fractions of oil which are resistant to weathering represent large volumes of environmentally toxic waste comprise complex mixtures of chemicals. Such fractions are unresolved by conventional analytical methods. A bacterial consortium enriched from Whitley Bay sediment, UK has been previously reported to degrade alkylcyclohexyltetralins proposed as model compounds for such oil fractions. In the present study, a monoaromatic hydrocarbon fraction isolated from Venezuelan TJP crude oil was subjected to biodegradation by the Whitley Bay consortium. Hydrocarbon degradation, and changes in the UCM composition were quantified using GC-MS and GCxGC-TOF-MS. Microbial community stability was assessed by PCR-DGGE of the 16S rRNA gene. Microtox studies of the isolated fraction were performed before and after exposure to the Whitley Bay consortium using a *Vibrio* strain. This characterised microbial consortium is thus able to aerobically degrade these hitherto recalcitrant toxic oil fractions.

### EM 03 Sulphate-reducing bacteria in Nigerian crude oil and produced water: characterisation and control strategies

**F.I. Akinnibosun**, S.K. Burton & H.M. Lappin-Scott

Hatherly Laboratories, School of Biosciences, University of Exeter, Exeter EX4 4PS

The quality of Bonny light crude oil (BLCO) and Bonny light produced water (BLPW) from Nigeria was assessed and compared by analysis of sulphate-reducing bacteria (SRB) using molecular methods. The concentration of hydrogen sulphide ($H_2S$) produced by SRB, was determined using a spectrophotometric sulphide assay. DNA was extracted from enrichment after incubation in Postgate's medium at $30^0$C or $55^0$C and amplified by Polymerase Chain Reaction using Eubacterial 16S rRNA primers. These products were analysed by Denaturing Gradient Gel Electrophoresis. DNA bands were excised and sequenced. BLCO was noted to contain little $H_2S$ compared to BLPW which may have been contaminated by subterranean water. Bacterial DNA sequence analysis resulted in detection of greater bacterial diversity for BLPW than BLCO. This implies that the innate bacterial community is not problematic with regard to $H_2S$ production. Thus adoption of these analyses may be useful in the determination of oil suitability for low cost production.

### EM 04 Pharmaceuticals in the environment: toxicity on aquatic microbes

**A.C. Mehinto**[1], S.K. Burton[1], E.M. Hill[2], C.R. Tyler[1] & H.M. Lappin-Scott[1]

[1]School of Biosciences, University of Exeter, Hatherly Laboratories, Exeter EX4 4PS; [2]Centre for Environmental Research, School of Life Sciences, University of Sussex, Chichester Building, Brighton BN1 9QJ

Pharmaceuticals are continuously released in the environment. Although detected in low concentrations in sewage effluents, pharmaceuticals are recognised to be persistent in the aquatic environment. An acute exposure may lead to long-term damage. This study aims to investigate the effects of the anti-inflammatory compound diclofenac on microbial communities.

Solid Phase Extraction and LC-MS-MS were optimised to detect diclofenac in water samples. A recovery rate of 57-69% was achieved. River water collected downstream sewage treatment plants was used in biodegradation studies. Results show a significant decrease in the microbial abundance. The observations suggest that diclofenac is not degraded but may be toxic. Similarly, the fungus *Cunninghamella elegans* cultured in the presence of diclofenac showed a stress response. Assessment of diclofenac toxicity on the main bacteria found in sewage treatment reveals their sensitivity to acute exposure. This lead to a new evaluation of the stability of microbial communities exposed to pharmaceuticals in the aquatic environment.

### EM 05 Evidence of the enhancing effect of interspecific competition on virulence in *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* in a cystic fibrosis model

**R.J. Goldstone**, S.K. Burton & H.M. Lappin-Scott

Hatherly Laboratories, University of Exeter, Prince of Wales Road, Exeter EX4 4PS

Cystic fibrosis (CF) is a debilitating life-threatening condition affecting primarily the lung epithelium. During illness, upwards of 90% of patients will become colonised by species of opportunistic pathogens notably *Pseudomonas aeruginosa* and members of the *Burkholderia cepacia* complex, of which *B. cenocepacia* is most prevalent. These pathogens constitute the major cause of morbidity in CF patients. Evidence of interspecies communication reflecting quorum sensing cross-talk has previously been demonstrated, effecting up-regulation

of virulence factors in *B. cepacia* in response to *P. aeruginosa*. To assess this in a CF background, quantitative reverse-transcriptase PCR was carried out on *P. aeruginosa* and *B. cenocepacia* in mono- and poly-culture, in an artificial CF media and control conditions (nutrient broth). This enabled dissection of the relative importance of i) CF conditions; and ii) interspecific competition on *P. aeruginosa* alginate *(AlgF)*, exotoxin A *(ToxA)*, and *B. cenocepacia* zinc metalloprotease *(ZmpA)* expression. The co-habitation of species was considered the greatest determinant of pathogenesis, causing considerable up-regulation of virulence gene expression. In contrast, no significant difference was observed between expression in CF and control conditions in either mono- or poly-culture. This indicates the development of quorum sensing inhibitors may have dramatic therapeutic benefit for patients of Cystic Fibrosis.

## EM 06 Comparison of bacterial communities degrading specific toxic oil fractions

**P. James**, M. Frenzel, S.K. Burton & H.M. Lappin-Scott

Hatherly laboratories, School of Biosciences, University of Exeter, Prince of Wales Road, Exeter EX4 4PS

An estimated 2.4 million tonnes of petroleum and petroleum fractions are introduced into the marine environment every year. Over time gas chromatographically resolvable hydrocarbons are removed or depleted via the action of weathering, leaving a mixture of hydrocarbons that cannot be easily resolved. The alkyltetralins are considered to be the highly toxic components of these fractions, which persist in the environment. These two factors demonstrate the need for a remediation procedure. Microbial consortia were isolated from the naturally coal contaminated shoreline at Whitley bay, UK, via enrichment culture with either 6-cyclohexyltetralin or 1-(3'-methylbutyl)-7-cyclohexyltetralin (model persistent oil fractions).

16S rDNA clone libraries were constructed from enrichment cultures shown to be degrading both model compounds. These clone libraries were screened by DGGE; DNA sequence analysis revealed the presence of *Bosea* spp, *Pandorea* spp, and Rhodococcus spp, common to both libraries, whilst *Rhyzobium* spp and *Ochrobactrum* spp, were unique to 6-cyclohexyltetralin enrichments.

## EM 07 2,4-D degrading bacterium *Burkholderia* sp. – molecular characterisation and functional gene flow within biofilms

**M. Mohseni**, S.K. Burton & H.M. Lappin-Scott

School of Biosciences, Hatherly Laboratories, University of Exeter, Exeter EX4 4PS

2,4-dichlorophenoxy-acetic acid (2,4-D) a chlorophenoxyalkanoate herbicide is extensively used to control weeds. Persistence within the environment is a serious concern due to impact on human health and environmental toxicity. The 2,4-D degradative genes (*tfdA-F+K*) are frequently located on a conjugative plasmid. However, sequence conservation of these genes and plasmid stability and mobility within natural biofilms is little understood. This study describes development of a system for real-time observation of the degradative plasmid prevalence within biofilms. The aims of this study were the isolation and identification of 2,4-D degrading bacteria from soil and horizontal degradative gene flow. Bacterial enrichments were performed from agricultural soils and degradation was monitored. Community analyses were undertaken using DGGE. PCR amplification of the 16S rRNA enabled identification of the isolates. A high homology to *Burkholderia hospita* was determined. DGGE analysis demonstrated bacterial community changes during successive subcultures. Plasmid profiling of *B. hospita* indicated the presence of *tfd* genes. *B. hospita* formed thick biofilms which were observed via flow cell microscopy. Furthermore we have developed a system to study *tfd* horizontal gene flow within degradative biofilms using *B. hospita* (pMM172::*gfp*). This allows *in situ* visualisation of degradative *tfd* gene movement within biofilms. GFP fluorescencing pMM172::*gfp* transconjugant microclony were detected with $8.68?10^{-6}$ transconjugant per donor within biofilm. This system is also suitable for the quantification of horizontal plasmid flow within mixed culture biofilms.

## EM 08 Biofilm polymicrobial infections on urinary catheters; quantification of pathogenicity gene expression

**N. Mohajeri**, S.K. Burton & H.M. Lappin-Scott

Hatherly Laboratories, School of biosciences, University of Exeter, Exeter EX4 4PS

Clinical observations suggest there may be inhibition of infection initiation of individual strains within a mixture of *Pseudomonas aeruginosa,* Methicilin Resistant *Staphylococcus aureus* (MRSA) and *Candida albicans*. Most biofilm studies have been undertaken using monocultures, however the importance of polymicrobial biofilms in determining pathogenicity of infection is not well studied. Therefore, this project aims to establish mixed community biofilms of clinically important pathogenic bacteria to study the effect of this communal existence on pathogenic gene regulation. All strains were grown as both planktonic and biofilm form in artificial urine media. Real time Q-PCR was used to determine expression of the genes encoding 16S rRNA, virulence, biofilm formation, and arginine biosynthesis (as a control). Total RNA was extracted from *P.aeruginosa* in planktonic and biofilm form and mixed culture using RNeasy minikit. cDNA was generated using Thermoscript RT-PCR kit. Random-hexamer primers were used. MiniOpticon system was used for real time PCR detection from cDNA synthesis using *toxA* primers. ToxA Expression was significantly increased within the biofilm form compared to the planktonic. In mixed culture, *P.aeruginosa* exhibited anti-Candida activity. This study elucidates the up-regulation of pathogenicity genes of individual strains comprising polymicrobial biofilms.

## EM 09 Microbial interactions with depleted uranium and its corrosion products in contaminated soil

**Alison Baxter**[1], Ian Thompson[2], Simon Jackman[1] & Sam Shaw[3]

[1]Oxford University, Earth Science, Oxford OX1 3PR; [2]Centre of Ecology & Hydrology, Oxford OX1 3SR; [3]School of Earth & Environment, University of Leeds, LS2 9JT

The environmental impact of depleted uranium (DU) in oxic soil is limited by information regarding uranium mobilisation. Micro-organisms have a significant effect on uranium mobility in natural environments. Microbial processes are affected by DU speciation, which must also be characterised within the soil. This project will focus on elucidating the microbial mechanisms contributing to DU behaviour within the soil matrix and place these within the context of biogeochemical processes.

In Britain DU test firing occurred in two sites. These sites have also been 'planted' with 240g DU pellets. The pellets have recently been excavated and the surrounding soil analysed geochemically and microbiologically. Initial findings show a correlation between the uranium concentration within the soil and the microbial metabolic diversity. However, there seems to be no correlation between the uranium concentration and bacterial numbers within the soil. This link will be further investigated by DGGE, and rank abundance, and the geochemistry elucidated by EXAFS analysis and SEM.

## EM 10 Reversible effects of desiccation on diversity and activity of bacterial communities in soil

Jane Jukes & **Richard J. Ellis**

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Soil bacterial communities are large and diverse, but the proportion contributing to ecosystem function is unknown. DNA-based techniques fail to account for variation in activity; cells remain amenable to PCR detection when inactive. The aim was to demonstrate that growth on agar plates is indicative of *in situ* activity. The temporal response of soil bacteria to desiccation was assessed by 16S rRNA-DGGE of both directly-extracted DNA, and DNA extracted from biomass on agar plates. Activity was monitored using culturable counts and direct counts of total and active cells. Desiccation resulted in a decrease of microscopic cell counts and CFU. DGGE profiles showed that the diversity of culturable organisms decreased as soil dried, whilst profiles from directly-extracted DNA remained similar throughout. Re-hydration of the desiccated soil increased culturability of the community. Whilst desiccation did not have a significant effect on total genetic diversity, activity was reversibly reduced, both in terms of the number and diversity of bacteria growing on agar plates.

## EM 11 Metagenomic analysis of human tongue using phage display

**Samantha Easton** & John Ward

Dept of Biochemistry & Molecular Biology, University College London, Gower Street, London WC1E 6BT

Microbial infections are dependent on a series of changes in both host and bacterium, in response to bacterial attachment. Commensal bacteria also develop and maintain an intimate relationship with mammalian cells, without triggering invasion mechanisms. Phage display is a simple genetic technique for the identification of protein-ligand interactions, such as microbial attachment to mammalian cells, and is currently used successfully in epitope mapping, antibody tailoring and receptor agonist and antagonist screening. It is well established that the adhesion of enteric, oral and respiratory bacteria is required for colonization and, once bound, the bacteria are less likely to succumb to host defences. This study aims to identify bacterial adhesins which are vital for bacterial colonisation of the human tongue, using Phage Display. Screening of the Phage Display library will be carried out using a technique called Bio-panning, against human IgA, and is expected to yield one or more bacterial adhesins.

## EM 12 Analyzing the oral metagenome

**S.J. Hunter**[1,2], B. Henderson[3], W. Wade[2] & J. Ward[1]

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The microbial community of the oral cavity is thought to be one of the most diverse in the human body. It is estimated that at least 800 bacterial species exist within the human oral cavity of which approximately half can be cultured. Bacteria adhere to both oral surfaces and each other forming biofilms. Generally bacteria exist in equilibrium with the host; however, some organisms are opportunistic and may cause disease. A comprehensive metagenomic study of the oral microflora will be used to investigate the diversity and adhesion mechanisms of this unique bacterial community.

High quality DNA is required for the production of metagenomic libraries and, therefore, a range of DNA extraction methods have been investigated including steps to eliminate contaminating human DNA. The DNA concentration and molecular weight of each sample have been determined and Q-PCR used to assess the amount of human DNA present. A DNA extraction using proteinase K and lysozyme gave the best results and fosmid libraries have been prepared from the resulting DNA samples.

## EM 13 Does the 'Enzymic Latch' occur in shallow organic soils?

**Katherine L. Chadwick**[1,2], Ken Killham[1], Julian J.C. Dawson[1], Brian Reynolds[2], Bridget Emmett[2] & Graeme I. Paton[1]

[1]School of Biological Sciences, University of Aberdeen, Aberdeen AB24 3UU; [2]CEH Bangor, Orton Building, Deiniol Road, Bangor, Gwynedd LL57 2UP

The 'Enzymic Latch' Hypothesis (Freeman *et al.*, 2001) identifies the importance of enzyme activity and inhibition in decomposition of soil bound C. In anaerobic conditions (as commonly found in upland organic soils), phenolic substances act as inhibitors to C-cycling hydrolase enzymes (e.g. β-glucosidase). However, as changes in oxygen availability occur (due to warmer, drier climatic conditions), phenol oxidase activity increases to remove these phenolics, opening a biological 'latch'. This causes increased C release from upland organic soils as they change from sink to source status. This work examines the enzymic latch hypothesis in shallow organic upland soil investigating the differences between organic and mineral horizons, providing a link between biological activity and the soil physico-chemical environment. Focus is given to the biological component of C decomposition in response to simulated climate change providing an assessment of rates of OM decomposition. The potential use of a phenol and analogue-specific, bioluminescent bacterial biosensor is hoped to enable quantification of hydrolase enzyme inhibition.

## EM 14 Application of a bioluminescence-based fungal bioassay to contaminated soils

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Bioluminescence-based microbial biosensors provide a biological tool for the assessment of metal bioavailability. Most of the biosensors used are bacterial and as such fail to represent wider soil ecological niches. The development of a bioluminescence-based fungal bioassay has extended the existing suite of biosensors available for ecotoxicity testing. The naturally bioluminescent fungi *Armillaria mellea* and *Mycena citricolor* were found to be sensitive to Cu and Hg, as well as to Zn and Cd, respectively. The fungal bioassay assessed the toxicity of pollutants in both laboratory-amended and historically contaminated soils. Chemical analysis quantified the pollutants and this was translated into a bioavailability model using contaminant partitioning ($K_d$) within the soil. The $K_d$ values explained the bioavailability of Cu and hence the bioluminescence response in historically contaminated soils. This study demonstrates the importance of integrated chemical and biological assessments in soil toxicity testing, and the value of bioluminescent fungi as biosensors in contaminated sites.

## EM 15 The impact on shellfisheries of runoff from land receiving organic waste and procedures to distinguish organic waste of animal and human origin in bivalve molluscan shellfish

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Several microbiological and chemical indicators have been used to distinguish between human sewage and agricultural run-off contamination of bivalve molluscan shellfish (BMS). Two methods were selected for an intensive study, adeno virus and F+RNA bacteriophage. The detection of adenovirus in polluted shellfish is indicative of the impact by effluents of human origin. Genotyping of F+RNA bacteriophage can be used to ascribe contamination from human or animal origin. Four synthetic oligonucleotide probes have been synthesised to F+RNA bacteriophage that correspond to serotypes I through IV, Strains isolated from human faeces are usually serotypes II and III, while serotypes I and IV are found in animal faeces. F+RNA bacteriophage plaques from shellfish samples were subjected to gene probing in a detailed examination of two catchments over a period of time to demonstrate whether run-off from the land impacted on the shellfisheries.

## EM 16 Investigation of an emerging *Yersinia ruckeri* biogroup responsible for outbreaks on fish farms in England & Wales using pulsed-field gel electrophoresis (PFGE)

**Richard Wheeler**[1,2], Robert Davies[2], David Verner-Jeffreys[1] & Sariqa Wagley[1]

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*Y. ruckeri* is a Gram-negative pathogen responsible for enteric redmouth disease (ERM) of farmed rainbow trout (*Oncorhynchus mykiss*). *Y. ruckeri* has been successfully controlled by vaccination, however recent outbreaks in England and Wales have been presumptively attributed to an emerging vaccine insensitive biogroup.

A (PFGE) protocol was developed to characterise closely related *Y. ruckeri* isolates in order to conclusively identify those responsible for recent disease outbreaks, and determine their relationship to other biogroups, which differ greatly in their virulence for rainbow trout. Analysis of isolates from infected farms using the restriction endonuclease *Not*I revealed a common pulsotype identical to that of a reference strain for the emerging biogroup. This pulsotype differs from a previously recognised serotype O1 clonal group by the presence of a single additional DNA fragment.

## EM 17 Identification of the pandemic O3:K6 clone in cases of *Vibrio parahaemolyticus* related illness in the UK

**Sariqa Wagley** & Rachel Rangdale

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Cases of food poisoning attributed to *V. parahaemolyticus* are commonly reported in countries with both a high ambient temperature and where seafood is consumed raw. In Europe, *V. parahaemolyticus* infections are rare and sporadic, usually travel related, and often attributed to inadequate hygiene standards leading to the recontamination of cooked foodstuffs. However, recently several outbreaks have been reported in Europe associated with indigenously produced seafood. In 2004, 76 cases of *V. parahaemolyticus*-associated gastroenteritis were reported in Spain. Molecular and serological characterisation of isolates collected from patients during these outbreaks demonstrated the presence of the clinically significant O3:K6 pandemic clone.

In 2004, 3 cases of *V. parahaemolyticus*-associated gastroenteritis were identified in the U.K. Characterisation of these strains using pulsed field gel electrophoresis showed highly significant homology with the pandemic O3:K6 clone linked to Spanish outbreaks. This article demonstrates the presence of *V. parahaemolyticus* O3:K6 in the UK and supports the hypotheses that the recently emergent O3:K6 pandemic clone in continental Europe has also been responsible for isolated *V. parahaemolyticus* cases in the UK.

## EM 18 DegU regulation of biofilm formation by *Bacillus subtilis*

**Taryn B. Kiley** & Nicola Stanley-Wall

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The Gram-positive soil bacterium *Bacillus subtilis* has been shown to form biofilms in response to environmental stresses such as osmolarity, high population density, and nutrient depletion. The transition from a free-living state to that in which multicellular behaviour is observed is controlled by a highly complex regulatory system. One key component of this network is the two component regulatory system, DegS-U. By conducting complementation studies, we identified that the phosphorylated form of DegU, DegU~P, was required for the formation of biofilms by *B. subtilis*. Two key operons which encode components of the *B. subtilis* biofilm matrix are the *epsA-O* operon and the *yqxM-sipW-tasA* operon. They are required for the biosynthesis of an exopolysaccharide and a protein component respectively, and therefore represent potential regulatory targets for DegU~P. Previous studies have shown that the transcription of these operons is highly controlled by various regulators, including the repressor SinR. The hypothesis which is being explored is directed at identifing whether DegU~P regulates the transcription of the *epsA-O* and *yqxM-sipW-tasA* operons, and if DegU~P does regulate the transcription of these loci, whether this is an indirect or direct regulatory event.

## EM 19 The complete characterization of plasmid pIJB1 from *Burkholderia cepacia* strain 2a reveals its evolution as modular and unique

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Bacterial genes for the degradation of xenobiotics are often located on mobile genetic elements, such as plasmids, transposons and insertion sequences (ISs). *Burkholderia cepacia* strain 2a is a soil-bacterium able to utilise 2,4-dichlorophenoxyacetic acid (2,4-D) and this ability is carried on a defective transposon Tn*5530* resident on an IncP1-β plasmid pIJB1. Here we report the complete sequence (99,448 nucleotides) of pIJB1 that consists of well-conserved IncP1-β backbone genes, a previously reported defective transposon Tn*5530*, a Tn*501*-encoded mercury resistance and a non-functional biphenyl operon. Phenotypic analysis of strain 2a harbouring pIJB1 revealed that the presence of *tfd* genes allows the host to utilise a wide range of phenoxyalkanoic acids as sole carbon and energy source. Transcriptional profiling of strain 2a, grown in media supplemented with those phenoxyalkanoic acids as carbon sources, showed that the *tfd* genes were slightly down-regulated with respect to their level of expression in media containing 2,4-D. This versatility in carbon source usage suggests that the regulation of the 2,4-D pathway is 'loose' and flexible. We also found that Tn*5530* was rich in remnants of ISs that are clustered near the *tfd* genes. The *tfd* genes GC content was similar to that of the genome of *B. cepacia* indicating that their origin is likely to be through IS-mediated capture of chromosomal genes rather than through lateral gene transfer. These observations are integrated into a model for the evolution of pIJB1 which is modular and unique in terms of the generation of a structure capable of utilising 2,4-D, and perhaps other xenobiotics.

## EM 20 Patterns in forest soil microbial community composition across a range of regional climates in western Canada

**B.F.T. Brockett**[1], S.J. Grayston[1], C.E. Prescott[1] & W.W. Mohn[2]

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Soil microbial communities can be characterized by community structure and function (community composition) across a spectrum of spatial scales, and variation in soil microbial composition has been associated with a number of environmental gradients. This study investigates the structure and function of soil microbial communities under mature, undisturbed forested sites across a range of regional climates in British Columbia and Alberta, and also examines the variation in community composition within sites.

Phospho-lipid fatty acid analysis was used to investigate the structure of soil microbial communities and total soil microbial biomass at each site. Extra-cellular enzyme assays established the functional potential of the soil microbial community at each site. The range of substrates chosen for the assays enabled us to relate enzyme activity to carbon, nitrogen, phosphorous and sulfur cycling in the ecosystems studied.

Multivariate analysis was used to examine the relationship between microbial community characteristics and regional climate variables such as annual precipitation and annual temperature, along with endogenous site characteristics such as slope, aspect, and soil particle size.

This study fits into a larger project which investigates the role of regional climate effects and stand characteristics on litter decomposition and nutrient cycling within British Columbia and Alberta.

## EM 21 Microbial community structure in soils subjected to different crop management practices

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Soil biota are essential for the maintenance of soil fertility and productivity, however, very little is known about the importance of microbial community structure in maintaining sustainability and functioning of soil ecosystems. Soil microbial diversity is influenced by a number of factors, including soil properties, environmental conditions and anthropogenic activities, such as land management systems. The objective of this study was to evaluate and compare the influence of past and current management practices on soil microbial diversity. Intact monolith lysimeters were taken from two sites of the same soil type that had been under long-term organic or conventional crop management and subjected to the same crop rotation (including a lupin green manure). Two fertiliser regimes were applied resulting in four treatments distinguished by farming history and short-term management. Microbial biomass C (fumigation extraction) and community composition of actinomycetes, fungi and eubacteria were determined (PCR-DGGE). After 30 months, DGGE profiles showed no differences between the treatments, indicating comparable microbial abundance and community structures. Results indicate that in the short term, microbial community size and composition are mainly influenced by crop type and rotation rather than fertilisation.

## EM 22 Future of colouration: colouring textiles with a microbial colour

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With the present awareness of ecology, the use of ecofriendly fibres and natural dyes has been increasing all over the globe. A dark pink pigmented microbe was isolated from mangroves for harnessing its ability to produce large quantity of the natural colourant for dyeing fabrics. The pigment, extracted with acidified methanol, was separated into fractions using HPLC and the major fraction collected and characterized using UV-Visible spectra, FTIR, $^1$H- NMR, EI-MS and ESI-MS. The pigment, found to be a close derivative of Prodigiosin pigment, was used for dyeing fibres such as silk, wool, cotton, polyester and polyester- wool blend and was capable of dyeing all fibres, except cotton. Although light fastness rating was 1-2 (poor), excellent (4-5) wash, rubbing and sublimation fastness were observed. The dyed fabrics showed antimicrobial activity (AATCC 100 method) against laboratory strains of *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Nocardia spps* and *Micrococcus luteus* and had a cidal effect on them. This thus increases its commercial value as an antimicrobial fabric and satisfies the need for a natural, ecofriendly dye.

## EM 23 Microbial degradation of dibenzothiophene by different bacteria species

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Dibenzothiophene (DBT) in one of the most important heterocyclic sulfur compounds which is found in petroleum and petroleum distillates, and used as model compounds for biodesulfurization studies

In this work, isolated species have the ability to co-metabolism DBT at different concentrations was isolated from sediment drawn of an open drain that receives refinery leakage. The significant isolates responsible for DBT degradation were characterized in minimal salts medium after several sub culturing techniques on agar plates supplemented with DBT. The isolates were nominated as $NPS_1$, $NPS_2$ &t$NPS_3$, however $NPS_1$ is considered to be *Pseudomonas* species and the other two genera are *Bacilli* species.

The experimental results of kinetic growth rate ($\mu._{max}$) at optimum growth conditions on DBT showed that ($NPS_1$) has the highest growth rate activity with (0.4 $h^{-1}$) and $NPS_2$ and $NPS_3$ were (0.3 $h^{-1}$, 0.275 $h^{-1}$) respectively.

Mass spectral analysis, confirmed that isolates are capable to degrade DBT and produce metabolism products that were identified as 1, 2 benzen dicarboxylic acids and benzoic acid with diethyl sulfate. However, these isolates could metabolize Dibenzothiophene via oxidative cleavage of the aromatic ring with a mechanism analogous to that which was described by kadoma pathway.

*Keywords* Biodegradation; dibenzothiophene;bacteria, Kadoma pathway

## EM 24 *Pseudomonas* degradation of normal and iso-alkanes in crude oil

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To study the impacts of micro-organism on crude oil chemical properties, a crude oil with different concentrations was subjected to *Pseudomonas* species at 37°C and various incubation periods. The results showed that Pseudomonas species grew faster at 1% (v/v) concentration of crude oil and exhibited high biodegradation ability within one week. The gas chromatography analysis of the saturated hydrocarbons of crude oil showed that, an increase in concentration

iso-alkanes in the range of $C_{15}$-$C_{20}$, and a bioconversion of heavy iso-alkanes in the range of $C_{21}$-$C_{22+}$ due to microbial growth, by-products and metabolites. Chemical analysis of crude oil by liquid chromatographic technique before and after growth showed that, the saturated alkanes were more degradable than aromatic and asphaltenic compounds, this was attributed to the capability of *Pseudomonas* species to degrade paraffinic fraction more than aromatic fraction.

*Keywords Pseudomonas*; crude oil; biodegradation, gas chromatography.

## EM 25 Co-contamination of soil with TNT and copper negatively impacts on the microbial community composition

**E.R. Travis** & S.J. Rosser

University of Glasgow

Heavy metals, such as copper, are often found as co-contaminants of trinitrotoluene (TNT). We investigated the resilience of TNT-contaminated soil to further perturbations through further amendments of the soil, in combination with a microtitre-plate based pollution induced community tolerance assay. High concentrations of TNT were seen to be toxic to the overall bacterial population, but caused an enrichment of the pseudomonad portion of the bacterial population. The addition of copper was seen to be toxic to the overall bacterial population, with a marked toxicity towards the pseudomonads. Soil amended with both TNT and copper exhibited low overall bacterial numbers and low pseudomonad populations. Similar results were observed with the microtitre plate toxicity assays showing that TNT-contaminated soil exhibited a pollution induced community tolerance to TNT, whereas soil contaminated with TNT showed a pollution induced community sensitivity to copper. This suggests that co-contamination of soil with organic and inorganic compounds can have a serious deleterious effect on the microbial population.

# Posters

## Education & Training Group

### ET 01 Development of a microbiology online learning resource for first year dental students

Andrew N. Jones & **Ruth Grady**

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At the University of Manchester, basic biosciences for dental students are taught largely as a problem-based learning course, where the students are given a clinical case and asked to define their own learning objectives in order to understand the science fully. Microbiology is embedded in some of the cases but is often only superficially covered, despite being a topic of enormous importance to dentists.

We have developed an online resource in WebCT that provides relevant microbiological topics and revision aids for the students' self-directed study.

The topics available have controlled access, such that students are not swamped with information from the outset but gradually build-up a full microbiology resource over the semester. The students are able to learn from the information presented and be guided to other relevant links on the internet and to further reading. Quizzes, with feedback for incorrect answers, are associated with each topic that can be attempted numerous times allowing students to learn from their mistakes and monitor their own understanding.

All the students that have used the site felt that the web resource has assisted their learning and understanding of microbiology and would recommend the web resource to future first year students. Microbiology resources delivered in this way for other years are currently being developed.

This project was sponsored by the SGM Education Development Fund

### ET 02 The use of formative assessment as a feedback tool in an undergraduate first year microbiology module

Pauline Fitzgerald

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The increased heterogeneity of the student cohort has meant an increase in the level of student support needed. Several studies have shown that early and relevant feedback from assessment activities is necessary to maintain student motivation and allow the student to reflect on and identify their strengths and weaknesses. The first year undergraduate module 'The Microbial World' is accessed by over 120 students from six different courses. A wide range of formative assessment methods, both on and off-line were used in this module. On line quizzes and self tests provided instant feedback, and allowed the tutor to view the student scores. Face to face support was in the form of a fortnightly tutorial- the large group was divided into 5 smaller groups. Diverse forms of assessment tasks were used, from preparing a poster about a particular micro organism, to data handling and problem solving. Student motivation and participation in task completion was high, enabling the tutor to monitor progress, and target any weak students requiring more support.

### ET 03 *Clostridium difficile*: is it increasing in the community?

**Angie Ghattas**[1] , Shobhit Baijal[2] & T.J. Elliott[3]

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*Background* Antibiotic prescribing has increased in the community. Is this reflected by the number of hospital admissions for *Clostridium difficile* infection? Has the number of community acquired *Clostridium difficile* increased over the last three years?

*Methods* Retrospective analysis of all the *Clostridium difficle* cases in the University Hospital Of Birmingham NHS Trust over a three years period (2004 – 2006). The cases were identified through the Hospital computer system. The date of diagnosis was correlated with admission date to determine whether this was a hospital aquired infection or not. Cases with an admission less than four weeks prior to developing *Clostridium difficile* were excluded from community acquired cases.

*Analysis* Six month period analysis of the data (January to June/ June to December). Chi-squared test used to determine statistical significance.

*Results* There has been a drastic increase in the total number of *Clostridium difficile* cases over the three years (193 in 2006 vs 114 in 2004). Over this period the absolute number of community acquired cases also increased, although the percentage remained the same (16%). The prevalence of admissions was greater during the winter months. In 2006 only 13% were admitted from nursing homes.

*How to use this data?* These results are very interesting as it encourages docotrs to look into their antiobiotics prescribing practices in order to reduce the rates of *Clostridium difficile* infections. The fact that only a minority of patients were from nursing homes (hence a low rate of cross infection between occupants) indicate that GPs need to prescribe less antiobiotics especially during the winter months for non-bacterial infections. This has very important educational potential for docotrs and pharmacits to ensure appropriate prescription. The public could also benefit from these data to understand their role in reducing a potentially lethal infection as not every 'snuff' and 'cold' requires an antibiotic.

# Posters

## Fermentation & Bioprocessing Group

### FB 01 Investigation of the use of chaperone proteins in the expression of soluble protein in recombinant *Escherichia coli*

**N. Walker**[1] & S.C. Rowe[2]

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The over expression of recombinant proteins in *E. coli* can lead to the formation of inclusion bodies. A number of strategies can be used to maximise the expression of soluble protein including expression at low temperatures, low IPTG induction concentrations and *E. coli* expression strains that encourage slow uptake of IPTG. These strategies however are not always successful. Co-expression of recombinant proteins with chaperone proteins has been shown to increase protein solubility.

Using the TaKaRa chaperone plasmid set we have investigated the co-expression of a panel of chaperone proteins with the insoluble protein BP4. The chaperone combination that yielded the most soluble protein was the simultaneous expression of groES-groEL-tig with BP4 at 15°C.



### FB 02 The large scale fermentation and bioprocessing of genetically engineered filamentous phage to underpin new therapeutic and industrial applications

**Steven D. Branston**[1], E.C. Stanley[1,2], E. Keshavarz-Moore[1] & J. Ward[2]

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Scientific and commercial interest in bacteriophage has increased in recent years, ranging from renewed attention to their well known role as bactericidal agents to new applications, such as vectors for vaccines, gene therapy delivery systems, and nano-architectural templates. In particular, the ability of Ff filamentous bacteriophages to accept large inserts into their genomes makes them popular candidates for phage therapeutics. In this work we describe the beginnings of a project for the large-scale production and purification of genetically engineered M13 filamentous bacteriophage. A custom-built rotating disk shear device was used to subject phage to shear forces with the intention of assessing their resistance to the shear environments found during the fermentation and bioprocessing stages. Our results indicate that despite the filamentous nature of M13, it is resistant to such environments; infectivity is retained and displayed foreign proteins appear to remain intact.

### FB 03 The large-scale fermentation and bioprocessing of genetically engineered bacteriophage for pharmaceutical applications

**E.C. Stanley**[1], S. Branston[2], E. Keshavarz-Moore[2] & J. Ward[1]

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Bacteriophage M13 and lambda are now being used for many new applications including therapeutics against pathogenic bacteria, delivery vehicles for protein and DNA vaccines, gene therapy vectors and the nanotechnology industry. However, many techniques used for the growth and purification of bacteriophage at small-scale are not transferable to large-scale production of bacteriophage in an industrial processes.

The final titre of M13 phage produced in 2 L fermentations was shown to be independent of the growth phase and the OD of the *E. coli* host inoculum. Lambda phage production has been optimised using an *E. coli* strain which produces DNAase and RNAase into its periplasm. Upon lysis of the *E. coli* by lambda the DNAase and RNAase are released into the surrounding media and successfully degrade the *E. coli* DNA and RNA. This action removes the need to add bovine DNAase/RNAase when producing pharmaceutical grade lambda bacteriophage.

### FB 04 Understanding the extracellular proteases to enhance heterologous protein production in a recombinant filamentous fungus, *Aspergillus niger* B1-D

**Qiang Li**, Linda M. Harvey & Brian McNeil

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Filamentous fungi have attracted great research interest recently, and several genetically modified organisms have been successfully constructed to express valuable heterologous proteins. Examples include industrial enzymes and gene products from higher organisms. The advantages of using filamentous fungi as hosts include, (1) the ability to secrete large amount of proteins, (2) post-translational modifications which are essential to proper protein function, but may be neglected in prokaryotic hosts, (3) fast growing and inexpensive cultures compared to insect, plant and mammalian cells.

In the formation of recombinant protein in fungi, one of the bottlenecks may be the extracellular proteases, which can digest secreted heterologous proteins at a high rate. In the present work, we cultivated a recombinant filamentous fungus, *A. niger* B1-D, genetically modified to secrete hen egg white lysozyme (HEWL), in a 15L fermentor, and investigated the properties of its extracelluar proteases. Our results suggest that extracellular proteases have negative effects on the HEWL production, and the proteases inhibitors studies show that cysteine, serine and metallo-protease type enzyes are present. Finally, the optimal temperature for the acid protease is around 45°C.

### FB 05 Challenging yeast in high throughput protein production

**Naglis Malys**, Jill A. Wishart, Douglas B. Kell, Stephen G. Oliver & John E. McCarthy

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During recent years, substantial breakthroughs have been achieved in the high throughput manipulation of nucleic acids including genome sequencing, gene cloning and expression analysis. The flood of information on genes and their expression has now expanded to studies of all existing gene products in an organism. A bottleneck in these studies is purifying the protein of interest efficiently and getting a sufficient amount of high purity product in a short period of time.

In an ongoing project we use baker's yeast as a protein production factory. In contrast to *E.coli*, the advantage of using eukaryotic cells is that it helps in making correctly folded protein with naturally existing modifications. The use of an affinity tag allows one to perform protein purification efficiently. In order to purify a large number of different proteins, we are in the process of developing a three-step procedure for high throughput purification. We also seek to conduct the purification process in the automated mode. In the systems biology approach envisaged, the purified proteins will be used in biophysical studies of networks and the kinetics of enzymatic reactions protein-protein and protein-ligand interactions in order to ultimately establish a genome-wide network of interactions.

Currently, we are working on proteins involved in glycolysis.

## FB 06 Development of homologous expression systems for producing recombinant soluble methane monooxygenase in methane-oxidising bacteria

**Tim Nichol**[1], Marc Dumont[2], Elena Borodina[2], J. Colin Murrell[2] & Thomas J. Smith[1]

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Soluble methane monooxygenase (sMMO) is a three-component enzyme system found in certain methanotrophic (methane-oxidising) bacteria, which catalyses the NAD(P)H- and $O_2$-dependent oxygenation of methane and many additional substrates, including the environmental pollutants trichloroethene and naphthalene. Mutagenic studies of sMMO are interesting in order to investigate the mechanism of biological methane oxidation and to tailor the very broad substrate range of sMMO for specific applications in synthetic chemistry and bioremediation. The active site-containing hydroxylase component of sMMO is not active when expressed in *Escherichia coli* and so we previously developed an expression system using an sMMO-minus methanotroph host, which was constructed by partial deletion of the active site-encoding *mmoX* gene. This host allowed us to construct and express the first active-site mutants of sMMO but was restricted to mutants within the first half of the *mmoX* gene. Here we describe construction and exploitation of a new methanotroph expression host from which the whole of the six-gene operon (*mmoXYBZDC*) that encodes the sMMO complex has been deleted. Thus, it is now possible to make mutations anywhere within sMMO. Using this new expression host a mutant deficient in methane oxidation has been characterised that could not have been made using the original system. A new plasmid vector designed to facilitate expression of site-directed mutants and to permit directed evolution of sMMO has also been constructed.

## FB 07 A high-throughput pipeline for human structural genomics

**Chris Cooper**, Nicola Burgess, Georgina Berridge, Shurene Bishop, Catrine Johansson, Nadia Pantic, Eidarus Salah, Pavel Savitsky, Binesh Shrestha & Opher Gileadi

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Structural genomics, the resolution of 3D structures of large numbers of proteins, has been made possible in recent years following the advent of whole genome sequencing and advances in crystallisation and data analysis techniques. Human proteins associated with disease are attractive targets for structural genomics, particularly in the field of drug discovery. This requires efficient, cost-effective strategies for cloning, expression, purification and crystallisation of proteins or individual domains, allowing high-throughput analysis of multiple targets with optimisation where necessary. The SGC approach is based on testing multiple truncated variants of each protein for soluble expression in bacteria and for crystallisation. Tractable variants can often be expressed and purified using fairly uniform procedures and success in crystallisation can be enhanced by including cofactors or ligands. This highly parallel yet adaptable modular approach has been extremely successful to date, with over 300 structures solved by the SGC in 2½ years.

## FB 08 Automated approaches for the expression of recombinant proteins using *Escherichia coli*

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*Escherichia coli* is the most widely used recombinant protein expression system.

Major advantages of using *E. coli* as a host for protein generation include cost effectiveness, high yields (up to 50% of total cell protein) and speed of production. However, sub-optimal culture conditions may result in the formation of insoluble aggregates. Rapid turnaround is important to the pharmaceutical industry where time lines are constantly under pressure. Rapid, parallel approaches supporting the identification of suitable clones and optimum expression conditions are clearly needed to improve protein production efficiency.

Piccolo® is a fully automated, high throughput system, specifically designed to support the rapid optimisation of protein production from both insect and microbial cells. The system offers an unparalleled capability to evaluate a large number of different conditions (up to a maximum of 1152) in a single experiment. Cell growth is monitored robotically and can be linked to inducer addition. Following expression, cells are centrifuged, lysed and the target protein is purified using affinity chromatography. Selected conditions can then be scaled up into fermenters. This automated approach is going some way towards our goal of reducing time lines and improving productivity.

# Posters

## Food & Beverages Group

### FdBev 01 Microflora diversity in raw ewes' milk cheese: from raw material to finished product

**Helen Davies**, Christine Dodd & Tim Aldsworth

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The natural microflora of raw ewes' milk has been found to add a complexity to cheese flavour that is absent from pasteurised milk cheeses. However, this very complexity of the raw milk flora causes difficulties for the manufacturer in controlling batch variation. To better understand these changes, we have assessed the microflora, particularly the lactic acid bacterial flora, using 16S rRNA-PCR-DGGE. Viable counts showed that the milk contained low numbers of microorganisms but the DGGE profile revealed that there was a high level of diversity. The microflora of the developing cheese interior contrasted with the milk, as it was heavily influenced by the starter culture. The microflora of the cheese exterior did, however, show some correlation with that of the milk. The substantial change in the bacterial microflora from raw milk to developed cheese demonstrates the impact of addition of starter culture which bodes well for the control of both batch variation and survival of possible pathogens from the raw milk.

### FdBev 02 Spatial distribution of yeasts in Stilton blue cheese related to the aroma profile

**Konstantinos Gkatzionis**, Christine Dodd & Robert Linforth

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*Background and objective of investigation* The yeast microflora and aroma profile in Stilton was studied regarding its three main parts: blue veins, white core and outer crust.

*Methods* Yeasts from the three different parts of the cheese were isolated on Rose Bengal Cloramphenicol agar and identified. The flavour profiles of these sections were analyzed by Atmospheric Pressure Chemical Ionisation (APCI). Principal Component Analysis (PCA) used to present and plot the relation of the aromas within the three regions.

*Results* Yeast communities in the three sections of Stilton consisted of combinations of five species common in blue cheeses. In addition, the PCA plot showed that specific groups of aroma compounds were related to these sections.

*Conclusions* There is a clear spatial distribution of the yeasts and flavour compounds in Stilton cheese. Interactions between the bacterial and yeast community might take place locally and form these flavour profiles. Such knowledge can be used for improving the starter cultures as well as producing flavourings and enzyme modified cheeses.

It is necessary to consider the microbial and flavour differentiation when studying cheeses with clearly defined structural areas as studies based on a homogenous sample might lead to misleading conclusions.

### FdBev 03 Both sporulation and growth NaCl concentration increase the lag phase during growth from individual spores of non-proteolytic *Clostridium botulinum*

Martin D. Webb, Michael W. Peck & **Sandra C. Stringer**

Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA

Spores formed by *Clostridium botulinum* are important in food safety as they can resist processes used to kill vegetative cells and lead to growth with associated production of an extremely potent neurotoxin. We studied the affect of NaCl concentration during sporulation (0% or 3.0% (w/v) added NaCl) and subsequent growth (0% or 2.0% (w/v) added NaCl) on the distributions of times to germination and later outgrowth events from individual non-proteolytic *Clostridium botulinum* Eklund 17B spores. The mean times for, and variability of, germination and later outgrowth events increased when NaCl was added to either the sporulation or subsequent growth medium. There was considerable variability at all stages of lag phase for each condition tested. Germination time did not correlate strongly with any of the times for later stages of lag. Suboptimal conditions during sporulation or growth also reduced the probability of germination and the probability of germinated spores developing into a mature cell. Spores formed in the presence of NaCl were no better adapted to growth in sub-optimal osmotic conditions than spores with no history of NaCl exposure.

### FdBev 04 Comparative genomic indexing of proteolytic *Clostridium botulinum*

**Andrew T. Carter**[1], David R. Mason[1], Carmen Pin[1], Catherine J. Paul[2], John W. Austin[2] & Michael W. Peck[1]

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A DNA microarray has been produced based on the genome sequence of the Hall A strain (ATCC 3502) of proteolytic *Clostridium botulinum*. It contains 3433 CDS (coding sequence(s)) probes (approximately 94% of those in the genome) in addition to 19 for those of the plasmid. Not all CDS were suitable templates for PCR-based probe synthesis, due to CDS duplication and/or low G+C content (28.2%). Additional features were incorporated to facilitate the genotyping of new strains isolated from disease outbreaks or bio-terrorism incidents. These include the N- and C- termini of type A-G neurotoxin genes (including the type $A_3$ neurotoxin sub-type), plus 17 probes for neurotoxin-associated genes (including haemagglutinins, NTNHs, cntRs, p47, lycA, ORFX1, ORFX2 and ORFX3). All tested strains of proteolytic *C. botulinum* (toxin types A, B, F and mixed toxin types) and *C. sporogenes* were closely related, but could be distinguished using the microarray. Proteolytic *C. botulinum* strains forming type A1 toxin appeared to be most closely related to the Hall A strain. The tested strains of proteolytic *C. botulinum* shared more than 89% of the Hall A CDS, while the *C. sporogenes* strains shared more than 84% of the Hall A CDS. DNA from strains of non-proteolytic *C. botulinum* and *C. difficile* failed to give meaningful results on the microarray, confirming the large phylogenetic distance between these clostridia and proteolytic *C. botulinum*. Two prophages found in the Hall A strain were absent from all other strains tested, although hybridization data suggests that many strains have related prophages of their own.

We are grateful to the competitive strategic grant of the BBSRC and a CRTI-IRTC operating grant for funding this work.

## FdBev 05 Antimicrobial and self-cleaning properties of $Ag/TiO_2$ and $CuO/TiO_2$ films deposited by chemical vapour deposition

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Glass surfaces coated with a thin film of $TiO_2$ are widely used as self-cleaning surfaces for windows. TiO2 is a semiconductor and Irradiation with long wavelength ultraviolet irradiation (UVA) promotes electrons from the valence band to the conduction band leaving a positively charged hole. The electrons and holes migrate and, at the surface react to give reactive oxygen species such as ·OH and $O_2^-$. These in solution can react to give $H_2O_2$. These act to convert contaminants to $CO_2$ and $H_2O$. The surfaces also have biocidal activity against bacteria and viruses but are dependant on irradiation for activity. The work will describe the activity of films which combine the activities of dual coatings which incorporate either Ag or Cu (as oxides), either as a top layer or as a bottom layer overlaid with a layer of $TiO_2$. The activity against *Escherichia coli* and bacteriophage T4 will be reported. These combinations have a much higher biocidal activity against bacteria and viruses than $TiO_2$ alone. There is also activity in the dark, albeit at a lower level than in the light. The coatings are suitable for self cleaning-self sterilizing surfaces for the food industry and medical applications.

## FdBev 06 Horizontal transmission of ABR plasmids in food preservation-stressed bacteria

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*Objectives* This study investigated the possibility that sub-lethal food preservation stresses (high/low temperature, osmotic and pH stress) can alter the rate of horizontal transmission of ABR plasmids between *E. coli* strains and between *E. coli* and *Salmonella* Typhimurium.

*Methods* Plasmid-bearing *E. coli* donor cultures (NCTC 50021, F1 plasmid R386 or NCTC 50338, Inc.gp. I1 plasmid TP307) and recipient *S.* Typhimurium cultures were mated under a range of sub-lethal environmental stress conditions (low/high temperature, low pH, high NaCl). A transfer rate was determined for each donor/recipient/stress combination.

*Results* The study found that the horizontal transmission rate of F1 plasmid R386 and Inc I1 plasmid TP307 is significantly increased ($p< 0.05$) when pre-stressed donor and recipient cells are mated under conditions of environmental stress.

*Conclusion* The use of bacteriostatic (sub-lethal), rather than bacteriocidal food preservation systems, may be contributing to the dissemination of ABR among food-borne pathogens.

## FdBev 07 Investigating the incidence of antibiotic resistance genes in Gram-negative bacteria isolated from retail poultry meat sold in Northern Ireland

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Antibiotics and other veterinary medicines can provide treatment and protection against disease causing bacteria within the farming industry.

However there are increasing concerns that overuse of such medication leads to an increased number of antibiotic resistant bacteria within the food chain. Traditional and molecular techniques have been utilised to investigate the incidence of a range of these genes found in Gram-negative bacteria isolated from retail poultry meat originating from Great Britain, Northern Ireland and Republic of Ireland. A range of antibiotic resistance genes were found in a number of samples from each of the areas, with, for example, Tetracycline resistant bacteria carrying more than one of the Tet resistance genes. To conclude, bacteria isolated from retail poultry meat have been shown to carry a range of antibiotic resistance genes which could potentially lead to an increase in the numbers of multi-resistant bacteria in the food chain.

## FdBev 08 Antimicrobial effect of dairy powders on *Streptococcus mutans*

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The antimicrobial properties of milk protein fractions, such as lactoferrin, have previously been reported. The aims of this study were to determine the potential of dairy powders to inhibit the growth of the dental caries inducing bacteria Streptococcus mutans. The effect of hydrolysis with porcine pancreatic lipase (2-18 hrs at 37°C) on the inhibitory effect of the powders was also assessed. With the exception of Cream powder, none of the unhydrolysed powders displayed antibacterial properties. Hydrolysed Acid WPC80, Sweet WPC35, Buttermilk powder and Whey Protein Isolate were ineffective as inhibitors. In contrast, hydrolysed Sweet WPC80, Cream Powder and an emulsion of butter in demineralised whey, inhibited S. mutans ($P\leq0.05$) at all concentrations >0.6mg/ml. Although demineralised whey, showed little or no inhibitory activity on its own, when emulsified with butterfat, significant and substantial inhibition was observed at concentrations as low as 0.08mg/ml. Enzyme treated dairy products may have potential for incorporation into various products as a natural antimicrobial.

## FdBev 09 Safety assessment, critical control of some traditional milk and cereal products in Nigeria

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Quality assessment of some local milk and cereal products were studied using hazard and critical control point concept. Milk samples were collected randomly from nomadic herds from southwest, northeast and north central zones of Nigeria and tested microbiologically at different stages of preparation, handling and storage. Toxicity of some of the pathogens was assessed. Most of the local milk products were contaminated with pathogenic and spoilage microorganisms. Although, the fermented cereal products analyzed were generally safe, risk increased due to faulty procedures, contamination during processing, handling and storage at ambient temperature. Contamination is also a function of the quality of raw materials used as starter cultures. The need to educate milk and cereal products processors as well as customers on the application of basic food safety principles to enhance food quality and safety is mandatory for healthy living.

Milk product pathogen includes; *Staphylococcus aureus, E. coli, Bacillus cereus*, and spoilage strains of yeast and mould. Cereal products contaminating agents includes; *Bacillus cereus, Staphylococcus aureus, Salmonella* sp. and *E. coli.*

*Keywords* fermentation, food safety, kunun-zaki, milk

# Posters

## Microbial Infection Group

### MI 01 Two component regulators of *Francisella tularensis*

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The Gram negative coccobacillus *Francisella tularensis*, the etiological agent of tularaemia, can cause serious disease in humans. The low infectious dose of *F. tularensis* has lead to concerns about the potential use of this organism as a bioweapon. A live vaccine strain, *F. tularensis* subspecies *holarctica* strain LVS, was developed but remains unlicensed due to a range of problems with the strain. Thus a licensable vaccine is required. The aim of the project is to produce a characterised defined two-component regulatory systems (TCS) mutant of *F. tularensis* that is attenuated and protective, that could form the basis of a new live attenuated tularemia vaccine.

TCS are widespread signal transduction devices in prokaryotes. TCS have been well studied in many pathogenic bacteria, and they are known to regulate expression of many key virulence factors. Inactivation of these genes is likely to result in gross effects, including attenuation.

There are currently three TCS annotated in the *F. tularensis* SchuS4 genome sequence determined by simple BLAST analysis. Therefore a more in-depth analysis of the genome sequence will be performed to ensure that these are the only TCS possessed by the pathogen. Isogenic mutants in the annotated TCS and in any we subsequently identify by this bioinformatic analysis will be produced and assessed.



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### MI 02 Characterisation of a *Francisella tularensis* subspecies *tularensis panCD* mutant

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*Francisella tularensis* is a Gram-negative, intracellular pathogen, which causes the debilitating disease tularemia in humans and a wide range of animals. The attenuated *F. tularensis* live vaccine strain (LVS) can induce protective immunity against tularemia in humans, but the basis of attenuation is not understood at the molecular level, thus preventing its licensing as a live vaccine. Therefore we aimed to construct a rationally defined mutant that would be suitable for licensing as a vaccine. The *panCD* gene, which is part of the pantothenate biosynthetic pathway, has been identified as a potential target for mutagenesis. The genes encode enzymes required for the production of vitamin B5. Inactivation of these genes has been shown to attenuate *Mycobacterium tuberculosis* and this attenuated mutant was able to induce a protective immune response in the mouse model. In order to create a *Francisella* mutant, a defined deletion was introduced into the *panCD* gene. The inactivated gene was then cloned into the suicide plasmid pSMP75 which contains the *sacB* gene and a kanamycin resistance gene as a means of selection. Conjugation was used to introduce the construct from *E. coli* S17λ*pir* into the virulent *F. tularensis* Schu 4 strain. A defined *panCD* mutant was isolated and was characterised *in vitro*.

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### MI 03 Generation of knock-out mutants in the LysR-type transcriptional regulators (LTTR's) of *Francisella tularensis*

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*Francisella tularensis* was first described in 1911 and is a facultative intracellular pathogen found primarily in the Northern hemisphere. It is transmitted by arthropod vectors and is one of the most infectious pathogens known. In the 1930's *F. tularensis* was weaponised and in the 1950's a live, attenuated strain (LVS) was isolated and used as a vaccine. This strain provides incomplete protection and its mode of attenuation remains undefined; it is therefore not yet licensed for use. Consequently the necessity to develop a well characterised, live attenuated vaccine strain still remains and can be achieved using methods that rely upon site directed mutagenesis and subsequent characterisation.

The LysR-type group of transcriptional regulators (LTTR's) are the most common family of transcriptional regulators found in prokaryotes. They are homo-di/tetrameric proteins that bind to DNA via a highly conserved winged helix-turn-helix binding motif. LTTR's are documented to control a variety of genes involved in metabolic functions, stress, virulence and quorum sensing. Eight putative LTTR's have been identified in the *F. tularensis* SCHU S4 genome, but it is not obvious what genes are being regulated. As a consequence of the involvement of this type of regulator in virulence, we hypothesise that mutations in these genes will produce a strain that is both attenuated and protective against challenge with live *F. tularensis* SCHU S4 in a mouse model. Knock-out mutations in *F. tularensis* are being generated utilising a suicide vector carrying a non-functional version of each of the LTTR-genes of interest; preliminary results are discussed.



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### MI 04 The expression and purification of immunoreactive proteins of *Burkholderia pseudomallei*

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*Burkholderia pseudomallei*, the causative agent of the disease melioidosis, is a micro-organism endemic in South-East Asia and Northern Australia, where it causes fatal pneumonia and septicaemia. These infections are difficult to treat as the bacterium is resistant to several antibiotics and there is currently no vaccine available.

Proteins located on the cell surface of bacteria have been used successfully as vaccine candidates against a number of different micro-organisms. A proteomics based approach was used to identify a panel of cell surface located immunoreactive proteins in *B. pseudomallei*. The genes encoding these proteins were then cloned into suitable expression vectors and experiments carried out to determine the optimal conditions for expression. Here, we present work on the expression and purification of these proteins. It is possible that one of these immunoreactive proteins could form a component of a new subunit vaccine to protect against *B. pseudomallei*.



## MI 05 Polysaccharides and virulence of *Burkholderia pseudomallei*

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*Burkholderia pseudomallei* is the causative agent of melioidosis, an infectious disease of humans and animals. Gene clusters which encode capsular polysaccharide (type I O-PS) and lipopolysaccharide (type II O-PS), both of which play roles in virulence, have previously been identified. Here we report the identification of two further putative clusters, type III O-PS and type IV O-PS. Mice challenged with type III O-PS or type IV O-PS mutants show increased mean times to death (7.8 days and 11.6 days) compared to mice challenged with wild type *B. pseudomallei* (3 days). To investigate the possible roles of polysaccharides in protection, mice were immunised with killed cells of wild type *B. pseudomallei* or killed cells of *B. pseudomallei* with mutations in the O-antigen, capsular polysaccharide, type III O-PS or type IV O-PS gene clusters. Immunisation with all polysaccharide mutant strains resulted in delayed time to death compared to the naïve controls following challenge with wild type *B. pseudomallei* strain K96243. However, immunisation with killed polysaccharide mutant strains conferred different degrees of protection, demonstrating the immunological importance of the polysaccharide clusters on the surface of *B. pseudomallei*.



## MI 06 Recombinant vaccinia proteins for use in a smallpox sub unit vaccine

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The causative agent of smallpox is *variola* virus. The current smallpox vaccine is comprised of live *vaccinia* virus, which is applied to the skin by scarification resulting in a localised infection. Whilst being very efficacious, inflammation and virus shedding occur at the resulting lesion, which represents a source of secondary transmission. Moreover, young children and immuno-compromised vaccinees are highly susceptible to complications from this live vaccine (Cono *et al*, 2003). Our aim was to produce a sub-unit vaccine replacement for the current live smallpox vaccine that would be safe and licensable.

Initial studies highlighted two *vaccinia* virus genes, that when administered to mice as DNA vaccines offered measurable levels of protection against an intranasal challenge with a lethal dose of *vaccinia* virus strain IHD (Galmiche *et al*, 1999). B5R and A27L were subsequently cloned to produce recombinant sub-units. In a murine model of lethal Othopoxvirus respiratory infection we have achieved protection equivalent to that of the current live vaccine when these two sub units were delivered together with CpG DNA (Coley Pharmaceuticals).



## MI 07 Effects of antimicrobial peptides against *Bacillus anthracis* spores

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Antimicrobial peptides can be found in all species of life; humans, amphibians, insects, reptiles, mammals and plants. They are effective against a broad host range including Gram negative and Gram positive bacteria, fungi and some viruses. Acquired resistance to the activity of antimicrobial peptides in target species is extremely rare in comparison to resistance to traditional antibiotics.

Antimicrobial peptides from a range of species were assessed for antimicrobial activity against ACDP Category 2 *Bacillus anthracis* spores in a time kill assay. The antimicrobial effects of these peptides were studied as singular peptides and in combination to determine compatibility or synergic effects.

Antimicrobial peptides are synergistic with conventional antibiotics. Hence, rapid acting antimicrobial peptide combinations may prove beneficial as a generic therapeutic against multiple potential Biological warfare agents.



## MI 08 Investigating the use of CpG as an adjuvant for an adenovirus-based vaccine against Venezuelan Equine Encephalitis Virus

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Venezuelan equine encephalitis virus (VEEV) is an important human and equine pathogen, especially in Southern America. Because it has potential for use as a Biological Weapon, there is a requirement to develop a vaccine which protects against airborne VEEV. Previously, we have developed a mucosally-delivered Adenovirus (Ad)-based vaccine which encodes structural proteins of VEEV. This was able to protect mice against airborne challenge with a number of VEEV strains. In the present study, we use a B-class CpG oligodeoxynucleotide in conjunction with the Ad-based vaccine with a view to enhancing both immune responses and protective efficacy. The results show that, in the mouse model, addition of CpG does not significantly enhance the immune response to the encoded transgene, but does increase vector-specific responses. Protective efficacy is not improved. Implications of these results are discussed.



## MI 09 Molecular characterisation of *Streptococcus pyogenes* two-component signal transduction systems

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Two-component signal transduction systems (TCS) play a key role in the regulation of virulence factors of various bacterial pathogens and have been proposed as targets for therapeutic agents. In this study, the genomic sequence of *Streptococcus pyogenes* strain MGAS2096 was interrogated using bioinformatic tools. Thirteen TCS have been characterised and their molecular architecture has been revealed. Protein sequence alignments of histidine kinases (HK) and response regulators (RR), shows that seven of the TCS have been characterised before. The domain-based structure of each HK and RR of the TCS has been examined and data strongly suggests that all RRs presented in this study are transcriptional regulators.

55 clinical isolates were screened, using PCR, for the presence of five of the uncharacterised TCS. All strains were positive for the TCSs, except one throat swab isolate for which TCS10 was missing. The DNA sequence conservation for these TCS has been investigated.

These uncharacterised TCS could form targets for investigations into the control of gene regulation in streptococci and act as targets for novel therapeutic agents.

## MI 10 Detection of bacterial DNA in clinical swabs using FTA paper and PCR

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The use of PCR based approaches is revolutionising detection of bacterial pathogens in clinical samples. However, sample preparation is often costly and time consuming. FTA paper cards have been used in forensic science for storage of human DNA and have recently been shown to kill pathogenic bacteria and stabilise their DNA for storage at room temperature. However, previous studies have used bacterial cultures for inoculation of FTA papers. We have assessed the utility of FTA paper cards for extraction of bacterial DNA directly from 50 clinical swabs carrying between one and four bacterial species. Good correlation was seen between results from PCR reactions with card plugs carrying DNA and culture based findings. This approach has significant implications for making safe samples that contain highly pathogenic organisms. It is possible that FTA paper cards could be introduced into routine molecular diagnostic assays to greatly reduce sample preparation time and make high risk samples safe for handling by lab personnel.

## MI 11 Identification and characterisation of peptide antibiotics showing activity against *Staphylococcus aureus*

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The emergence and wide geographic spread of multidrug-resistant *S. aureus* has initiated a search for alternative chemotherapeutic agents active against these bacteria. One class of agent that has received increasing attention recently are the basic polypeptides, or bacteriocins. Gram positive bacteria have attracted the focus of many reserchers as bacteriocin producers. These bactriocins have not yet been used in the setting of chemotherapy on the same scale as traditional antibiotics.

In the present study, 513 *Staphylococcus aureus* and 250 *Staphylococcus epidermidis* strains were investigated for activity against *S. aureus* using simultaneous and deferred antagonism methods. The investigation process has revealed 14 bacteriocin-like agents that have activity against methicillin resistant *S. aureus* (MRSA). Amongst the 14 isolates 2 have shown lysin-like inhibitory activity, whereas 12 isolates have been confirmed as potential bacteriocin producers. Initial characterisation of the spectrum of activity of some of these agents suggests that they may have potential as topical therapeutic agents for treating staphylococcal infections, including those caused by MRSA.

## MI 12 Screening for antimicrobial peptides active against biofilm forming coagulase negative staphylococci

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In recent years the coagulase negative staphylococci (CNS) have become one of the most important causes of medical device related infections due to their ability to form bacterial biofilms. The increasing resistance of biofilm forming bacteria to currently available antimicrobial chemotherapy enforces the requirement for more effective agents. Antimicrobial peptides provide a potential alternative group.

The aim of this study was to screen over 800 organisms including CNS, *Staphylococcus aureus* and other gram positive strains for activity against clinically isolated strains of biofilm forming CNS. Simultaneous and deferred antagonism methods were used to identify potential inhibitors. Two strains exhibiting peptide activity inhibited the growth of biofilm producing strains of CNS, inhibiting 100% of 19 biofilm forming clinical CNS isolates.

The results clearly indicate the peptide substances produced by these two isolates could potentially represent novel agents that could be utilised to impede bacterial proliferation and biofilm formation on medical devices.

## MI 13 Conserved lipoproteins of *Staphylococcus aureus*

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*Staphylococcus aureus* is a major cause of nosocomial infections and community-associated MRSA infections are increasing in incidence. Secreted proteins e.g.toxins play major roles in pathogenesis. The final destination of a protein is governed by the presence or absence of signal peptides and/or retention signals. These are signal sequences at the N-terminus which direct proteins for transport through the cytoplasmic membrane. Secreted proteins in Gram-positive bacteria can be targeted to three distinct cellular locations: the cytoplasmic membrane, the cell wall and the outer leaf of the membrane or can be excreted into the surrounding medium. Whilst the role of excreted proteins and cell-wall anchored proteins is well characterized, the role of lipoproteins has not been established.

Using bioinformatic and molecular techniques a group of seven conserved lipoproteins were identified. Three proteins were involved in housekeeping functions of signal peptide processing of lipoproteins, diacylglyceryl transferase and a protein involved in localisation of membrane proteins. One protein had an RGD motif which may be involved in protein-protein interactions. There was no close match for this protein in other bacteria. Three other conserved lipoproteins were analogues of iron transport proteins which may be linked to virulence. The proteins may provide novel targets for interference with pathogenicity.

## MI 14 Presence of non-haemolytic pneumolysin in serotypes of *Streptococcus pneumoniae* associated with disease outbreaks

**Calum Johnston**, Johanna Jefferies, Lea-Ann Kirkham, Graeme Cowan, Andrew Smith, Stuart Clarke, Angela Brueggemann, Robert George, Bruno Pichon, Gerd Pluschke & Tim Mitchell

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*Streptococcus pneumoniae* is a human pathogen causing serious diseases including meningitis, pneumonia and bacteraemia. The haemolytic toxin pneumolysin (Ply) is an important virulence factor of this organism. Sequence analysis of the *ply* gene from a number of isolates revealed 14 Ply alleles, with varying degrees of haemolytic activity. This level of variation is higher than expected for a cytoplasmic protein. A non-haemolytic Ply allele was found to be harboured by the prominent clones of serotypes 1 and 8, classified by Multi-Locus Sequence Typing (MLST). These isolates retained the

ability to cause invasive pneumococcal disease despite lacking the haemolytic activity of the toxin. Both serotype 1 and 8 pneumococci are known to be associated in particular with outbreaks of invasive disease, and neither is present in the current 7-valent pneumococcal vaccine. This non-haemolytic Ply allele is therefore associated with the dominant clones of outbreak-associated serotypes of *S. pneumoniae*.

## MI 15 Analysis of a putative glycosylation locus from *Streptococcus pneumoniae*

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The genome of human pathogen *Streptococcus pneumoniae* strain TIGR4 contains a 37kb DNA island not present in strain R6. This contains 18 putative genes: glycosyl-transferases, alternative secretion genes and an ORF encoding a large serine rich repeat protein annotated as SP1772 in the TIGR4 genome. A homologue of SP1772 in *Streptococcus gordonii* is involved in adhesion to platelets. Three of four unfinished pneumococcal genomes, sequenced in collaboration with the Sanger Centre, contain this locus. We have annotated the loci and found a naturally truncated homologue of SP1772 in a serotype 14 strain. Microarray and southern blotting are being used to examine the distribution of this locus among disease causing pneumococcal isolates. Analysis of a SP1772 null mutant in the TIGR4 background shows this putative gene plays no role in pathogenesis by single strain infections in systemic and respiratory infection models. We are investigating whether this locus plays a more subtle role *in vivo* by use of competitive index methods and models of nasopharyngeal colonisation.

## MI 16 Novel adjuvant properties of pneumolysin, a toxin produced by *Streptococcus pneumoniae*

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*Streptococcus pneumoniae* is a human pathogen that causes both mucosal and invasive diseases, including pneumonia, bacteremia, otitis media, and meningitis, throughout the world. Currently available vaccines are based on the capsular polysaccharide. As polysaccharide vaccines are poorly immunogenic in those most at risk of disease new protein/polysaccharide vaccines have been developed. These vaccines are expensive to produce, limiting their use in the developing world where the burden of disease is greatest. New strategies are required that provide capsular and serotype independent protection. Here we show that proteins genetically fused to pneumolysin (Ply) become capable of generating an antibody response that is not seen when they are administered as a mix. In this study, pneumococcal surface adhesin A (PsaA) was fused to the N terminal of Ply and administered intranasally to mice. Serum IgG responding to PsaA and Ply were at a level comparable to those produced by parental administration. We also report the protective efficacy of these responses.

## MI 17 Comparison between the reliability of serologic and bacteriologic tests and incidence of Brucellosis in Kerman (Iran)

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Brucellosis is a zoonotic disease caused by gram negative bacteria Brucella that are pathogenic for a wide variety of animals and human beings. Recent studies have shown that prevalence rate of Brucellosis varies in different parts of Iran. This study has been carried out in two separate parts:

I) Comparison between the reliability of serologic and bacteriologic tests. This test was preformed on 119 blood and serum samples taken from patients. A sample of blood for culture and serum samples of the patients were tested with Rose Bangal, Wright test by tube method, 2ME, Coombs Wright and ELISA.

The results of the above tests were as follows:

A. Twenty four (20%) out of 119 patients tested with Rose Bangal and Wright had anti brucella antibody titre (> 1:80).

B. Seventeen (14.2%) patients had positive antibody titre in 2ME test titre.

C. In those patients who had negative Rose Bangal and Wright test, (95 patients) five of them (0.2%) showed positive Coombs Wright test.

D. Twenty eight patients had positive IgG (23.5%), and forty patients had positive IgM titre (33.6%) in ELISA test. Only one case of positive culture was detected in bacteriologic study, the serum of this patient showed positive Rose Bangal, Wright and 2ME test (> 1:40).

As a result of the above study, Wright test was chosen as the diagnostic test of Brucellosis in the second part of our study because of feasibility, higher sensitivity and reliability.

II) Six hundred serum samples from age group 20-40 years old residents of city of Kerman were analysed. Fifteen (2.5%) individuals had antibody titre equal to or higher than 1:80 (8 males and 7 females). The difference in the rate of infection among males and females wasn't significant. The highest rate of infection (4%) was seen in age group of 25-29 years old. Based on occupation, the highest rate of infection (10%) was among workers and farmers and the lowest rate was among business men.

Therefore the prevalence of Brucellosis in the city of Kerman seems somewhat lower than the overall figure of the country (3.5%).

## MI 18 An investigation into the biological properties of spore surface component(s) from the opportunistic pathogen *Aspergillus fumigatus*

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*Aspergillus fumigatus* is a saprophytic fungus responsible for 90% of aspergillosis in humans. This study focused on the effect of diffusible spore surface component(s) from *Aspergillus fumigatus* (termed *Af*D) on the function of human neutrophils. Neutrophils are an important first line of defence and have a short life-span that involves an orchestrated process of migration, phagocytosis and killing. Human neutrophils were treated with *Af*D and migration was assessed by the ability of neutrophils to change shape (polarise) and transverse a polycarbonate filter. Phagocytosis was measured by the ability of neutrophils to ingest FITC-labeled *E. coli* and the respiratory burst was measured by the release of superoxide anion. Further investigations looked at *Af*D-induced programmed cell death (PCD). Results showed *Af*D to reduce neutrophil polarisation (76±6%), migration (57± 29%), phagocytosis (31±6%) and respiratory burst (95±2%). *Af*D also induced PCD through a heat-sensitive component(s); therefore, it is likely that the anti-inflammatory properties are explained by the *Af*D-induced PCD. Furthermore, *Af*D has heat-sensitive and heat-stable active compounds suggesting multi-component activity.

## MI 19 Exopolysaccharide production in the *Burkholderia cepacia* complex

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The *Burkholderia cepacia* complex (BCC) was first identified as the cause of onion rot, but has recently emerged as a major pathogen in cystic fibrosis (CF).

Exopolysaccharide (EPS) could be an important virulence factor of the BCC and has been compared to the role of alginate in mucoid *Pseudomonas aeruginosa*. Animal models show a correlation between conversion to a mucoid phenotype and persistence in the murine lung. However, most BCC isolates are phenotypically non-mucoid, and the molecular basis of EPS biosynthesis and regulation in BCC are unclear. When grown on onion extracts, the original host, the majority of strains tested produced copious amounts of EPS. The aim of this study was to identify the plant factors responsible for EPS production. Paper electrophoresis, chromatography, acid hydrolysis and HPLC of onion extract revealed a number of sugars. Collectively, and individually some of these sugars induced EPS production. This study highlights the metabolic potential of the BCC and reveals a phenotype on 'plant host extract' which would be missed in routine culture.

## MI 20 Use of suppression subtractive hybridization to characterise genome diversity in *Campylobacter jejuni*

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Improved knowledge of the variable genome of *Campylobacter jejuni* may uncover links between variable gene distribution and strain phenotypes such as virulence and host preference. Known variable genes currently come from only human or chicken strains and may be unrepresentative of strains from potential disease reservoirs such as wildlife and ruminants. We carried out five subtractions using strains from alternative hosts (cow, wild bird, rabbit), both between and within MLST clonal complexes.

Using BLASTX 97% of 195 subtracted sequences matched *Campylobacter* proteins already in the database. The distribution of eight sequences was analysed by screening isolates from diverse sources and clonal complexes, using PCR, dot blots and Southern blots. Variation in sequence distributions existed both within and between clonal complexes.

There was no evidence to link the distribution of subtracted sequences with host species. However there was a clear correlation between subtracted sequences and clonal complex, suggesting minimal lateral transfer of these genes between bacteria from different clonal complexes.

## MI 21 Over-production of pyocyanin is widespread amongst isolates of a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa*

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Infection by *Pseudomonas aeruginosa* plays an important role in the morbidity and mortality associated with cystic fibrosis (CF). In the Liverpool adult CF unit, it has been shown that patients infected with the transmissible Liverpool epidemic strain (LES) of *P. aeruginosa* suffer greater morbidity than those infected by non-LES strains. In some LES isolates, an unusual hypervirulence phenotype has been identified. Using assays for the quorum sensing-regulated exoproducts pyocyanin and LasA, we have demonstrated that this unusual phenotype is widespread amongst LES isolates. Sequential isolates from six individual patients revealed a wide variation in exoproduct secretion, antimicrobial susceptibility, morphology, motility, auxotrophy and hypermutability. Some LES isolates secreted no pyocyanin or LasA, due to mutations in the *lasR* gene. Isolates with the same colony morphology were found to have very different phenotypic characteristics. These studies suggest that the unusual hypervirulence phenotype may play a major role in the greater morbidity of CF patients colonized by the LES.

## MI 22 RTX toxin ApxIV of *Actinobacillus pleuropneumoniae* is secreted *in vitro*

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Contagious pleuropneumonia, caused by *Actinobacillus pleuropneumoniae* (App), is a severe bacterial respiratory disease of the pig. Apx toxins are pore-forming toxins of the RTX group (Repeated in Toxins). The fourth toxin, ApxIV was discovered by Schaller *et al.* (1999) using recombinant ApxIV produced in *E. coli* to detect anti-ApxIV antibodies in the infected pigs. It was shown to be a large protein (180 kDa), and like anthrax holotoxin, to be produced only *in vivo*. A defined ApxIV-negative mutant was derived in App serotype 15 using natural transformation and allelic exchange by insertion of *kan*$^r$ cassette into the *apxIVA* gene. To mimic the conditions needed for expression of ApxIV, we tested a range of culture conditions, including divalent cations. $CaCl_2$ was shown to cause production in culture, of a protein of 180 kDa which was absent from the mutant strain. The optimal concentration of $Ca^{2+}$ for ApxIV production was 10 mM. The role of this toxin in the presence and absence of other Apx toxins is under investigation.

## MI 23 Polar flagella glycosylation in *Aeromonas caviae*

**M.J. Day**[1], S.M.B. Tabei[1], P. Hitchen[2], A. Dell[2] & J.G. Shaw[1]

[1]University of Sheffield, Sheffield; [2]Imperial College London

*Aeromonas* cause disease in poikilothermic animals and in humans. They are motile by the action of a single polar flagella, the flagellins of which are post-translationally modified with pseudaminic acid, a nine-carbon sugar related to sialic acid, that is also found on the flagella of *Campylobacter* and *Helicobacter*. The genes required for this modification are located in the *flm* locus. Downstream of this locus has been sequenced by inverse PCR to ensure the full extent of the locus had been reached. The role of the gene products of this locus are under investigation by their effect on flagella expression. Moreover, a potential role for the flagella glycosylation in their interaction with the immune system is also under investigation. His-tag recombinant flagellin proteins have been overexpressed in *E.coli* and purified and his-tag glycosylated flagellins have been overexpressed in *Aeromonas*. These flagellins were used in an IL-8 stimulation assay and it was found that the unglycosylated flagellins highly stimulate IL-8 production. It is expected that the glycosylated flagella will have similar effects.

## MI 24 DNAase activity of aerobic bacteria from the human tongue

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The human tongue harbours about 100 species of bacteria which must bind tightly to the tongue surface to prevent the bacteria being

washed off. We are using metagenomic DNA libraries of these tongue bacteria to search for adhesins by phage display. One major problem in preparing metagenomic DNA samples by scraping the tongue is the presence of human cells and thus human DNA in the tongue scrapes. We have developed a method for isolating the bacterial DNA and removing the human DNA before bacterial cell lysis. During this method development we observed some bacterial isolates secreted DNAase activity which possibly contributed to the destruction of the DNA released from lysed human cells.

We have carried out 16s ribosomal DNA sequencing of the DNAase producing aerobic bacteria and partially characterised the DNAase enzymes from some of these isolates.

## MI 25 Design of quantitative assays for trichomonads

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*Trichomonas vaginalis,* the most prevalent form of non-viral sexually transmitted disease is detected traditionally using culture and microscopy methods. Limitations of these techniques such as low sensitivity with respect to number of organisms and culture period can be overcome by molecular techniques such as PCR and Real – Time PCR. In this study a quantitative PCR was developed, with the primers targeting the *cysteine proteinase* regions of the *T.vaginalis* genome.

The newly designed CP1cd, CP1ab and ND CP2 primer sets were highly specific to both pure *T.vaginalis* DNA and trichomonad DNA isolated from urine. In combination with BTUB, a beta tubulin primer set, CP1cd, CP1ab and NDCP2 primer sets gave amplicons of 154bp, 179bp, 202bp and 110bp respectively as expected. Therefore the multiplex of CP primer sets with the BTUB primer set can provide more valuable and reliable information for diagnostic purposes. These novel primers when tested with quantitative Real – Time PCR, proved to be highly specific with a minimum sensitivity of one trichomonad cell. Combination of primer multiplexing with Real-Time PCR, if successful in identifying various strains of *T.vaginalis* could reduce the number false positive results associated with other primers.

## MI 26 'Vomocytosis' of live pathogenic yeast by macrophages

**Hansong Ma**, Joanne E. Croudace, David A. Lammas & Robin C. May

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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 neoformans*. This expulsive process, which we have termed 'vomocytosis', does not result in host cell death. Expulsion is extremely rapid and can occur many hours after phagocytosis of the pathogen in both cultured cell lines and primary macrophages. Moreover, we found the expulsion occurs independently of the initial route of phagocytic uptake and does not require phagosome maturation. After the expulsive event, both the host macrophage and the expelled *C. neoformans* 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.

## MI 27 Antimicrobial immunity in the nematode, *Caenorhabditis elegans*

Elizabeth K. Marsh, Maaike C.W. van den Berg & **Robin C. May**

School of Biosciences, University of Birmingham, Birmingham

The nematode *Caenorhabditis elegans* has been used as a model laboratory organism for almost forty years and has recently been established as an alternative host for studying a variety of infectious organisms.

Since nematodes have no cell-mediated immunity, they rely entirely on secreted antimicrobial molecules to resist infection. Evolutionary theory predicts that selective pressure from pathogens will lead to the diversification and specialisation of such molecules. We now show that this is indeed the case for one family of antimicrobial molecules, the lysozymes.

Using microarray analysis and genetic knockouts, we demonstrate that:

1) different lysozyme combinations are expressed in response to infection by different micro-organisms.

2) loss of single lysozyme genes dramatically reduces resistance to some pathogens whilst leaving immunity to others unimpaired.

3) lysozyme genes are reciprocally regulated, such that any increase in resistance to one pathogen comes at the cost of increased sensitivity to another.

The nematode model therefore represents a powerful system with which to probe the evolution of immune mechanisms at both the molecular and species level.

## MI 28 The mucin utilisation ability effects the regulation of virulence gene expression in *Streptococcus pneumoniae*

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*Streptococcus pneumoniae* colonises the mucin-rich environment of the nasopharynx. As colonisation may be the first stage of infection, we investigated whether the pneumococcus could utilise mucin as a source of nutrient and whether its virulence gene expression is influenced by this glycoprotein. It was found that when grown in Siccard's defined medium supplemented with 1% w/v mucin, the organism grew at a rate similar to that in rich medium. We also report the presence of sialate *O*-acetylesterase activity, an enzyme implicated in glycoprotein degradation, was shown in pneumococcal cell extracts. We hypothesised that Neuraminidase A (NanA), which is linked to pneumococcal virulence, plays an important role in mucin utilisation. Mucin leads to an increase in *nanA* transcription and a NanA$^-$ deficient strain of pneumococcus exhibited an extended lag period when grown in medium supplemented with mucin.

## MI 29 Interaction of HCV NS5B with endoplasmic reticulum resident proteins

**Ahmed Mohamed**, A.R. Walmsley & R.P. Yeo

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*Background* Hepatitis C virus (HCV) infection is an important health problem worldwide. It infects approximately 170 million people and causes a wide range of liver diseases such as, acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Currently, there is no vaccine for HCV and the present treatment is limited to the use of interferon either alone or in conjunction with Ribavirin, but the rate of sustained virologic response remains suboptimal. Therefore,

there is an urgent need for the development of novel therapeutics or at least identify potential targets that may aid in protection.

Protein-protein interactions play a critical role in regulating HCV replication by the non-structural protein 5B (NS5B) (the virus RNA-dependent RNA polymerase) that forms the main component of the virus replication complex. In our attempt to further our understanding of HCV replication and the contribution of host factors to the replication process, yeast two-hybrid screening of a mammalian cDNA library was used to look for cellular protein-protein interactions with NS5B. Interestingly, a number of proteins have been identified to interact with NS5B within the yeast two-hybrid system. The biological function and the location of these proteins on the plasma membrane or endoplasmic reticulum, the site of HCV replication, strongly suggest that they may have a role in the virus replication.

## MI 30 Genetic analysis of bacterial biofilm formation

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A biofilm is an aggregation of microorganisms forming a complex microbial community. They impact on a wide variety of fields ranging from biotechnology to medicine. Much remains to be learned about their formation and dispersal, and how they contribute to antibiotic resistance. *Klebsiella pneumoniae* is a gram-negative, biofilm forming bacteria, and is a common pathogen implicated in a variety of infections. *K. pneumoniae* is clinically the most important member of the *Klebsiella* genus and as well as being a cause of pneumonia, is frequently implicated in urinary tract infections (UTI), specifically catheter related UTI. Treatment can become difficult due to this bacteria's biofilm forming capacity. The process behind the phenotypic shift from a planktonic to an attached biofilm state is little understood and we aim to identify the genes involved using molecular genetic techniques. We will discuss the development of the genetic technique RIVET (recombination-based in vivo expression technology) to identify genes induced during biofilm formation. This research will go some way to elucidate areas at which treatment can be targeted.

## MI 31 Functional genomics of *Photorhabdus asymbiotica* – Rapid Virulence Annotation (RVA) of pathogen genomes using invertebrate models

**María Sánchez-Contreras,** Ioannis G. Eleftherianos, Richard H. ffrench-Constant & Nicholas R. Waterfield

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*Photorhabdus asymbiotica* is an emerging human pathogen, also capable of infecting insects. The genome sequence is almost completed and the annotation is underway. We are particularly interested in the functional annotation of toxins and virulence factors.

We have developed a screening method using three model organisms: *Manduca sexta*, *Caenorhabditis elegans* and *Acanthamoeba polyphaga*. Screening a genomic library of *P. asymbiotica* with these three organisms has allowed the identification of toxins, secondary metabolites, secretion systems and completely novel virulence factors. Mapping these regions in the genome and comparing with *P. luminescens* TT01 (an insect pathogen sequenced strain) gave us clues about virulence regions that are exclusive to the human pathogen and play a role in infection.

This Rapid Virulence Annotation (RVA) is a powerful tool to identify virulence genes in other sequenced bacterial pathogens, thus helping the annotation proccess. Finally, in addition to providing an alternative to mammalian testing, RVA identifies new targets for the development of novel insecticides, nematicides and anti-protist drugs.

## MI 32 Cold water strawberry disease of rainbow trout (*Oncorhynchus mykiss* Walbaum)

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Coldwater strawberry diseases (CWSD), also known as Red Mark Syndrome, is a severe skin disease that has recently emerged in farmed rainbow trout in the UK. CWSD has severe commercial impact, as affected fish are down-graded at harvest. A multidisciplinary investigation was instigated to uncover the likely causes of the condition, assess its impact and recommend potential control measures.

The syndrome presents histopathologically as a full thickness dermatitis with extensive lymphocytic infiltration into the dermis, and limited epidermal involvement. Epidemiological investigations showed there was live fish-associated spread of CWSD from affected to unaffected stocks. Transmission studies provided further evidence that that the condition is infectious, with 114/157 naïve rainbow trout cohabited at 10 °C with CWSD-affected fish displaying characteristic external lesions 96 days later.

Results from a range of analyses of field and laboratory infected material (including histopathological examination, inoculation onto a variety of cell lines and bacteriological media, direct PCR amplification and cloning of bacterial 16S rRNA genes), deployed to identify the aetiological agent, are presented.

## MI 33 Enzyme-modified and unmodified buttermilk powder and cream powder can inhibit cariogenic streptococci adhering to hydroxylapatite

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Adhesion and colonisation of the tooth surface by *Streptococcus mutans* is the first step in pathogenesis of dental caries. *Streptococcus salivarius* has also been associated with progression of caries. Recent studies have shown that enzyme-modified whey products can inhibit adhesion of *S.mutans*. In the present study, the effect of enzyme-modified and unmodified buttermilk powder (BMP) and cream powder (CP) on the adhesion of both of these cariogenic streptococci to hydroxylapatite (HA) was examined, using a fluorescence-based microtitre plate assay. Enzyme-modified cream powder (EM-CP) was found to be the more effective ($P$=<0.05) inhibitor of adhesion at concentrations >0.125mg/ml and >0.25mg/ml for *S.salivarius* and *S.mutans*, respectively. CP and BMP, in both their enzyme-modified and unmodified states, were found to cause 100% inhibition of adhesion at 1mg/ml. Both unmodified BMP and CP caused complete inhibition at 0.5mg/ml also. Thus, prevention of dental caries may be a novel use for these dairy products.

## MI 34 Protein networks of *Rhodococcus equi*

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The intracellular pathogen *Rhodococcus equi* causes severe pyogranulomatous bronchopneumonia in young foals. Virulent strains possess an 80-90kb plasmid, which is required for survival within host macrophages. Virulence-associated proteins (Vaps) are encoded by a pathogenicity island (PI) within this virulence plasmid, including the

lipid-modified, surface-expressed VapA protein. Deletion of *vapA* results in attenuation and rapid host clearance of a *vapA* mutant strain in mice, showing that VapA is a virulence factor. Using the yeast two-hybrid system, we detected protein-protein interactions involving pathogenicity island-encoded proteins. Interactions were found between VapA and a number of other PI-encoded proteins, suggesting a central role for this protein. Radiolabelled HA or c-Myc-tagged proteins were produced *in vitro* and interactions were verified using co-immunoprecipitation. To determine the amino acids involved in VapA interactions, N- and C-terminal deletions were made in the VapA protein. Deletion of N-terminal residues of VapA was found to affect the detected interactions. This work provides the first evidence of VapA interacting with other *Rhodococcus equi* plasmid-encoded proteins and may help in determining a virulence mechanism for this organism.

## MI 35 Theatre footwear use outside theatres – is it a serious risk?

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*Background* Although there is no formal evidence that outdoor shoes are a source of infection within the operating theatre (Hughes *et al.*), it has been suggested that the process of changing shoes or putting on overshoes can result in contamination of hands of theatre staff. Studies of bacterial contamination of operating theatre corridor floors also indicate that a change of footwear should be as far as possible from the operating theatre (Nagai *et al.*). Both of these imply footwear as a possible culprit in increasing infection rates in the operating theatre.

*Aim* It is a frequent practice amongst theatre staff and clinicians to walk out of theatre to wards and other hospital departments in their theatre shoes, without overshoes, and then return back to theatre. This practice theoretically increases contamination in theatre. It is to prove or disprove that this audit was carried out.

*Material and methods* We performed a prospective audit and concluded that incidence of bacterial contamination in the footwear is similar in the control group (theatre footwear worn only in theatre) and test group (theatre footwear worn outside and inside theatre). Swabs from both the sides and soles of theatre shoes, of staff, from a control group (theatre footwear worn only in theatre) and test group (theatre footwear worn outside and inside theatre) were taken and cultured on agar plates. 20 samples of each were taken at around the same time of the day in theatre, during preparation time in anaesthetic room in the control group and the test group samples were taken randomly outside the theatre ( shoes from surgeons and anesthetists in the wards) and the results were then analysed.

*Results* The incidence of bacterial colonization in both the groups were similar and was not statistically significant.

*Conclusion* We feel that though changing footwear helps theoretically to reduce infection, our study doesn't provide conclusive evidence to support it.

## MI 36 Ers, a new transcriptional regulator involved in the virulence of *Enterococcus faecalis*

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*Enterococcus faecalis* is a human commensal of the lactic acid bacteria group but is also associated with nosocomial infections. This makes *E. faecalis* a very 'ambiguitous' micro-organism. Thus it appears important to understand why this 'safe' bacterium can become a dangerous pathogen. In this study, we have identified a new transcriptional regulator of *E. faecalis*, named Ers. This protein shares strong homologies with PrfA the major regulator of virulence in *Listeria monocytogenes*. Quantitative real-time PCR was carried out to identify genes potentially regulated by Ers. The results reveal that *ers* itself and *ef0082* are positively regulated by Ers. Phenotypical analysis of the *ers* mutant reveals that Ers is important for pathogenicity and oxidative stress response of *E. faecalis*. Ef0082 does not appear to be implicated in pathogenicity, but based on sequence homologies, could be an efflux pump involved in the quinolone resistance. In conclusion, the Ers protein was characterized as a new virulence factor in *E. faecalis* and the identification of the Ers regulon by global transcriptional analysis is in progress.

## MI 37 The *Legionella pneumophila* plasminogen activator: cloning and functional analysis

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*Legionella pneumophila,* a Gram-negative facultative intracellular pathogen, is the causative agent of a severe form of pneumonia called Legionnaires' disease. As outer membrane proteins (Omps) are temporally and spatially the first proteins to interact with the host cell, Omps are important determinants for virulence. In a search for *L. pneumophila* Omps involved in bacterial virulence, we identified a gene encoding a homologue of the plasminogen activator of *Yersinia pestis* belonging to the class of omptins. The omptins are a family of surface proteases/adhesins that share high sequence identity but individual omptins exhibit differing virulence-associated functions.

We describe here the cloning of the plasminogen activator homologue of *L. pneumophila* and show that the protein has the capacity to convert plasminogen into plasmin. The relevance of the plasminogen activation for *Legionella* virulence will be discussed.

A better understanding of the function of *L. pneumophila* Omps and their role in infection, is very important in order to get a profound insight in the *legionella*-host interaction and in its pathogenicity.

## MI 38 A study on tinea gladiatorum in young wrestlers and dermatophytes contamination of wrestling mats from Sari-Iran

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*Objective* For the first time we studied the prevalence of tinea gladiatorum among young wrestlers and dermatophytes contamination of wrestling mats from Sari city the capital of Mazandaran, a northern Province of Iran.

*Design* We examined 324 wrestlers (age range 9-20 years) from 7 active clubs in Sari city and obtained skin scrapings from 135 of suspected wrestlers to tinea gladiatorum. The scraped skin samples were evaluated with potassium hydroxide examination. Pleated carpet sterile fragments (5x5 cm) were used for survey of wrestling mats contamination. Sabouraud's dextrose agar with and without chloramphenicole and cyclohexamide (SC and SCC) was used to culture scrapings and wrestling mats samples. The dermatophytes were identified by routine laboratory techniques.

*Results* Our study showed that of the 324 wrestlers, 65 (20.1%), presented tinea gladiatorum. The most lesions have been on the trunk and head. All of wrestling mats samples was positive for dermatophyte. *Trichophyton tonsurans (T. tonsurans)* was isolated from all of scrapings and wrestling mats samples.

*Conclusion* Considering that the isolation of many number colonies

of *T. tonsurans* from all of wrestling mats and from involved wrestlers to tinea gladiatorum as the only dermatophytes species, we think the contamination of wrestling mat to *T. tonsurans* has a crucial role to catch tinea gladiatorum among wrestlers.

*Keywords* Tinea gladiatorum, *Trichophyton tonsurans*, Wrestler

## MI 39 Cytological findings of *Helicobacter pylori* related gastric mucosal abnormalities in touch imprint smears

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*Background Helicobacter pylori* is the major causative agent of peptic ulcer, chronic gastritis, gastric dysplasia and even adenocarcinoma and lymphoma. Early detection of precancerous changes of gastric mucosa is mandatory for the preventive goals. Touch imprint cytology is a simple and fast method for this purpose.

*Materials and methods* Touch imprint cytological smears of 500 patients underwent upper GI endoscopy prepared from fresh gastric mucosal biopsy specimens. The smears stained with Giemsa and modified Papanicolaou methods and studied for abnormal cytological changes.

*Results* Data collected and compared with the results of histopathology, PCR, ELISA and cultures of the specimens for H.pylori. All the specimens were infected by *H. pylori* organism. The cytological features of the precancerous changes were described thoroughly and accuracy, sensitivity and specificity of the method for the detection of those changes estimated which was 85%, 90% and 88% respectively. There was only one case of gastric adenocarcinoma which was detected by touch imprint method.

Conclusion: Touch imprint cytology is an accurate, simple and cost effective method for the early detection of *H. pylori* related precancerous lesions of gastric mucosa.

## MI 40 Identification of proteins induced at low pH or low oxygen tension conditions in *Mycobacterium tuberculosis* H37Rv

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*Mycobacterium tuberculosis* likely reside within phagocytic vacuoles of macrophages, where it encountered a moderately acidic and hypoxic environment. These stress conditions induce *M. tuberculosis* to latency and nonreplicating persistent (NRP) state.

The influence of low pH and low oxygen tension levels on the expression of proteins of *M. tuberculosis* H37Rv were analysed by 2-dimensional polyacrylamide gel electrophoresis (2-DE). The protein samples were collected from mycobacterial culture filtrates under conditions of variable oxygen tension and pH. *M. tuberculosis* H37Rv culture filtrates and lysates were analyzed by isoelectric focusing and SDS-PAGE.

It was found that new proteins were intensively expressed in culture filtrates under low pH and hypoxic conditions. By MALDI-TOF analysis, PhoS1 (Rv0934) and ScoB (Rv2503c) were identified in culture filtrate results. PhoS1 is putative phosphate binding protein, and ScoB is succinyl-CoA:acetoacetate CoA-transferase (SCOT) β subunit.

These results suggest that phosphate transport protein and SCOT are essential for mycobacterial survival in a mildly acidic and hypoxic environment such as putative conditions within the phagosome of macrophages. These results indeed extend our understanding of hypoxic and acidic response in *M. tuberculosis* and provide an important insight into physiology of the latent bacilli.

## MI 41 Monitoring of microbial growth curves by Laser Nephelometry

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Different analytical approaches in clinical immunology and drug discovery take advantage of two closely related techniques based on light scattering. Turbidimetry is the measurement of light transmitted through a suspension of particles. In contrast, nephelometry is a direct method of measuring light scattered by particles suspended in solution at right angles to the beam, or preferably, at a forward angle. In dilute solutions, where absorption and reflection are minimal, the intensity of the scattered light is a function of the concentration of scattering particles.

The most common application of laser-based nephelometry in microplate format is the fully automated solubility screen in HTS laboratories. Determining aqueous compound solubility has become an essential early measurement in the drug discovery process.

Herein we present growth curves of *Corynebacterium glutamicum* monitored with the NEPHELOstar. The basic for that kind of measurements is a good correlation between absorbance and nephelometry measurements which is also demonstrated.

The described application reveals that laser nephelometry is a reliable technique for monitoring microbial growth besides the classical applications like compound solubility testing and immunoprecipitation. Studies show that the nephelometric assay, compared to the turbidimetric assay, is not only comparable, but clearly superior regarding sensitivity. The key advantage of nephelometry is the ability to detect scattered light, even if the concentration of scattering particles is very low, which is the case during the lag phase and beginning of the log phase. Using the NEPHELOstar, instead of a traditional transmission reader, this early part of the growth curve can be monitored much more accurately.

## MI 42 High-Throughput determination of bacterial growth kinetics using a FLUOstar OPTIMA microplate reader

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**Background** *Salmonella enterica* serovar Typhimurium is a leading causes of gastrointestinal disease in the UK. Regulations preventing the use of antibiotics for prophylactic treatment of animals have been introduced in an attempt to reduce the emergence of antibiotic resistance in the food chain. This has necessitated in increase in the application of biocides to reduce microbial contamination of animal houses. In this study mutants of salmonella resistant to biocides were selected and characterised.

**Methods** Previously a panel of *Salmonella* Typhimurium were used to select biocide resistant mutants. The rate of growth in Luria-Bertani broth ± biocide for each mutant was determined using a FLUOstar Optima (BMG LABTECH, UK).

**Results** No biocide selected mutant was compromised for ability to grow relative to parent strains in the absence of biocide. The biocide selected mutants were more resistant to the addition of biocides to the media than parent strains.

**Conclusion** These data clearly indicate that biocide exposure selects for strains with increased tolerance to biocides.

# Posters

## Physiology, Biochemistry & Molecular Genetics Group

### PBMG 01 A putative DiaA homologue is involved in cold and pressure adaptation in the deep-sea bacterium *Photobacterium profundum* SS9

**Ziad El-Hajj**[1], Federico Lauro[2], Douglas Bartlett[2] & Gail Ferguson[1]

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The deep sea is characterised by pressures that can reach 110MPa and an average temperature of 3$^0$C. High pressure disrupts a variety of essential cellular processes, but pressure-loving bacteria (piezophiles) have adapted to grow optimally at high pressure. One such piezophile, *Photobacterium profundum* SS9, grows optimally at 28MPa and 15$^0$C but can grow over a range of temperatures (2-20$^0$C) and pressures (0.1-70MPa). The ability to grow under atmospheric pressure makes it an ideal model organism to study the molecular basis of piezophilic growth. We are characterising a *P. profundum* SS9 mutant that lacks a homologue of DiaA, which in *E. coli* is involved in timely initiation of DNA replication. The putative *diaA* mutant is cold and pressure-sensitive in liquid culture but exhibits a general growth defect at atmospheric pressure on agar and is unable to adapt to high pressure on agar. Although SS9 is rod-shaped at 28MPa, the putative *diaA* mutant filaments under this condition. Further investigations with this mutant could lead to a better understanding of the role of initiation of DNA replication in pressure and cold adaptation.

### PBMG 02 Serine palmitoyl transferase; the gateway to sphingolipid biosynthesis

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Sphingolipid biosynthesis is an essential pathway in mammals, plants and fungi. However, no homologous pathway has been identified in bacteria. The outer membrane of the Gram negative bacteria *Sphingomonas paucimobilis* contains glycosphingolipids (GSL), in contrast to other Gram negative bacteria which have lipopolysaccharide (LPS, endotoxin). The presence of complex sphingolipids in the outer membrane of *S. paucimobilis* indicates that these bacteria must contain a sphingolipid pathway. The first enzyme in all sphingolipid pathways studied to date is Serine Palmitoyl Transferase (SPT) but its isolation from eukaryotes has proved difficult since it is membrane bound and heterodimeric. In contrast, the *S. paucimobilis* SPT is a soluble homodimer.

Here, we present a high resolution (1.3 Å) crystal structure of the cofactor-bound form of this important enzyme. Eukaryotic SPTs are targets for the development of novel drugs and the bacterial SPT structure provides a model for the design of new inhibitors.

### PBMG 03 Investigation of the factors regulating the activation of the alternative sigma factor, $\sigma^B$, in the human pathogen *Listeria monocytogenes*

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The Gram-positive food-borne pathogen *Listeria monocytogenes* exhibits the capacity of surviving harsh environmental conditions, which is achieved in part by the activation of a group of genes known collectively as the *sigB* ($\sigma^B$) regulon. Access to this regulon is provided by the alternative sigma factor, $\sigma^B$, but the factors that influence the activation of $\sigma^B$ are poorly understood in *L. monocytogenes*. This project seeks to elucidate the molecular mechanism involved in regulating the activity of $\sigma^B$, in particular how RsbW (a putative anti-$\sigma$ factor) and RsbV (a putative anti-anti-$\sigma$ factor) act to control the availability of $\sigma^B$. Each gene (*rsbV*, *rsbW*, and *sigB*) was expressed in *E. coli* and recombinant proteins were purified by Nickel affinity chromatography and used for generation of polyclonal antisera capable of detecting each protein in *L. monocytogenes*. Initial western blotting experiments have looked at cellular levels of each of the proteins in response to growth phase and osmotic stress. Levels of $\sigma^B$ have been shown to accumulate in exponential phase and diminish in stationary phase while levels of the putative anti-sigma factor, RsbW, have remained consistent throughout growth. Future work will look at a comparison of $\sigma^B$ levels versus activity throughout growth.

### PBMG 04 Investigation of the inhibitory mode of action of homocysteine in *Escherichia coli*

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The enzyme threonine deaminase (TD) is involved in the conversion of threonine to $\alpha$-ketobutyrate and ammonia during isoleucine biosynthesis in *Escherichia coli*. *In vitro* studies have indicated that TD is inhibited by the sulphur-containing amino acid homocysteine (Hcy). TD was cloned and over-expressed. The allosteric enzyme was then purified by $Ni^{2+}$ affinity chromatography. Kinetic studies were undertaken to determine the mode of inhibition of Hcy on this enzyme. TD appears to be inhibited by competitive inhibition with increasing substrate concentration providing relief from inhibition by Hcy. It was determined that strains expressing increased levels of TD may in turn give rise to Hcy resistance. Mutations that lead to increased TD activity were examined. The *ilvA* gene, which encodes TD, is known to be expressed at increased levels in some valine resistant *E. coli* strains. One such valine resistant mutant was isolated. This mutant possesses a frameshift mutation in the *ilvG* gene and displayed greater Hcy resistance compared to the wildtype. These data suggest a model which accounts for the inhibitory effect of Hcy in *E. coli* and may also suggest a novel target for Hcy toxicity in humans.

### PBMG 05 Using microarray analysis to determine the effect of salt on transcription in *Listeria monocytogenes* and characterisation of the specific role played by $\sigma^B$

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The human pathogen *Listeria monocytogenes* has the ability to grow and survive in a range of stressful conditions such as high salt concentrations, acidic conditions and low temperatures. The alternative sigma factor, sigmaB ($\sigma^B$) plays an important role in redirecting transcription under stress conditions and also, in regulating known virulence genes. In this study gene microarrays were firstly used to determine the effect of salt on gene expression

in a wildtype strain *L. monocytogenes* 10403s and secondly in elucidating fully the role of $\sigma^B$ in modulating transcription in this strain. Gene transcription was studied in different phases of growth either with or without the presence of osmotic stress (0.5M NaCl). During growth in exponential phase without osmotic stress very few $\sigma^B$-dependent genes were identified. In contrast, $\sigma^B$ appeared to play a significant role in transcription during stationary phase or when osmotic stress was present. Furthermore, many salt inducible genes were found to be $\sigma^B$ dependent, confirming the central role for $\sigma^B$ in regulating the response of *L. monocytogenes* in osmotic stress.

## PBMG 06 Investigation into the molecular responses to weak organic acids in the human pathogen *Listeria monocytogenes*

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Contamination of processed food by micro-organisms, such as the pathogen *Listeria monocytogenes*, is a major human health risk. *L. monocytogenes* causes the gastro-intestinal food infection listerosis. Food preservation methods include the use of weak organic acids to reduce microbial growth. The response of *L. monocytogenes* to strong acids has been investigated, however there is limited knowledge of the effect of weak organic acids on *L. monocytogenes*, particularly at the molecular level. Growth of *L. monocytogenes* in response to five food-grade acids, lactic, sorbic, acetic, benzoic and citric acid, was explored. The levels of acid that caused a 50% inhibitory effect on the growth of *L. monocytogenes* were established and found to be strongly dependent on the pH of the medium. Growth was more inhibited at lower pH values suggesting that it is the undissociated form of the acid that causes the inhibitory effect. In order to elucidate the molecular responses of *L. monocytogenes* to organic acid stress DNA microarray analysis is currently being used to investigate the impact of these acids on global gene expression.

## PBMG 07 Identification of sensor kinase interacting with VirS encoded by the virulence plasmid of *Rhodococcus equi*

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Two-component systems are widely distributed in bacteria and enable the organisms to respond to environmental stimuli. *Rhodococcus equi* is an important pathogen of foals, causing severe pyogranulomatous pneumonia. Virulent *R. equi* grows within macrophages, a process which remains poorly characterized. A *R. equi* chromosomal DNA library was constructed; an orphan response-regulator protein VirS encoded by the *R. equi* pathogenicity island was used as bait in a yeast two-hybrid screen of the genomic library. We identified a novel sensor kinase protein VirX which interacts with VirS. Radiolabled HA or c-Myc-tagged VirX and VirS were produced *in vitro* and the interaction was verified by co-immunoprecipitation. We measured gene-expression and virulence in VirX and VirS knock-out mutants. Reverse-transcription real-time PCR shows that virulence gene-expression at low pH and high temperature decreased rapidly in the VirS-mutant while it only slightly decreased in the VirX-mutant. The VirS-mutant was fully attenuated. These studies provide the first identification of the cognate chromosomally encoded sensor kinase which interacts with its virulence plasmid encoded response regulator.

## PBMG 08 Mutational characterisation of the enterotoxigenic *Escherichia coli* transcriptional regulator Rns

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhoea amongst children and travellers in the developing world. An important early step in the establishment of infection is the adherence of ETEC to the host intestine and this is mediated by fimbriae on the bacterial surface. Expression of CS1 and CS2 fimbriae in ETEC is activated by Rns, a member of the AraC family of transcriptional regulators. This project aims to characterise Rns in order to learn more about its structure-function relationship.

Pentapeptide insertion mutants of Rns were generated by performing scanning linker mutagenesis on a plasmid encoding *rns*. The resulting mutants were co-expressed with a reporter plasmid containing the CS1 promoter cloned upstream of a promoterless green fluorescent protein gene. This enabled the isolation of mutants with insertions that reduced the activity of Rns and insertions that had no effect on Rns activity. Characterisation of representative mutants by band shift analysis identified areas of Rns in both the N and C-terminal domain that affect the binding of the protein to its sites at the CS1 promoter.

## PBMG 09 Purification and partial characterisation of *Tritrichomonas foetus* enzymes and determination of some biochemical characteristics of DNases

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Proteins from the protozoan *Tritrichomonas foetus* were purified by ammonium sulphate precipitation and lectin column chromatography using Con- A and Lentil lectins. DNase activity was detected in the starting material and the concentrated enzyme precipitate. It was found that DNases were specifically released by binding to Con A lectins. This was confirmed by detecting DNase activity in the enzyme fractions purified after it was allowed to dissociate from the Con A lectin column using selected elution conditions, which also suggests that these enzymes are glycosylated. Although No DNase activity was detected in the enzyme fractions purified using the Lentil column, proteins were detected in these fractions using Bradford assay, suggesting that lentil lectin have purified other *T. foetus* proteins. Furthermore, different elution conditions affect the degree of purification. DNase activity detected in the enzyme fractions that were eluted using concentrated (.5 M) α-methyl D (+)-glucoside was significantly higher than those eluted using 1.5M NaCl/PBS. In addition higher protein concentrations obtained in the fractions eluted using sugar than in those eluted using salt. Twenty one different biotinylated lectins were tested for binding to the purified enzymes using Enzyme Linked Immunosurbent Assay (ELISA). Eleven lectins of these appeared to bind to *T. foetus* – enzymes. The highest spectrophotometer reading was found to be for *Lycopersicon esculentum* (tomato) lectin, which indicates that this lectin might have the highest binding affinity to *T. foetus* enzymes among the 21 tested lectins including Con-A and LCA lectins. Being purified using lectins and binding to lectins indicates that some *T. foetus* enzymes are glycosylated. The DNase enzymes isolated showed higher activity at low pH points when its activity was tested against different solutions with different pH values. Interesting results were obtained when experiments were carried out to determine the effect of different metal ions on DNase activity. The experiments showed that the Activity of DNases appear to be affected with the presence of different metal ions. Among the metal ions studied ($Mn^{2+}$, $Ca^{2+}$, $Mg^{2+}$ and $Zn^{2+}$) the highest rate of activity of DNases was obtained in presence of manganese while the lowest was in presence of zinc. The findings in this project is supporting the suggestion that *Tritrichomonas foetus* enzymes are glycosylated and put forward the future challenge in explaining their stability despite the presence of glycosidases enzymes. Furthermore exploring the biochemical features of *T. foetus* enzymes helps us in providing better conditions to increase their degree of purification in future experiments.

## PBMG 10 Lrp, NtrC, TfdR, ppGpp and sigma factor competition form a network that orchestrates the metabolism of 2,4-D in *Burkholderia cepacia* strain 2a

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The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is catabolised by a number of bacteria and amongst these, the soil-bacterium *Ralstonia eutropha* JMP134, which harbours a plasmid pJP4 encoding the 2,4-D degradation pathway (*tfd* genes), is the best-studied. Whilst the genetics and the biochemistry of the process have been established, little is known about the regulatory mechanisms that govern the expression of the *tfd* genes. Here we show that the catabolism of 2,4-D in *Burkholderia cepacia* strain 2a is achieved through a network of dynamic interactions involving *cis*- ($\sigma^{70}$-$\sigma^{54}$ dependent promoter) and *trans*- (NtrC, TfdR, sigma factor competition, ppGpp, Lrp) regulatory elements. Transcriptional profiling of strain 2a (and various mutant derivatives) grown in a mixture of succinate and 2,4-D revealed that high levels of expression of *tfd* genes requires an intact $\sigma^{70}$-$\sigma^{54}$ dependent promoter, the enhancer protein NtrC and the lysR-type transcriptional activator TfdR. In addition, expression of *tfd* genes is influenced by $\sigma^{S}/\sigma^{70}$ factor competition for RNA polymerase. Strain 2a has an *lrp* gene (a global regulator in *E. coli*) which is essential for up-regulating the expression of the *tfd* genes. We also observed that the signal molecule ppGpp is able to stimulate the expression of both *lrp* and *tfd* genes when succinate was present in the growth medium. Our results suggest that this network allows strain 2a to fine tune the expression of the catabolic pathway and we believe that Lrp might be the central hub that integrates the 2,4-D pathway into global cellular physiology.

## PBMG 11 Identification and characterization of the *O*-acetyltransferase gene in *Enterococcus faecalis*

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Over the last decades, *E. faecalis* has emerged as major cause of nosocomial infections. In their infection processes, bacteria have to overcome the host's immune responses. We investigated how *E. faecalis* can evade one of the most important and widespread compounds of the constitutive defence system: the lysozyme. Based on the sequence analysis we identified one gene locus, potentially involved in lysozyme resistance of *E. faecalis*. This gene named *ef0783* shares strong homology with a *Staphylococcus aureus* gene encoding *O*-acetyltransferase (OatA) enzyme implicated in peptidoglycan modification. RP-HPLC and MALDI-TOF-MS peptidoglycan structural analysis of *ef0783* mutant and its parental strain showed that the *ef0783* product has *O*-acetyltransferase activity and can be renamed OatA. However, lysozyme resistance analysis failed to show strong impact of the absence of *ef0783* in *E. faecalis*. Theses results demonstrated that on the contrary to *S. aureus*, OatA is not the major determinant for lysozyme resistance in *E. faecalis*.

## PBMG 12 Histidine-containing phosphotransfer protein B-mediated signalling pathways in *Pseudomonas aeruginosa* PAO1

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The histidine-containing phosphotransmitter (Hpt) domain is known to be required for certain bacterial two-component sensor kinases to carry out multistep phosphotransferring reactions. The aim of this study is to elucidate the signaling pathway mediated by three Hpt proteins found in *Pseudomonas aeruginosa* PAO1. A phosphorelay profiling assay was performed on the three Hpt proteins, twelve orphan sensor kinases, and nine response regulators. Six of the recombinant sensors possess an autokinase activity and four of them (PA1611, PA1976, PA2824 and RetS) could transfer the phosphoryl group to HptB (PA3345), which in turn relays the signal to response regulator PA3346. We also demonstrated that PA3347, PA3346 and *hptB* are organized as an operon. PA3346 gene product contains a Ser/Thr phosphatase domain, could dephosphorylate PA3347, which has an anti-sigma factor antagonist domain. Finally, analysis of PA3346 and PA3347 gene knockout mutants revealed that these genes are associated with motility and biofilm formation. Together, these results provide a novel multistep phosphorelay system, and therefore offer a new insight about how bacteria response to a wide spectrum of environmental signals.

## PBMG 13 Regulation of type 3 fimbriae in *Klebsiella pneumoniae* CG43

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A lambda phage clone containing type 3 fimbriae gene cluster *mrkABCDF* was isolated from a genomic library of *Klebsiella pneumoniae* CG43, and the sequences determined and analyzed. Upstream of the *mrk* gene cluster are *phgS* and *phgM*, homologs of *pecS* and *pecM* virulence regulatory genes of *Erwinia chrysanthemi*, followed by the *fim* gene cluster encoding type 1 fimbriae. In LB and GCAA media, expression of type 3 fimbriae was observed in the bacteria using western blotting hybridization and immunogold-labeling transmission electronic microscopy (TEM), wereas, the expression of type 1 fimbriae was evident only in the *mrkA* deletion mutant as assessed using the analysis of western blotting hybridization and mannose-sensitive yeast agglutination. The overexpression of *fimB* in *K. pneumoniae* CG43 appeared to switch on the expression of type 1 fimbriae while the expression of type 3 fimbirae was diminished. The subsequent assay using LacZ as a promoter reporter revealed that the deletion of *phgS* or *phgM* reduced the expression of $P_{S\text{-}mrk}$ but did not affect the activity of $P_{L\text{-}mrk}$ The presence of PhgS/PhgM-dependent and PhgS/PhgM-independent regulation for the expression of type 3 fimbriae was investigated.

# Posters

## Plenary

### PS 01 Copper homeostasis in *Salmonella*

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Copper is essential for infection by *Salmonella* as a cofactor for the periplasmic copper-zinc superoxide dismutases which protect against reactive oxygen species generated by the host respiratory burst oxidase. However, copper is also highly toxic and elaborate copper homeostatic mechanisms are required to maintain essentially no free cytosolic copper whilst allocating copper to copper-requiring proteins. Analysis of the *S. typhimurium* genome has revealed genes corresponding to the *cue* and *cus* systems in *E. coli*. These include *sctR* (transcriptional regulator), *cuiD* (copper oxidase) and *copA* (copper-exporter). However, *S. typhimurium* possesses an additional cluster of genes, *copB*/*copR*/*copZ*, for a deduced copper-transporter, transcriptional regulator and copper-metallochaperone, respectively. Expression from the *cuiD*, *copA* and *copB*/*copR*/*copZ* promoters is copper responsive, and *cuiD*, *copA* and *copB* contribute to copper tolerance. However, while copper-responsiveness of *copA* and *cuiD* is controlled by SctR, *copB* is regulated by both SctR and CopR. We reveal that copper induced gene expression increases during *Salmonella* infection of macrophages, consistent with elevated bacterial copper load. Work is continuing to establish the roles of these proteins in copper transport and trafficking and their contributions to intracellular survival.

### PS 02 Genomes and the intracellular life of *Rhizobium*

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Nitrogen fixation is the process by which nitrogen is made available to plants. Both free-living and symbiotic bacteria are capable of this process. Symbiotic rhizobial bacteria carry out nitrogen fixation and are harboured in root nodules of leguminous plants.

The recently published genome sequences of *Rhizobium legumuinosarum bv vicie* 3841 and *Rhizobium etli* CFN42 provide us with an opportunity to investigate the symbiotic lifestyle of *Rhizobium* at sequence level. The genomes of both of these organisms consist of a chromosome and a set of six plasmids. Crucially, at the sequence level, these bacteria reveal a core gene set that is largely chromosomal and conserved, whilst the organisms appear to have recruited an accessory gene set that is largely plasmid-based and unique to the species.

### PS 03 An analysis of the metabolic status of *Salmonella* Typhimurium inside macrophages and the role of central metabolism in intracellular replication

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To survive and replicate within macrophages, *Salmonella* Typhimurium must adapt to utilise the limited carbon sources and other nutrients available within the *Salmonella* containing vacuole (SCV). However, very little is known about the nutrients available to *S.* Typhimurium when it is inside a host cell.

Using a microarray-based approach, the Molecular Microbiology group at the IFR have generated transcriptomics data for all the *S.* Typhimurium genes during infection of macrophages. These data have been used to identify the genes encoding enzymes involved in central metabolism that show differential expression during infection.

Our findings have led us to hypothesise that specific nutrients sustain the growth of intracellular *S.* Typhimurium within the SCV. We are testing our hypotheses by deleting key genes involved in the transport and metabolism of nutrients potentially utilised by intracellular *S.* Typhimurium. These experiments will help define which nutrients and metabolic pathways are utilised by intracellular *S.* Typhimurium and whether these play a role in virulence.

### PS 04 The bacterial signal molecule ppGpp mediates the environmental regulation of both the invasion and intracellular virulence gene programs of *Salmonella*

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During infection of mammalian hosts, facultative intracellular pathogens must adjust rapidly to different environmental conditions encountered during passage through the gastrointestinal tract and following uptake into epithelial cells and macrophages. Successful establishment within the host therefore requires the co-ordinated expression of a large number of virulence genes necessary for the adaptation between the extracellular and intracellular phases of infection. In this study we show that the bacterial signal molecule, ppGpp, plays a major role in mediating the environmental signals involved in the regulation of both the extracellular and intracellular virulence gene programs.

Under oxygen-limiting conditions, we observed a strong ppGpp-dependence for invasion gene expression, reflecting severe reductions in expression of the *Salmonella* pathogenicity island 1 (SPI1) transcriptional regulator genes *hilA*, *C*, *D* and *invF* in mutants lacking ppGpp. Over-expression of the non-SPI1-encoded regulator, RtsA, restored *hilA* expression in the absence of ppGpp. SPI2-encoded genes, required for intracellular proliferation in macrophages, were activated in the wildtype strain under aerobic, late-log phase growth conditions. The expression of SPI2 genes was also shown to be ppGpp-dependent under these conditions.

The results from this study show that ppGpp specifically mediates *Salmonella* virulence gene expression on a global scale. Since ppGpp is found in nearly all bacteria, the results suggest that it may play a much broader role in mediating bacterial virulence gene expression.

## PS 05 Molecular cloning, characterisation and transgalactosylation properties of galactosidases from *Bifidobacterium bifidum* (NCIMB 41171)

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The observation that galacto-oligosaccharides (GOS) stimulate the growth of bifidobacteria and other health-promoting intestinal bacteria has boosted the interest in the transferase reaction of galactosidases and in their ability to synthesise GOS. This study focuses on the isolation and characterisation of galactosidases from a *B. bifidum* strain (NCIMB 41171) and their transgalactosylation properties. One α-galactosidase (*mel*A) and four β-galactosidase genes (*B1, P1, P2* and *P11*) were isolated by shotgun-cloning, the genes were sequenced and the gene expression products purified and finally characterised.

Pure enzyme preparations were used for synthesis of GOS with 40% substrate (lactose for β-galactosidases and melibiose for α-galactosidase). The comparison of the synthesis products with each enzyme (analysed with HPAEC) showed that P11 and B1 were synthesising the highest amount of high degree of polymerisation GOS. Moreover, especially B1 but as well P1 and P2 except of the β-GOS were also forming an α-galactobiose, showing their ability to synthesise GOS with opposite orientation of the glycosidic bond (namely, α- or β-).

## PS 06 Exploring intracellular *Mycobacterium tuberculosis* transcriptomes from human dendritic cell and macrophage infection models

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The ability of human dendritic cells to restrict *Mycobacterium tuberculosis* growth compared to the permissive environment of the macrophage may be a key step in the control of *M. tuberculosis* infection. This scenario also provides an opportunity to study the interactions between infecting bacilli and human-derived immune cells where the outcome of infection is different. Here we extracted monocytes from five healthy donors and matured them into macrophage or dendritic cells. Mycobacterial RNA was extracted using the differential lysis method after infection of each cell type, amplified using an oligo-dT T7-based system and hybridised to a whole genome *M. tuberculosis* microarray. Gene expression pathways involved in the oxidation of fatty acids, respiratory state, and maintenance of the cell wall were induced amongst others on infection of both cell types. Comparison of the *M. tuberculosis* transcriptomes from macrophage and dendritic cell infections identified changes that confirm differences in the intracellular environments encountered, and reveal possible mechanisms of *M. tuberculosis* pathogenicity.

## PS 07 Nitric oxide metabolism in *Salmonella enterica* Typhimurium and its role within macrophages

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A potent anti-microbial defence mechanism of macrophages is the production of nitric oxide (NO). The survival of several strains of *Salmonella* in the murine macrophage cell line, J774.2 was investigated in the absence and presence of IFN-γ. The NO-producing capacity of J774.2 cells was assessed by accumulation of nitrite and nitrate. Survival of the *fur* mutant was attenuated compared to an isogenic wild-type strain in both non-stimulated and stimulated macrophages, presumably a consequence of exacerbated oxidative stress sustained by the mutant. In contrast, the flavohaemoglobin (*hmp*) mutant was attenuated only in stimulated macrophages, indicating an insufficient nitrosative response in non-stimulated macrophages. Survival of the *hmp* mutant was negatively correlated with accumulation of nitrite and nitrate in media. A mutant in the major aerobic repressor of *hmp*, NsrR, was also attenuated in stimulated macrophages, despite over-expression of flavohaemoglobin in this mutant, presumably due to superoxide formation by Hmp.

## PS 08 Immunological recognition of expressed proteins of *Lawsonia intracellularis*

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*Lawsonia intracellularis* is the aetiological agent of proliferative enteropathy, a commercially significant disease in pigs with a worldwide distribution. Very little is understood regarding the pathogenesis of this Gram-negative, obligate intracellular bacterium due to its fastidious nature. Major *L. intracellularis* proteins were identified in whole cell extracts from heavily infected *in vitro* cultures by a shotgun proteomic analysis employing LC-ESI-MS/MS and subsequent searching of mass spectra against a *L. intracellularis* MASCOT database. ORFs of several identified proteins were amplified by PCR and cloned into pRSET A expression vectors to produce recombinant fusion proteins with a polyhistidine tag. Purified recombinant proteins were probed on Western blots with a panel of sera from pigs naturally infected with *L. intracellularis*. The immunological recognition of these proteins provides an insight into the interaction of this unusual bacterium with its mammalian host as well as identifying putative diagnostic or vaccine candidates.

## PS 09 Characterisation of a *Corynebacterium pseudotuberculosis* chromosomal locus containing a homologue of the C. diphtheriae *dtxR* gene

**C.A. Walker**[1], W. Donachie1, D.G.E Smith[1,2] & M.C. Fontaine[1]

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As part of a larger study into the effects of environmental iron on the regulation of gene expression in *Corynebacterium pseudotuberculosis*, an investigation of a homologue of the *C. diphtheriae* Diphtheria toxin repressor (DtxR) was initiated, based on the observation that this transcriptional regulator is known to regulate virulence gene expression in response to changes in the concentrations of iron/manganese in a variety of bacterial species. In the first instance, it was necessary to confirm its presence and subsequently characterise the chromosomal locus in which the *dtxR*-homologous gene was resident. Using inverse PCR a 6.5 kb DNA fragment was amplified, cloned and sequenced, novel sequences were identified by BLAST analysis. The *dtxR*-homologue corresponded to a predicted ORF encoding a 226 aa protein; immediately upstream was an incomplete predicted ORF encoding a protein sharing homology with *C. diphtheriae sigB* and immediately downstream was a predicted ORF encoding a 327 aa protein sharing homology with *C. diphtheriae galE*. Observation of a *sigB-dtxR-galE* chromosomal locus in

*C. pseudotuberculosis* is consistent with other actinomycetes, including *C. diphtheriae* (DIP1413 to DIP1415). Further work is now underway to identify *C. pseudotuberculosis* genes which may be regulated by the DtxR-homologue.

## PS 10 Investigation into iron-regulated exported proteins from the facultative intracellular pathogen *Corynebacterium pseudotuberculosis*

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*C. pseudotuberculosis* is a Gram positive facultative intracellular pathogen and the causative agent of caseous lymphadenitis (CLA). CLA is a well established disease of sheep in the UK and of major economic importance worldwide. Currently there is limited knowledge regarding virulence determinants and mechanisms of gene regulation in *C. pseudotuberculosis* largely due to the lack of a genome sequence for the bacterium. Using 1-D PAGE, MALDI-ToF and LC-ESI-MS/MS we investigated the exported protein profile of *C. pseudotuberculosis* grown in iron-replete and iron-deplete conditions in order to identify novel iron-regulated proteins which may have potential uses as vaccine candidates or for use in diagnostic tests. Using BLAST analysis we identified two proteins which are known virulence determinants of *C. pseudotuberculosis* that have available sequence, however there were no other homologues with significant mowse scores when the databases were searched, indicating the exported proteins from *C. pseudotuberculosis* are divergent from other species within the genus.

## PS 11 An investigation into the extent and function of sigma factors in the genome of *Rhodococcus equi*

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*Rhodococcus equi* is an intracellular pathogen of macrophages, causing pneumonia in foals and immuno-suppressed humans. Research into its virulence mechanisms have been greatly enhanced by the sequencing of the genome, currently being annotated at the Welcome Trust Sanger Institute, U.K. Work so far has included the analysis of sigma factor expression levels, throughout the life cycle of *R. equi* using the real time PCR method. This has been enabled by the identification of putative sigma factors in silico, followed by the construction of various knock out mutants. SigB, similar to the principal alternate sigma factor in *M. tuberculosis*, has been shown to be highly expressed in the early stages of growth, pointing to possible importance during infection of the host. Pending their identification and investigation, the aim of my work is to present a clearer picture of the interplay between several sigma factors and known virulence genes within *R. equi*, using both in silico analysis and real time expression methods.

## PS 11 Symbiotic rhizobia use pathogenic-like protein secretion systems to establish an intracellular life within plant roots

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The symbiosis between rhizobia and leguminous plants begins with an exchange of molecular signals between the two organisms. Flavonoids exuded by plant roots induce the synthesis of Nod factors (NFs) by rhizobia. NFs allow rhizobia to enter root cells and induce the formation of new plant organs (nodules), into which rhizobia are released. Within nodules rhizobia reduce atmospheric nitrogen to ammonia, which is taken up by the host plant in return for photosynthates. Other signals such as protein secretion systems also influence the symbiosis. *Rhizobium* species NGR234 possesses a functional type III secretion system (T3SS) that can improve or reduce the symbiotic ability of NGR234. At least 8 proteins, called nodulation outer proteins (Nops) are secreted by the T3SS. Comparing Nop mutants on the same plant species shows that NGR234 secretes a mixture of positive and negative acting Nops. Thus the T3SS of NGR234 can aid the process of nodulation, although parts of this system may betray NGR234 as a potential pathogen to some plant species, initiating a defensive response that blocks the symbiotic interaction.

## PS 13 Use of bioluminescent *Neisseria meningitidis* to study invasion and survival within mammalian cell lines

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*Neisseria meningitidis* is the major causative agent of bacterial meningitis, with over 500,000 cases occurring globally each year. The lack of animal models for this human specific disease has hampered our knowledge of exactly how this pathogen enters cells and breaches the blood-brain barrier to cause fulminating meningitis.

Bacterial bioluminescence has been shown to be an accurate real-time reporter of metabolic activity of bacteria. A strain of *N. meningitidis* C751 has been genetically modified with *luxCDABE* from *Photorhabdus luminescens* on the plasmid pGLITE to give a self-bioluminescent reporter construct. This self-bioluminescent strain of *N. meningitidis* was used in the development of an assay to study invasion and survival within monolayers of ECV-304 endothelial cells, C6 glioma cells and Caco-2 human colon adenocarcinoma cells.

At present, this study has not produced any robust evidence of internalisation of *N. meningitidis* C751 pGLITE within these cell lines. This may be due to internalisation being short-lived or paracytosis being used as the predominant mode of movement across cells.

# Posters

## Systematics & Evolution

### SE 01 Comparative genomics of *Francisella tularensis* using a DNA microarray

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Four subspecies of *F. tularensis* have been identified, each characterised by differences in virulence and in geographical area. To investigate the genetic basis for these differences we have constructed an oligonucleotide microarray based on the genome sequence of *F. tularensis* subspecies *tularensis* strain Schu S4. We performed genome-wide analyses by hybridizing DNA from representing each of the four subspecies to the microarray. DNA from each strain gave a unique pattern of differentially hybridizing probes (DHPs). Using this technique we were able to identify 18 regions of difference (RD) that distinguish the highly virulent *F. tularensis* subspecies *tularensis* from the moderately virulent *F. tularensis* subspecies *holarctica*. Of these RDs, 11 have been previously identified and seven are novel.

*This work has been supported by UK Ministry of Defence.*

### SE 02 Three new spore-forming and moderately halophilic bacteria, isolated from soil in Japan

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Aerobic, spore-forming, Gram-positive, rod-shaped and moderately-halophilic bacteria are taxonomically diverse and have been isolated from various environments. Phylogenetic analyses and chemotaxonomic characteristics have been revealed that the halotolerant and moderately halophilic bacteria formed several phylogenetically distinct lineages scattered within genus *Bacillus*, which were described as the genera *Halobacillus, Virgibacillus, Gracilibacillus, Oceanobacillus, Lentibacillus, Thalassobacillus* and *Pontibacillus*.

We have isolates five strains were isolated from field soil in Japan. Based on partial 16S rRNA gene sequence analyses, the strains were found to be closely related to spore-forming, moderately halophilic bacteria. In addition to the phylogenetic data, physiological and chemotaxonomical analysis, suggested that these isolates are new member of spore-forming, moderately halophilic bacteria. Based on this substantial data, it is proposed that the isolates represents a novel species of genus *Virgibacillus*, *Virgibacillus halophilus* sp. nov., and two novel species within a new genus, *Terribacillus saccharophilus* gen. nov., sp. nov. and *Terribacillus halophilus* sp. nov.

### SE 03 New actinobacterial isolates from soil and lichen in Japan

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*Introduction* Five strains of actinobacteria designed as SK15, SK60, DS66, DS472 and RC825 were isolated from soil and lichen samples in Japan. Polyphasic study was undertaken to establish the taxonomic positions of these trains.

*Methods and results* Phylogenetic analysis based on 16S rRNA gene sequences revealed that five strains form novel evolutionary lineages within the genus *Kitasatospora* (SK15, SK60, and DS66), *Rhodococcus* (DS472) and *Nocardioides* (RC825), respectively. DNA–DNA hybridization experiment demonstrated they were distinct from their closest phylogenetic neighbors. Chemotaxonomically, strains SK15, SK60 and DS66 contained *meso*- and LL-diaminopimelic acids in the cell-wall peptidoglycan, and rich in sugar galactose, mannose and ribose, major cellular fatty acids iso-$C_{15:0,}$ anteiso-$C_{15:0}$, $C_{16:0}$ and iso-$C_{16:0,}$ menaquinone MK-9($H_6$) and MK-9($H_8$). Combination of phylogenetic analysis indicated that they represent three novel species of genus *Kitasatospora*. Meanwhile, strains DS472 and RC825 were also found to have morphological and chemical properties consistent with the genus *Rhodococcus* and *Nocardioides* respectively. Therefore, five novel species are proposed based on phenotypic and phylogenetic analyses.

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# Systematics & Evolution Group session

## *Alphaproteobacteria*: unifying a diverse class

### Genomes, phylogenies and evolution

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The Class *Alphaproteobacteria* is united by similarities in ribosomal RNA and in the sequences of many other ubiquitous genes. In other respects, however, the members of this Class are very diverse. Genome size varies from under a megabase to almost ten megabases, reflecting lifestyles that range from intracellular pathogens to versatile soil-dwellers. Most free-living species have a G+C content of 60% or more, but reduced genomes with low G+C have arisen at least twice in intracellular specialists. The high-G+C bacteria have diverse capabilities that extend their ecological niches and make them of interest to humans, such as photosynthesis, nitrogen fixation, methylotrophy, plant symbiosis, biodegradation and metabolite synthesis. Many of these properties are distributed with little phylogenetic coherence; they are encoded by accessory genes with a history of horizontal gene transfer. Parts of this accessory gene pool have equilibrated to a DNA composition close to that of the corresponding core genomes, but a substantial component retains a distinctive lower G+C composition. By analysing and comparing complete genome sequences, we begin to discern the organisation and evolution of the core and accessory gene pools.