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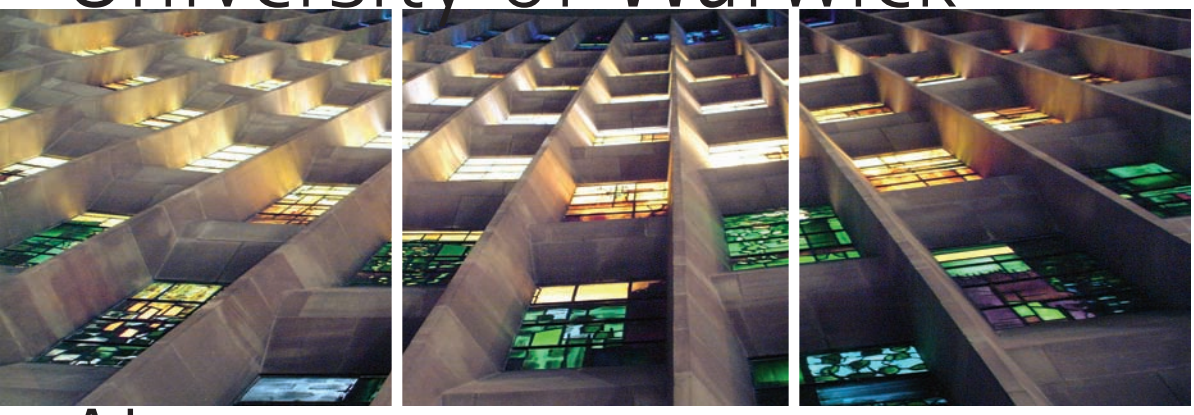

Abstracts

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Contents

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Plenary session

Prokaryotic diversity: mechanisms and significance

Microbial diversity in the era of genomics

Rita R. Colwell

University of Maryland College Park and Johns Hopkins University Bloomberg School of Public Health, USA

In this era of genomics, the perspective of microbial ecologists has been expanded, and the panorama that the world of microbial diversity comprises is only beginning to be known. In the decade ahead there will be revealed a grand tapestry of life, reflecting the richness and abundance of the microbial world. With this new knowledge, there will be a powerful reinforcement of the complexity of life on this planet: life that is not only complicated but also integrated, and both genetically and functionally interwoven. The sustainability of our planet rests unmistakably and irrevocably on the vast and intricate microbial diversity that we should come to appreciate and, hopefully, understand.

Patterns in biodiversity: are prokaryotes really different?

B. Bohannan

University of Stanford, USA

Abstract not received

Archaeal diversity

E. Koonin

National Centre for Biotechnology Information, Bethesda, USA

Comparative-genomic analysis of Clustered Regularly Interspaced Short Palindrome Repeats (CRISPR) and the CRISPR-associated (*cas*) genes, which are widely represented in prokaryotic genomes, leads to the hypothesis that the CRISPR-Cas system (CASS) is a mechanism of defense against invading phages and plasmids that functions analogously to the eukaryotic RNA interference (RNAi) systems. Specific functional analogies are drawn between several components of CASS and proteins involved in eukaryotic RNAi, including the double-stranded RNA-specific helicase-nuclease (dicer), the endonuclease cleaving target mRNAs (slicer), and the RNA-dependent RNA polymerase. However, none of the CASS components is orthologous to its apparent eukaryotic functional counterpart. It is proposed that unique inserts of CRISPR function as prokaryotic small interfering RNAs (psiRNA), by base-pairing with the target mRNAs and promoting their degradation or translation shutdown. Specific hypothetical schemes are developed for the functioning of the predicted prokaryotic RNA interference system and for the formation of new CRISPR units with unique inserts.

The significance of prokaryote diversity in the human gastrointestinal tract

Harry J. Flint

Rowett Research Institut, Aberdeen

The human large intestine represents one of the most densely colonized microbial ecosystems. While recent culture-independent analyses indicate that a majority of the diverse micro-organisms found in the human intestine are not represented by known cultured species, culturability appears high relative to most non-gut communities. Research that combines cultural microbiology, molecular ecology, genomics and metabolite tracking is starting to relate molecular phylogenetic signatures to function. This is serving to identify the roles of even highly oxygen-sensitive species in processes including the fermentation of dietary substrates, the formation of short chain fatty acids, and interactions with host tissues, that have a major influence upon human health. The little studied low % G+C Gram-positive Firmicute bacteria, for example, have been shown to include the major butyrate-producing bacteria of the human intestine. Metabolic interactions and competition between these and other members of the gut community, with respect to dietary carbohydrate substrates that escape digestion in the small intestine, is central to understanding the effects of diet upon gut health.

The evolution of phenotypic diversity

Hubertus J.E. Beaumont, Stefanie M. Gehrig, Rees Kassen, Christopher G. Knight, Jacob G. Malone, Andrew J. Spiers & **Paul B. Rainey**

University of Auckland, New Zealand and University of Oxford

The diversity of life posses a myriad of problems for biologists; foremost among these are the causes of phenotypic diversity. Concepts that stem from Darwin's theory of evolution by natural selection have produced an ecological theory of diversification, but a corresponding genetic theory remains under developed. Necessary for progress is an understanding of how genotype, phenotype and fitness are connected through evolutionary time: in short, what is necessary is a genetical theory of evolutionary development. One of the most profound phenotypic innovations is the kind that promotes cooperation among individual biological units (e.g. genes, cells, organisms) in order to create higher-level units (e.g. chromosomes, multicellular organisms, societies). During my talk I will focus on the evolutionary transition from single cells to simple groups. During this transition the unit of selection shifts from individual cells to groups of cooperating cells. Such a transition occurs readily in experimental populations of *Pseudomonas fluorescens* propagated in a spatially heterogeneous environment and thus is amenable to empirical analysis. Cooperation is mediated by simple mutations at different genetic loci that decrease the activity of negative regulatory elements that control the activity of diguanylate cyclases (GGDEF domain proteins). The net effect of these mutations is the over-production of an adhesive polymer. The adhesive polymer causes the interests of individuals to align with those of the group. Consistent with predictions from theory cooperation is costly to individuals, but beneficial to the group. Defecting genotypes evolve in populations founded by the cooperating type and are more fit in the presence of this type than in their absence. In the short term defectors sabotage the viability of the group; nevertheless, these findings show that evolutionary transitions are readily achievable, provide insights into the genetic and selective conditions, and facilitate experimental analysis of the evolution of individuality.

Minimal genomes required for life

Rosario Gil, Vicente Pérez-Brocal, Amparo Latorre & **Andrés Moya**

Universitat de València, Spain

The minimal cell field is partly anchored in solid experimental facts, and partly still belongs to the sphere of theoretical research. Projects designed to scan entire microbial genomes for essential genes have revealed a remarkably compact and conserved, but not universal, set of genes whose functions are necessary for survival or reproduction. A minimal cell cannot be sharply defined, since different essential functions can be defined depending on the environmental conditions.

Nevertheless, it is possible to try to delineate which functions should be performed in any modern living-cell, and list the genes that would be necessary to maintain such functions, although numerous alternative minimal genomes can be conceived to fulfill such functions even for the same set of conditions.

Remarkably, the different approaches used to define a minimal genome converge on the conclusion that genes dealing with RNA biosynthesis are common to all cellular life, while some of the main components of the DNA-replication machinery are not universal.

The development of more sophisticated techniques for genomic engineering, together with the continued efforts in defining the minimal genome, will help to achieve the exciting goal of experimentally constructing a modern-type minimal living cell in a perhaps no so distant future.

Evidence of core genes – what can be and are laterally transferred?

V. Daubin

CNRS, Lyon, France

The extravagant diversity of microbes has only been fully apprehended with the development of comparative genomics. Comparisons of gene repertoires among prokaryotes have revealed striking differences among species, and even among strains of the same species. For example, the genomes from three *Escherichia coli* strains have been shown to share only 40 % of their genes, with most of the remaining genes being strain-specific. More generally, although most prokaryotic genomes contain thousands of genes, only a handful can be identified as truly ubiquitous in modern organisms. This so called 'core' of universal genes has received much interest from evolutionary biologists because it likely represents a relic of the last universal common ancestor (LUCA) and provides valuable information for reconstructing the tree of life. It has also been viewed as the *sine qua non* condition of life because no living organism seems to be able to survive without it. However, perhaps more interesting is the paucity of these ubiquitous genes, as it shows the formidable evolutionary plasticity of biological systems, and points at the mechanisms necessary for acquiring and generating new genes.

Biogeographical diversity of archaeal viruses

K.M. Stedman

Portland State University, USA

Viruses of thermophilic Archaea are unique both in their morphology and genomic sequences. The best-studied of these viruses are the Fuselloviruses, Spindle-Shaped Viruses (SSVs), of the hyperthermophilic and acidophilic crenarchaea *Sulfolobus*. Fuselloviruses are present worldwide in 70+ °C, pH 3 volcanic hot springs and mud pots in which *Sulfolobus* is found. The 15 kbp double stranded DNA virus genomes are relatively small, making them attractive for biogeographical studies. The complete genome sequence of four SSVs from Japan, Iceland, the USA and Russia has been determined. They were found to be only 55 % identical. To answer the question of whether these differences are due to geographical isolation two parallel studies were performed. In one, the complete genome sequence of an additional SSV isolate from Iceland was determined. In parallel, culture-independent studies were performed in Yellowstone National Park and Lassen Volcanic National Park in the USA. Massive diversity was observed in the culture-independent data set. By contrast evidence for endemism was seen in the complete genome study.

Is there a link between *Chlamydia* and heart disease?

Lee Ann Campbell & Cho-chou Kuo

University of Washington, Seattle, USA

Chlamydia pneumoniae is a human respiratory pathogen that causes acute respiratory diseases. *C. pneumoniae* has also been associated with atherosclerosis, a disease of chronic inflammation and the leading cause of morbidity and mortality in the western world. This association has been based on several lines of evidence including: 1) seroepidemiological studies demonstrating an increased risk of coronary heart disease and myocardial infarction in patients with antibodies to *C. pneumoniae* in comparison to controls; 2) detection and isolation of the organism from atheromatous tissue; 3) *in vitro* studies demonstrating upregulation in expression of proatherogenic factors by *C. pneumoniae*; and 4) studies in animal models demonstrating acceleration of atherosclerotic plaque formation, promotion of endothelial dysfunction, and effects on plaque destabilization. A few of the early small clinical treatment trials using macrolide antibiotics for prevention of cardiac events in humans were promising; however, none of the three recent large scale trials of secondary prevention in the US demonstrated a prolonged benefit of antibiotic treatment. Because of the difficulty in treating chronic chlamydial infections, if the organism does contribute to advancement of atherosclerosis, basic science studies focused on the identification of targets or strategies for earlier intervention or prevention are critical.

Unculturable oral bacteria

William Wade

King's College London

The human mouth is the most easily and frequently accessed of body cavities. It is surprising therefore that the micro-organisms found there are largely unknown. To date, over 800 oral bacterial species have been detected, together with viruses, protozoa and fungi. Oral bacteria include representatives of 13 phyla from the domain *Bacteria* and representatives of the genus *Methanobrevibacter*, from the *Archaea*. Comparison of microscopic and viable counts reveals that 50 % of oral bacteria cannot be cultured using conventional bacteriological methods. Unculturable taxa fall into two groups: those that are relatives of culturable taxa and those belonging to entire lineages without culturable representatives, such as the phyla TM7 and OP11 and major branches of the *Bacteroidetes* and *Firmicutes*. Many taxa considered unculturable, because they are unable to grow in mono-culture, are able to grow in co-culture with other oral bacteria. This suggests that nutritional or signalling interactions between species are important *in vivo*. Unlike the normal microflora found at other body sites, oral bacteria have to be controlled to prevent the commonest bacterial diseases of man: dental caries and periodontal disease. The investigation of the pathogenic potential of the oral microflora needs to consider unculturable species; metagenomic approaches will be important.

Comparative genomics – what do such studies tell us about the emergence and spread of key pathogens?

Richard W. Titball & Melanie Duffield

Dstl, Porton Down

Prior to the availability of genome sequence information, it had generally been assumed that pathogens causing severe diseases would have evolved by acquiring additional DNA encoding virulence determinants. Genome sequence information has allowed us to test this hypothesis and has revealed that the situation is much more complex. It is true that pathogens like *Bacillus anthracis* have evolved solely by the acquisition of DNA. However, many other pathogens have evolved by the simultaneous acquisition and loss of DNA (like *Yersinia pestis*) or even by the loss of DNA alone (like *Mycobacterium leprae* and *Burkholderia mallei*). It is clear that the emergence of pathogens merely reflects the myriad of genetic interchanges between bacterial species and genetic changes within species which are occurring at all times.

On the basis we can be certain that in the future other pathogens will emerge.

Spread of genomic islands between clinical and environmental strains

J. Klockgether, O.N. Reva & **B. Tümmler**

Medizinische Hochschule, Hannover, Germany

Despite substantial horizontal gene transfer the phylogenetic relationships between taxa are robust as indicated by the congruence of gene trees based on rDNA sequence, gene contents or average amino acid identity of shared genes. Genomic islands that spread among taxa, but occur only in a subset of strains in each taxon, are an exception from this rule. The most prominent example is an evolutionarily ancient genomic island that is widespread among β- and γ-*Proteobacteria* and has been identified in isolates from clinical and environmental habitats. This genomic island consists of two modules: one module endows strain-specific features, the other module consists of a set of conserved syntenic genes. Sequence identity of the syntenic set is less than that of vertically transmitted genes indicating that these genes and likely also the encoded functions are more diverse than those encoded by orthologs of core genomes. Future research will unravel whether or not the syntenic gene set is not only essential for the maintenance of the genomic island, but also influences the expression, regulation and function of gene products of the core genome.

Evolving gene clusters in soil bacteria

E.M.H. Wellington, A. Morningstar, W.H. Gaze, S. Tolba & A. Ul-Hassan

Dept of Biological Sciences, University of Warwick, West Midlands CV4 7AL

Clustered genes are a common phenomenon on microbial genomes and often form part of mobile or mobilizable genetic elements such as transposons, integrons, pathogenicity islands and plasmids. They frequently have co-ordinately regulated genes with adaptive functions such as symbiosis, virulence, biodegradation of xenobiotics, biosynthesis of antibiotics or multiple antibiotic resistances. Clustering of genes provides an effective way of mobilizing a key function and clearly genomic islands have played a major role in the adaptive evolution of prokaryotes. Rapid changes in phenotype can be achieved by horizontal transfer of a gene cluster which may further evolve by gene duplication and loss. We are studying adaptation in environmental bacteria and present here our data on the evolution of antibiotic biosynthetic gene clusters and antibiotic resistance. Using the streptomycin gene cluster we have evidence of several key evolutionary patterns including decay of an ancient gene cluster coupled with extensive horizontal gene transfer, with deletion and duplication. These events could help in explaining the extensive metabolic diversity of antibiotic biosynthetic origins observed within actinobacteria. Genome rearrangements involving large fragments of DNA should avoid core regions which are conserved and maintain vital housekeeping functions. Variable chromosomal regions such as IS elements, integrons, plasmids etc provide genome plasticity and the role of integrons in bacterial adaptation to specific environments will be discussed.

Unusual micro-organisms from unusual habitats: hypersaline environments

Antonio Ventosa

University of Sevilla, Spain

Hypersaline environments are typical extreme habitats in which the high salt concentration is not the only environmental factor that may limit their biodiversity; the two main groups of micro-organisms that predominate are the moderately halophilic bacteria and the extremely halophilic micro-organisms (archaea and bacteria). Archaea have been associated to extreme environments and although today they are also recognized as normal inhabitants of other non-extreme environments, they constitute a large proportion of the microbial biota of hypersaline environments. Most are haloarchaea included in the order *Halobacteriales,* family *Halobacteriaceae;* they are currently represented by 20 different genera and a large number of species. Direct observations of brines of salterns and other hypersaline environments suggest that square cells, described by A.E. Walsby in 1980 for the first time, are very abundant in such habitats, especially in the most concentrated ponds with salinities higher than 3-4 M NaCl; many attempts have been carried out to isolate these square micro-organisms but only recently have two independent studies reported their isolation and cultivation. Besides, the presence and characteristics of other halophilic micro-organisms, such as the extremely halophilic bacterium *Salinibacter ruber*, the moderately halophilic bacteria as well as eukaryotic organisms, will be reviewed.

Genomic islands and evolution of catabolic pathways

Stéphan Lacour, Muriel Gaillard & **Jan Roelof van der Meer**

University of Lausanne, Switzerland

Genomic islands can be defined as discrete regions on the bacterial chromosome, which can be flanked by direct repeats, are potentially unstable and/or transferable to other bacteria. The most 'complete' genomic island is probably the one which can excise from its chromosomal location, self-transfer to another host and reintegrate at one or more specific sites, but defects in one or more of these capabilities are common. Pathogenicity islands are probably the most well-known version of genomic islands; they carry functions contributing to the pathogenic character of its host. Some genomic islands, however, provide the host with the capacity to metabolize aromatic compounds; functions which traditionally have been thought to be solely associated with catabolic plasmids. Here we will introduce the so-called *clc* element originating in *Pseudomonas* sp. strain B13, a 105-kb self-transferable genomic island. We will show that parts of this mobile element are well-distributed among a variety of proteobacterial hosts, but that it became specialized in providing genes for aromatic compound metabolism, notably chlorocatechols and 2-aminophenol. Furthermore, we will describe some of the more peculiar properties of the *clc* element with respect to its excision and transfer.

Horizontal gene transfer and its role in the emergence of new phenotypes

A. Mark Osborn

University of Sheffield

This review discusses the role and importance of horizontal gene transfer (HGT) in prokaryotic adaptation and evolution. HGT operates via the classically described mechanisms of transformation, transduction and conjugation. The field of HGT has undergone a recent renaissance with the emergence of a plethora of novel mobile genetic elements (MGE) including integrative and conjugative elements (ICE) and mobilizable transposons. Whilst our understanding of HGT was for many years driven by analysis of plasmids and bacteriophage, comparative genomics and more recent metagenomic analysis has revealed that many prokaryotic chromosomes show considerable evidence of HGT-mediated evolution. This review discusses the emergence of perhaps surprising new HGT-mediated phenotypes in particular in environmental systems. Examples include the discovery of phage-mediated transfer of photosynthesis genes in marine cyanobacteria, and promotion of biofilm formation by conjugative plasmids. The transfer of ICE between bacteria has additionally been shown to be promoted by environmental stimuli including the presence of antibiotics or high UV levels. Such examples suggest considerable interaction between HGT mediated prokaryotic evolution and their environment.

Cells & Cell Surfaces Group session

Surface-anchored molecules: sticky fingers

Assembly of bacterial capsules – translocation of polysaccharides to the cell surface of *Escherichia coli*

Chris Whitfield

Dept of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

The surfaces of bacteria are complex arrays of proteins and glycoconjugates. Capsular polysaccharides form surface layers that often protect bacterial pathogens from phagocytosis, and in some cases confer resistance to complement-mediated killing. The systems involved in the biosynthesis and assembly must be highly efficient because the extent of surface coverage and optimized polymer chain length are both crucial protective properties. Significant progress has been made in identifying gene products involved in capsule assembly and there is an emerging biochemical understanding of the initial steps in capsular polysaccharide biosynthesis. In contrast, there is relatively little information available concerning mechanisms by which synthesis and export is coordinated for polymers whose molecular weights can exceed 106 Da. In Gram-negative bacteria the export processes are complicated by the need to cross the inner and outer membranes, and the peptidoglycan-containing periplasm. Emerging evidence for prototype capsule assembly systems points to the involvement of an envelope-spanning complex that couples capsular polysaccharide biosynthesis to a multimeric 'secretin'-like outer membrane channel. I will discuss the current structural and functional data concerning these complexes and their relationship to other macromolecular systems involved in efflux and secretion of diverse substrates in Gram-negative bacteria.

Bacterial lipopolysaccharides – structure, biosynthesis and function

Otto Holst

Division of Structural Biochemistry, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Parkallee 4a, D-23845 Borstel, Germany

Lipopolysaccharides (LPS, endotoxins) are unique cell wall constituents and major virulence determinants of Gram-negative bacteria. They comprise three regions which can be distinguished by they structure, genetics, biosynthesis and function: a glycolipid, termed lipid A, which anchors the molecule in the outer leaflet of the outer membrane of the cell wall and which in the case of endotoxic LPS represents the endotoxic moiety, a core region which is a (phosphorylated) oligosaccharide of not more than 15 sugars, linked to the lipid A, and the O-specific polysaccharide (the O-antigen) which in most cases is a heteropoly-saccharide built up from repeating units of 2–8 sugar residues. Regarding LPS genetics and biosynthesis, quite a number of (rather recent) investigations have provided a significant body of information which enables us to visualize LPS assembly and transport of LPS to the bacterial cell surface. Despite their function as bacterial cell wall components, LPS play a major role in gram-negative infections and septic shock. LPS interact with cells of the innate immune system, in particular with their receptor complex TLR4-CD14-MD2, leading to the initiation of intracellular signaling processes which result in the production of (high amounts of) pro-inflammatory cytokines and thus in various subsequent pathopysiological effects.

Lipoteichoic acids and teichoic acids

Andreas Peschel

University of Tubingen, Germany

Most of the Gram-positive bacteria with low G+C content contain peptidoglycan-anchored wall teichoic acids (WTA) and membrane-anchored lipoteichoic acids (LTA) in their cell envelopes. The two types of polymers differ in structure, biosynthetic pathways, and biological activities. An in-depth characterization of WTA and LTA has been hampered by the great variability of structures and complicated chemistry. Bacterial mutants with altered LTA glycolipid anchor have recently enabled functional studies on LTA function using isogenic bacterial mutants. However, most of the LTA biosynthetic genes are still unknown. We have recently identified a number of genes involved in WTA biosynthesis and generated mutants with lacking or altered WTA in Staphylococcus aureus. Their characterization indicated an essential role of WTA in human nasal colonization and in specific interaction with epithelial and endothelial cells. Our data demonstrate critical roles of WTA and LTA in bacterial physiology and virulence. Further analyses of structure, function, and biosynthesis of these prominent surface structures may lead to new approaches to prevent and treat bacterial infections.

The regulation of type 1 fimbriation in *Escherichia coli*: how and why?

I.C. Blomfield

University of Kent at Canterbury

Type 1 fimbriae of *E. coli* promote bacterial attachment to, and invasion of, host cells. The adherence is proinflammatory, activating TLR4 signalling. Like many adhesins, type 1 fimbriation is regulated by phase variation (cells switching between expression and non-expression states). Phase variation of fim involves inversion of a DNA element (*fimS*) that contains a promoter for the fimbrial structural genes. Inversion of *fimS* is catalyzed by FimB and FimE and is controlled by DNA bending proteins IHF and Lrp. FimB, by catalysing off-to-on switching, generates fimbriate cells. fimS inversion is regulated by temperature, osmolarity, pH, and leucine and alanine, as well as by sialic acid (Neu_5Ac) and *N*-acetylglucosamine (GlcNAc). Whereas GlcNAc and Neu_5Ac inhibit *fimB* expression, the amino acids control the inversion directly. Factors that suppress fim off-to-on phase variation (elevated temperature, free Neu_5Ac and GlcNAc) are hallmarks of inflammation and oxidative stress. Moreover, both *fimB* expression, and the biosynthesis of the branched-chain amino acids, are inhibited by superoxide stress. A small (~2-fold) decrease in *fimB* expression reduces FimB recombination substantially (~12-fold), and we propose that this effect, coupled to direct control of FimB inversion, suppresses fim off-to-on phase variation with increasing sensitivity if inflammation escalates.

Lipoprotein sorting in *Bacillus subtilis*

Jan Maarten van Dijl[1], Mark J.J.B. Sibbald[1] & Harold Tjalsma[2]

[1]Laboratory of Molecular Bacteriology, Dept of Medical Microbiology, University Medical Centre Groningen and University of Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, The Netherlands (Tel. +31-50-3633079, email j.m.van.dijl@med.umcg.nl); [2]Dept of Clinical Chemistry, Radboud University Nijmegen – Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

One of the most commonly used bacterial sorting (retention) signals for proteins that are exported from the cytoplasm is an amino-terminal lipid-modified Cys residue. In Gram-positive bacteria, lipid-modified proteins (lipoproteins) are retained in the cytoplasmic membrane. In Gram-negative bacteria, these proteins are retained in the cytoplasmic or the outer membrane. The numbers of putative lipoprotein-encoding genes per bacterial genome seem to range from ~18 in *Mycoplasma genitalium* to ~94 in *Escherichia coli* and up to 114 in *Bacillus subtilis*. Thus, lipoproteins appear to represent about 1-7 % of the proteome of bacteria. Bacterial lipoproteins are involved in a variety of processes, such as the uptake of nutrients, resistance to antibiotics, protein secretion, sporulation, germination, cell wall biogenesis, and bacterial targeting to different substrates, bacteria and host tissues. Furthermore, surface-exposed lipoproteins have been implicated as important mediators of the inflammatory response in human hosts during infections of Gram-positive and Gram-negative pathogenic bacteria. In this presentation, several aspects of lipoprotein sorting in Gram-positive bacteria will be discussed using examples from our protein secretion research in *B. subtilis*. These include the cellular machinery for lipid-modification, and a proteomic view on lipoprotein export from the cytoplasm and retention in the membrane.

Surface-anchored proteins of *Staphylococcus aureus* involved in nasal colonization and pathogenesis

T.J. Foster

Microbiology Dept, Trinity College, Dublin 2, Ireland

Nasal colonization by *Staphylococcus aureus* is an important risk factor for infection. Bacteria adhere to desquamated epithelial cells in the nares. Several proteins including clumping factor B (ClfB) that are expressed on the bacterial cell surface promote adherence to squames. In addition to binding fibrinogen, ClfB also binds strongly to cytokeratin 10, one of the major proteins in squames. Molecular analysis identified the binding region for ClfB in keratin 10 as C-terminal 'tail' region that is composed of quasi repeats of Tyr–$(Gly/Ser)_n$-Tyr. A synthetic peptide mimicking a typical glycine loop (YGGGSSGGGSSGGY) blocked adherence of ClfB-expressing bacteria to immobilized keratin 10. A ClfB–defective mutant colonized the nares of mice less readily than the wild-type. Active immunization with rClfB and passive immunization with anti-ClfB monoclonal antibody reduced nasal colonization in mice.

Clumping factor A is structurally related to ClfB. It binds avidly to fibrinogen but not to keratin 10. The mechanism of binding to the C-terminus of the γ-chain of fibrinogen likely occurs by a 'dock latch and lock' mechanism. ClfA is an important virulence factor in septic arthritis and endocarditis infection models. Vaccination with recombinant ClfA stimulated protective immunity.

S-layers

M. Sára

Universität für Bodenkultur Wien, Austria

Abstract not received

The principles of N-linked protein glycosylation in bacteria

Markus Aebi

Institute of Microbiology, Dept of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

N-linked protein glycosylation is the most frequent protein modification in eukaryotic cells. This conserved process initiates at the membrane of the Endoplasmic Reticulum, where an oligosaccharide, $Man_5GlcNAc_2$, is assembled on the lipid carrier, dolichylpyrophosphate, translocated across the membrane and completed to $Glc_3Man_9glcNAc_2$. This oligosaccharide is then transferred to selected asparagine residues of nascent polypeptide chains.

N-linked protein glycosylation does also take place in archaea and in bacteria. The recently discovered N-linked protein glycosylation process in *Campylobacter jejuni* was transferred into *Escherichia coli*, enabling a genetic and biochemical analysis of the prokaryotic pathway. As in eukaryotic cells, an oligosaccharide, $GlcGalNAc_5Bac$ in the case of *C. jejuni*, is assembled at the cytoplasmic side of the plasma membrane on an isopreonoid carrier, bactoprenyl-pyrophosphate. After translocation across the membrane, the oligosaccharide is transferred to protein. The membrane protein PglB catalyses this transfer. D/E-X-N-X-S/T was found to be the acceptor for glycosylation (X can be any amino acid except proline).

The high sequence similarity of the bacterial oligosaccaryltransferase with one subunit of the eukaryotic enzyme, the very similar protein acceptor sequence as well as the finding that bactoprenyl-pyrophosphate- and dolichylpyrophasphate-linked oligosaccharides serve as substrates in the reactions suggest that the bacterial and the eukaryotic N-linked protein glycosylation are homologous processes.

Clinical Virology Group session

Viral central nervous system infections

Persistence of HTLV-1 and cell-cell spread through the virological synapse

Charles R.M. Bangham

Dept of Immunology, Wright-Fleming Institute, Imperial College, Norfolk Place, London W2 1PG (Email c.bangham@imperial.ac.uk)

Three broad questions have driven research into the immune response to HTLV-1. First, how does HTLV-1 persist in the individual host? Second, why do some HTLV-1-infected people develop a consequent disease such as HAM/TSP or leukaemia, whereas the majority remain asymptomatic carriers of the virus? Third, how is the inflammatory lesion in HAM/TSP initiated and maintained, and how can the inflammation be halted? Evidence on the immune response to HTLV-1 will be summarized from recent work in host and viral genetics, DNA expression microarrays, T-cell function and cell biology. This evidence favours two main conclusions: first, that the efficiency of a person's cytotoxic T-lymphocyte (CTL) response to HTLV-1 plays a dominant role in determining that person's proviral load of HTLV-1 and the risk of the associated inflammatory diseases; second, that HTLV-1 is persistently transcriptionally active and spreads directly between lymphocytes by a specialized cell-cell contact known as the virological synapse. These conclusions have implications for therapeutic approaches both in the inflammatory diseases and in the HTLV-1-associated syndrome of adult T-cell leukaemia, and in the immune response to persistent viral infections.

Herpes simplex and the brain: from encephalitis to gene therapy

Richard Whitley, MD

The University of Alabama at Birmingham, USA

Herpes simplex virus (HSV) is a known pathogen of neuronal tissue, especially the central nervous system. Being one of the most common causes of sporadic and oft times fatal encephalitis, HSV, historically, had not been considered an appropriate vector for gene therapy. Recently, the engineering of HSV has allowed for the ablation of neurovirulence. The deletion of a diploid gene which maps in the inverted repeat unique long segment, namely, the $\gamma_1$34.5 gene, ablates the ability of the virus to replicate in post-mitotic neuronal cells. As a consequence, HSV constructs deleted in this gene have been utilized both for the development of a genetically engineered vaccine as well as for gene therapy. Replication competence is established in non-neuronal, dividing cells. Utilization of viruses deleted in $\gamma_1$34.5 in animal models of human tumors, indicate long survival with no evidence of disease.

Because it is unlikely that genetically engineered HSV alone will be useful as a single therapeutic approach to brain tumors, it can serve as a platform for the expression of foreign genes, including cytokines, receptors, enzymes and paracrines. For example, the expression of the cytokine interleukin 12 (IL 12) in HSV has been accomplished. Expression of IL-12 in an HSV vector deleted of $\gamma_1$34.5 has a similar beneficial effect, if not enhanced long term survival in animal murine systems. The induced inflammatory response indicates a Th-1 driven effect.

Proof of principal has been established in a Phase I clinical trial. An HSV construct, G207, a $\gamma_1$34.5 deletion, has been tested in 21 human volunteers with glioblastoma multiformi refractory to standard therapies. In this dose escalating Phase I clinical trial there was no evidence of encephalitis. Furthermore, there was a suggestion of a beneficial effect on tumor regression. These data indicate the possibility of utilizing HSV as a vector for foreign gene expression.

Entry of HIV into the brain; host and viral determinants

M. McCrossan[1], S. Willey[1], M. Marsden[1], F.W. Carnie[2], S. Minnis[1], B. Hansoti[1], I.C. Anthony[2], R.P. Brettle[3], J.E. Bell[2] & **P. Simmonds**[1]

[1]Centre for Infectious Diseases, University of Edinburgh, Summerhall, Edinburgh EH9 1QH; [2]Dept of Pathology, Western General Hospital, Crewe Road, Edinburgh EH4 2XU; [3]Regional Infectious Diseases Unit, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU

The brain is targeted by HIV during the course of untreated infection, leading to cognitive impairment, neurological damage and encephalitis. To study the dynamics of HIV entry into the brain, we examined an autopsy series of samples obtained from untreated individuals who died in presymptomatic stages of infection from non-HIV causes, and from individuals who died while receiving highly active antiretroviral therapy (HAART). Brain samples from pre-symptomatic individuals were characterized by a complete absence of actively expressing HIV-infected cells detectable by immunohistochemistry, and variable and generally extremely low levels of proviral DNA sequences. All brain regions showed varying degrees of infiltration of CD8 lymphocytes in the brain and associated expression of activation markers which correlated with the presence of HIV infected cells (proviral load; $R = 0.608$; $P < 0.05$) and genetic segregation of brain variants from populations in lymphoid tissue (AI value, $R = -0.528$; $P < 0.05$). The inflammatory process found in pre-symptomatic individuals was comparable in extent among individuals receiving HAART, despite the effectiveness of treatment in controlling viral replication in the brain and periphery. While HAART is capable of preventing AIDS development, the ongoing inflammatory process in the CNS may lead to progressive neurological damage and cognitive impairment among long term-treated individuals.

Antigen identification in multiple sclerosis (MS)

Donald H. Gilden

University of Colorado, Denver, USA

The CSF of many MS patients contains inflammatory cells, and nearly every MS CSF contains increased amounts of IgG and oligoclonal bands. The cause of MS is unknown, but epidemiological evidence suggests that MS is acquired, a notion supported by the fact that when an identical twin develops MS, only 30 % of second twins develop disease. The most important evidence suggesting an infectious etiology of MS derives from studies of CSF from patients with chronic infectious CNS diseases. In these individuals, oligoclonal IgG in CSF is directed against the agent that causes disease.

Molecular analysis of the specificity of IgG in MS brain and CSF has the potential to identify an infectious antigen in MS. This talk will describe application of these techniques to single B-lymphocytes and plasma cells in the CSF of MS patients, which reveal features of an antigen-driven response. More importantly, recombinant antibodies prepared from overexpressed sequences in single plasma cells from the brain of a patient with SSPE were shown to be specific for measles virus, the cause of SSPE. Identical strategies and techniques can readily be applied to identify the causative antigen of MS. The search for a viral cause of MS must be continued.

Varicella-zoster virus latency in human ganglia

Peter G.E. Kennedy

University of Glasgow, Dept of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51 4T

Varicella-Zoster Virus (VZV) is human herpesvirus which causes varicella (chickenpox) as a primary infection, and then establishes a latent infection in trigeminal and sensory ganglia. Following a latent period of years, the virus may reactivate to cause a painful vesicular skin eruption called herpes zoster (shingles) which is followed by post-herpetic neuralgia in about 50 % of patients over the age of 65 years. VZV infections are more common in immunosuppressed individuals, especially those with AIDS, and may be followed by a variety of neurological complications. The health and economic burden of VZV infections is considerable.

The molecular mechanisms underlying VZV latency are not yet understood. Both rat and simian models of VZV latency have been developed and hold promise as tools to study latency. The cellular location of latent VZV in human ganglia was controversial for a decade but is now known to be predominantly neuronal. The nature and extent of VZV gene expression during latency is also important, and studies to date have indicated that it is restricted to 6 VZV genes. There is also evidence for several VZV gene-encoded proteins being expressed during latency. Microarray technology is also being used to address both lytic and latent viral gene expression.

Mosquitoes and the risk of West Nile fever in Britain

C.A. Malcolm

School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS (Email c.a.malcolm@qmul.ac.uk)

There are 33 species of mosquito in Britain; almost half bite humans and at least ten are a source of recurrent human biting nuisance. Several may be potential 'bridge vectors' capable of transmitting West Nile virus (WNV) from birds to humans causing fever and the more severe neuroinvasive disease. These mosquitoes are relatively uncommon, or limited in their distribution. The most prevalent mosquito in Britain is *Culex pipiens*, which is the most widely distributed WNV vector around the world, but its vectorial dynamics are complicated by morphologically near identical forms that vary in their biting behaviour and the extent to which they interbreed. Two distinct non-interbreeding forms exist in Britain: the more common one only bites birds; the other has a strong preference for humans, but will also bite birds and could be a moderate to poor bridge vector. More dangerous variants, for example a novel 'hybrid' that contributed to the severity of the recent and unprecedented outbreak in the USA could pose a risk in Britain if transported here by accident. This possibility was illustrated by the discovery of an isolated rural population of the human biting form in central Scotland. This was derived from a single inseminated female passively transported from southern Europe, where there is a long history of sporadic and isolated outbreaks of West Nile diseases.

Education & Training Group session

What does an undergraduate microbiologist need to know?

In 1994/1997, the ASM produced the Curriculum Guidelines 'Core themes and concepts for an introductory microbiology course (module)'. This information has proved valuable for US institutions developing new courses and/or reviewing existing courses/modules/units – as a consultation document rather than regulatory.

We may assume that such core themes are likely to be global, but this has never been considered in the UK and Ireland, and more widely in Europe. With the advent of 'benchmarking' and the Bologna process (which aims to harmonize degrees across Europe and enhance mobility), and the changing nature of microbiology within University departments/schools (encapsulated within biology, molecular biology etc), perhaps it is timely to consider what we believe to be the key requirements of an (early years) undergraduate microbiology curriculum – and how the currency of such information can be retained.

This workshop will aim to produce a draft outline curriculum and discussion document to carry forward to for further consideration.

Microbiology educators from Europe will outline their activities and concerns. ASM guidelines will be circulated to enable discussion after the presentations. Information will be collected via a plenary session, and findings will be circulated for comments before they are disseminated more widely.

The Bologna process

A.J. Vickers

University of Essex

The establishment of the European Higher Education Area (EHEA) is a reality. What it will be is down to those involved in its creation. The Bologna Process will be discussed in this presentation along with the implications for Higher Education in the EHEA area, and in particular the impact on Institutions in the UK

The ASM undergraduate microbiology curriculum

Neil R. Baker, ASM Undergraduate Education Committee Chair

Associate Professor of Microbiology, The Ohio State University, Columbus, Ohio, USA

The ASM Undergraduate Education Committee has established curriculum guidelines to aid educators in the design and implementation of a stand-alone introductory microbiology course, major programs and other specialty areas such as biotechnology. The motivation to develop the recommendations came from individual faculty and programs seeking assistance in the design of courses and programs. To establish a core curriculum for an introductory course in microbiology faculty from community colleges through research universities met at the first Annual ASM Conference for Undergraduate Educators in 1994. The product of this meeting was a set of core themes and concepts that define a common foundation for an introductory microbiology course at any institution. Since 1994 faculty attending the annual ASM Conference for Undergraduate Educators have defined guidelines for microbiology major programs, allied health programs, biotechnology programs, and microbiology content of biology courses for non-sciences and general education majors. The conference also serves as a valuable platform to evaluate and enhance learning based on these guidelines.

The European undergraduate microbiology curriculum: views from across the Community:

What does an undergraduate microbiologist need to know?

Hans Utkilen

Norwegian Institute of Public Health

Valuable information for undergraduate microbiologist should be focussed on subjects that will awake the interest and curiosity of the students and show them that there are many possible careers paths to follow as a microbiologist.

The student should learn that understanding microbes will help them to do well in such fields as business, sociology, food science, pharmaceutical and health sciences, medicine and agriculture. This may be achieved by learning about the place of microbes in ecology and the environment, and the use of microbes in biotechnology. Additionally, knowledge of the role of microbes in food production, and the numerous others ways that microbes contribute to the quality of our lives will further increase the interest. This all builds on the knowledge about microbial growth and physiology, which therefore should have a high priority, but this is probably not the subjects that would initiate enthusiasm among students. However, done in the right way and showing how metabolites or enrichment of special bacteria can change the environment from being destructive to useful. I am sure that the value of this basic 'boring' knowledge can be shown to be crucial for success in the mentioned fields.

In Norway the plan is to start a course in microbiological techniques and simple/illustrative class experiments for biology teachers in secondary schools. This will be performed at the Bø College. An idea of introducing science and mathematics in kindergartens has been brought forward, an initiative that has obtained support from the government. Microbiologists should engage themselves in these discussions and propose that microbiology also can be introduced at this stage. Because there are so many interesting examples in the daily live of a child that can illustrate how micro-organisms affect them. An initiative has also been taken to write a book on microbiology for children.

Gosse Schraa

Laboratory of Microbiology, Wageningen University and Research Centre

Dutch students have little knowledge of microbiology when they start a B.Sc. program at universities in The Netherlands.

Microbiology in the BSc. study at Wageningen University: There are eight Dutch universities with microbiology departments. Several programs in these universities lead to a B.Sc. degree with a substantial, but variable amount of (medical) microbiology knowledge (bacteriology, mycology, phycology, protozoology and virology).

All students of Wageningen University in the programs Biology, Biotechnology, Food Technology, Molecular Sciences, and Nutrition and Health have to take an introductory microbiology course in their first or second year. This course consists of 6 ECTS credits and is partly based on the ASM's curriculum recommendations for an introductory course in microbiology.

Advanced microbiology courses in the second and third year of the B.Sc., that are compulsory for specific specializations or that can be taken optionally, are Microbial Physiology, Bioinformation Technology, Applied Molecular Microbiology, an advanced practical microbiology course and several Food Microbiology courses. The

program committees and the departments/professors are responsible for the content of these courses.

Additional theoretical and practical microbiology knowledge is also obtained in courses such as Enzymology, Cell Physiology and Gene Technology, which are offered by the Biochemistry and the Molecular Biology Department.

David J. Adams

Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT

In the UK, GCSE students (age 14-16) and their equivalents in Scotland must study only the rudiments of microbiology. Some examining bodies offer additional microbiology at this level while microbiology is offered as an optional part of the A-level (age 16-18) syllabus. Nonetheless, most students arrive at UK universities with very little knowledge of the fundamentals of microbiology. There is therefore a need for a comprehensive grounding in bacteriology, mycology and virology with consideration of environmental, industrial, food and medical microbiology during Level 1 studies. Most students also lack a basic understanding of the immune system and must be taught the fundamentals of microbial immunology at an early stage of their university career. Microbiology is a hands-on subject that provides great scope for the development of a wide range of skills ranging from microscopy to microbial culture/identification to data handling. A practical-based approach at Level 1 is recommended as it helps enthuse students in the study of what is for many an exciting new subject. UK universities adopt a range of approaches for the teaching of microbiology. The establishment of a core curriculum with key skills, new knowledge and techniques should help establish a more thorough and consistent approach.

Markus Aebi

Institute of Microbiology, Dept of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

The Zurich microbiology curriculum: Due to the introduction of the Bachelor-Master system in the course of the 'Bologna process', the University of Zurich and the Swiss Federal Institute of Technology (ETH) Zurich have coordinated their teaching programs in Biology. The complementary expertise of the two universities made it possible to offer the students a broad and exciting spectrum of courses.

Based on a profound knowledge in Mathematics, Physics, but particularly in Chemistry and in Biology (including Microbiology) students can focus their studies in the third year of the BSc Biology program on Microbiology. More than 20 Microbiology-specific courses, covering very different topics such as Molecular Microbiology, Microbial Physiology, Microbial Ecology, Parasitology, Medical Microbiology, Cellular Microbiology, Virology, Mycology or Plant Pathology are offered.

In a research-oriented Master program in Biology with focus on Microbiology, research projects are performed in the different research groups, accompanied by specialized courses in the different areas of Microbiology.

B. Pérez-Uz, S. Serrano & C. Vázquez

Dept Microbiología III. Facultad CC. Biológicas. Universidad Complutense de Madrid. 28040 Madrid, Spain

The microbiology curriculum in the current undergraduate education context in Spain is included as a core course in the second or third year of the degrees on Biology, Pharmacy, Veterinary or Medicine. The changes happening with the advent of the convergence on European Higher Education encouraged the Spanish Microbiology Society (SEM) to propose the creation of a Microbiology degree; however, it seems this proposal will not be considered in the degrees catalogue produced by the government that will rule the professional proficiency of those degrees included in it. This is important since it will define a different landscape for the future curricular developments to be considered. In this framework, the microbiology curriculum design will still depend on the degree bias where the course is included.

The microbiology program in the present Biology degree is included in the 2nd year during two consecutive semesters. The course content has 6 credits (10 h/credit) plus a 1.5 credits (15 hours) seminar and 3 credits (30 hours) laboratory modules. Core themes include an introduction to microbiology including microbial methods, five sections dedicated to bacterial structure, metabolism and growth, genetics, interactions and diversity; there is also a section on virology and finally another one on eukaryotic microbiology. This program is probably too comprehensive but it must supply the future Biology Graduate with a complete view of the microbiology. The condensed insight of the subjects considered however, might be supplemented with further specific elective courses that would allow the graduate to have certain unofficial specialization.

Julian R. Marchesi

Dept of Microbiology, University College Cork, College Road, Cork, Ireland

Irish students take a wider range (typically 7 subjects) in their final secondary school year, which is similar to the Scottish system, but different to the English/Welsh system, hence they specialize later in the education. Accordingly, BSc programmes in Irish Universities are typically 4 years with the first year devoted to providing a broad grounding in physical, chemical and biological sciences. At the end of this year they will be asked to choose three 'degree outlets' which they will study in their 2nd year and after this year they will choose their degree subject for years 3 and 4. In order to attract students to study microbiology, the first year is viewed as a recruitment exercise. The students are provided with a comprehensive overview of the subject and shown the exciting and 'sexy' side of the subject, with a key aim of 'hooking' them into the area. The proposal to establish a core curriculum for first year students, would not be so easily implemented in Ireland since each University decides how students enter courses and may require a change of the system to allow students to specifically choose microbiology when they enter college.

Franc Viktor Nekrep

University of Ljubljana; Biotechnical Faculty, Slovenija

At the University of Ljubljana (UL) we are expecting in 2006 the 14th and last generation of students entering our present microbiology curricula: 4 (5) years' 'Diploma' programme; 2 years' MSc programme and 3 years' PhD programme. In 2007 we are planning to enrol our first students into new 'Bologna inspired' curricula: 3 years' Bachelor programme; 2 years' Master programme and 3 years' PhD programme. Several improvements will be connected: considerable transition to active and e-learning practices; enlargement of practical training share; enforcement of the visiting professorship culture; enforcement of the tutorial support for students and engagement of larger HE public budget inputs. Our provisions abroad are: enhancing students' international mobility (under Erasmus & similar schemes) and enlarging the international research and edu collaboration of the faculty. Concerted European activities should be a notable support to this. We see them as 'European macro tasks': assuring for European microbiology HE an outstanding position in the EHEA; discussing and harmonizing European microbiology syllabi along some basic lines without unifying them; linking EU HE schools tightly to Socrates parallel activities (Tempus, Erasmus Mundus etc.); destining FEMS and member societies for establishing a Microbiology Education Network and organizing a European Microbiology OpenCourse Initiative. As 'European micro tasks' we see: shaping comparable students' assessment practices; fostering teachers' professional growth; advising schools on teachers' promotional criteria and advising national authorities on institutional verification (certification) solutions.

Environmental Microbiology Group / NERC Environmental Genomics joint session

Environmental genomics: metagenomics workshop – metagenomics principles, practice and progress

Genomes to metagenomes

To begin at the beginning: challenges and strategies for determination and analysis of genome sequences from single bacteria

Julian Parkhill

The Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA

With nearly 350 complete prokaryotic genomes in the public databases, the sequencing of genomes from single strains of bacteria has come to seem almost routine. However, this apparent facileness masks a number of challenges that still arise in individual genome sequencing projects. Firstly, and perhaps most interestingly, even clonally growing bacteria can exhibit variation in their genome sequences. Secondly, many areas of genomes can be refractory to standard cloning and sequencing processes, and these often contain biologically interesting sequences. These will, of course, pose equally interesting problems for metagenomic analyses. Beyond standard Sanger capillary sequencing, new systems for very-high throughput sequencing are now coming into use. These will initially be used for individual genome sequencing, but may also have a role in metagenomic analyses. These systems may solve some of the problems described above, but will have their own types of limitations. The increased throughput promised by these machines sets a challenge to the ability of human annotators to annotate and analyze this potential flood of data.

Testing the water: origins and achievements of marine metagenomics

O. Beja

Technion-Israel Institute of Technology, Haifa, Israel

Abstract not received

Getting our hands dirty: challenges and success in interrogating the metagenome of soil

J. Handelsman

University of Wisconsin, Madison, USA

Abstract not received

Many environments, many genomes

Quite a mouthful: metagenomics of dental biofilms

Peter Mullany

Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD

In order to begin to understand the extent to which the total bacterial flora present in digestive tract of humans contributes as a reservoir of antibiotic resistance one must understand the role of the organisms that cannot be cultivated in the lab (and which are estimated to comprise 50 % of the total flora). To get round this problem we constructed metagenomic libraries of DNA isolated from saliva, plaque or faeces from volunteers from different European countries. The libraries were estimated to provide complete coverage of the metagenomes and, by end sequencing, to contain DNA from representatives of all the major bacterial groups expected in each microcosm. A large number of clones contained inserts that were not homologous to anything in the databases indicating that this approach enables cloning of novel DNA. The libraries were screened for the expression of tetracycline resistance genes. The complete sequence of novel genes were obtained and the sequence flanking some known genes were obtained to determine the genetic support of these genes. Novel resistance genes and genetic elements were discovered and the libraries can be further interrogated to find a range of novel functional elements.

Competitive metagenomic DNA hybridization identifies host-specific genes in human fecal microbial communities

Orin C. Shanks, Jorge Santo Domingo, Jingrang Lu & Catherine A. Kelty

US Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, 26 West Martin Luther King Drive, MS-387, Cincinnati, OH, USA

Although recent technological advances in DNA sequencing and computational biology now allow scientists to compare entire microbial genomes, the use of these approaches to discern key genomic differences between natural microbial communities remains prohibitively expensive for most laboratories. Here we report the application of a genome fragment enrichment (GFE) method that identifies genomic regions that differ between the metagenomes of two fecal microbial communities. In this study, human fecal microbial community DNA was hybridized against a pig fecal DNA background. Three hundred fifty one individual clones were sequenced and screened for redundancy. Dot blot analysis of 296 non-redundant sequences confirmed that 98 % of the sequences were specific for the human fecal microbial community. Functional annotation of the enriched metagenomic sequences suggests that the majority of the genetic variation between the human and pig fecal DNA communities resides in genes encoding for unknown (33.1 %), hypothetical (10.7 %), and mobile (8.8 %) functions. In addition, secretion and transmembrane prediction analyses indicated a preponderance of DNA sequences (42.6 %) predicted to encode membrane-associated and secreted proteins. Oligonucleotide primers capable of annealing to 26 enriched DNA sequences did not amplify pig fecal DNA and exhibited different levels of specificity with fecal DNA from other animal sources. Five PCR assays were human-specific and demonstrated a broad distribution of corresponding genetic markers among different human populations. These data demonstrate that direct metagenomic DNA analysis using GFE is an efficient method for identifying useful microbial community-specific genetic variation, and for characterizing differences between metagenomes.

Keywords Metagenomics, Genome Fragment Enrichment, GFE, Microbial Source Tracking, MST, *Homo sapiens*

Metagenomics; going down the drain

Margaret Wexler

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ

Waste water treatment plants are complex microbiological systems in which macromolecules are broken down by a variety of micro-organisms. They represent a rich source of genetic diversity for the construction of metagenomic libraries. Metagenomics offers much promise for isolating pharmacologically useful compounds. Numerous obstacles must be overcome before this potential can be fully realised. One of the most fundamental is the commonly reported failure of heterologous gene expression in *E. coli*. To investigate and overcome this problem we used both narrow and broad host-range cosmid vectors to allow screening of metagenomic libraries in different Gram negative bacterial hosts. Gene libraries were constructed using DNA from various stages of a waste water treatment plant, and from a laboratory scale waste water treatment reactor inoculated with activated sludge. Using functional screening, various metagenomic genes were identified that are differentially expressed in different hosts. These included genes specifying an unusual alcohol/aldehyde dehydrogenase, which enables *R. leguminosarum* to grow on ethanol as a sole carbon source, and an enzyme which results in blue pigmentation in *E. coli*. The characterization and differential expression of these genes in different hosts and the benefits and disadvantages of using broad host-range vectors will be discussed.

Beyond the metagenome

Beyond the metagenome; functional analysis of metagenome libraries

Rolf Daniel

Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstr 8, D-37077 Göttingen, Germany

In recent years, many studies have involved the mining of metagenomic gene libraries, which have been constructed from environmental DNA isolated directly from microbial communities without prior culture. Genes of interest have been identified from these libraries by functional screens, usually in the *Escherichia coli* surrogate host, or by sequence-driven approaches. Gene mining based on function, rather than sequence homology, has the potential to isolate genes from metagenomes that are truly novel. This has been demonstrated by isolation of novel genes encoding degradative enzymes, antibiotic resistance, and antibiotics. Although function-driven screens usually result in identification of functional gene products a limitation of this approach is its reliance on the expression of the cloned genes and the functioning of the encoded protein in a foreign host. Therefore, low gene detection frequencies or the inability to recover active proteins during function-driven screening of complex metagenomic libraries are often due to the fact that many genes and gene products are not expressed and inactive, respectively, in the host strain. Strategies to expand the range of biomolecules that can be detected and to increase gene detection frequencies during functional screens of complex metagenomic libraries will be presented and discussed.

Analysis of transcriptome dynamics of the nitrifying bacterium *Nitrosomonas europaea* after pH perturbation using a genome-wide oligonucleotide DNA-microarray

T.E. Freitag & J.I. Prosser

Plant & Soil Science, School of Biological Sciences, University of Aberdeen, St Machar Drive, Aberdeen AB24 3UU

Autotrophic nitrification, the conversion of ammonia to nitrate via nitrite, plays a major role in global biogeochemical cycling of nitrogen. Betaproteobacterial ammonia oxidizers (AOB) are considered to be the key organisms controlling ammonia oxidation, the first step in nitrification processes. Autotrophic ammonia oxidation is highly pH dependent and optimal conditions are limited to pH values between 7–8. To understand the molecular mechanisms associated with pH adaptation of AOB, we have constructed a genome-wide microarray for two AOB, *Nitrosomonas europaea* and *Nitrosospira multiformis*. By including the genomes of representatives from both genera of AOB, which often appear concurrently in natural communities, the array allows comparative transcriptomic studies. To optimize protocols, a test array has been constructed containing 384 70mer oligonucleotide probes for 361 selected coding sequences of the *N. europaea* genome. This covers a sufficient range of the *N. europaea* genome for initial validation of whole-genome microarray studies and is suitable for the low-cost application in expression analysis of the included genes. Preliminary analysis on late log-phase cultures demonstrates highest expression of mRNAs coding for putative RNA- polymerases, cytochromes, sensory kinases (Cen-W) and ammonia monooxygenase genes. The results demonstrate the suitability of oligonucleotide DNA microarrays for successful whole-genome expression studies on *N. europaea* and *N. multiformis*.

DNA microarrays in biogeochemical applications: the promise and pitfalls of array analysis in the environment

Bess B. Ward

Princeton University, New Jersey, USA

DNA and RNA microarrays have great potential for high throughput analysis of microbial community composition, diversity and activity in a variety of biogeochemical and environmental applications. Several different formats and quantification methods are currently in use and under development. A major attraction of the approach is the ability to detect many different gene targets in a single sample. Even if absolute quantification of individual targets is not possible, quantification relative to internal standards or to other genes and mixtures makes it possible to compare DNA or RNA levels among samples. Thus one can evaluate complex assemblages much more rapidly than can be accomplished with the sequencing of clone libraries, and more specifically than can be attained with, e.g. TRFLP or ARISA analysis. The main uncertainty in array analysis arises from the necessity to hybridize all targets simultaneously under the same hybridization conditions. It is not practical to perform extensive calibration tests with each probe on large arrays, but characterization of target hybridization behaviour is necessary at several levels, ranging from hybridization conditions to ground truthing of expression dynamics in different systems. Examples will be drawn mainly from recent experience with functional gene DNA microarrays applied in aquatic environments.

The promise and pitfalls of proteomic analyses in the environment

Philip L. Bond[1,2] & Paul Wilmes[1]

[1]School of Environmental Sciences; [2]School of Biological Sciences, University of East Anglia, Norwich, Norfolk NR4 7TJ

The application of post-genomic techniques is mainly limited to laboratory studies of pure cultures and, hence, does not provide information on gene expression in complex mixtures of micro-organisms. However, transcriptomic and proteomic analyses offer great potential for functional investigation of environmental samples. Proteomic analyses are presently being employed to investigate mixed microbial communities of low complexity, such as activated sludge wastewater treatment biomass, and biofilms in the extreme environment of acid mine drainage. While providing insight, these

applications face challenges that include aspects of: preparation of protein extracts, protein fractionation and separation, protein quantification, mass spectrometry, and protein identification. Although not essential, the availability of metagenomic sequence data from a particular environment vastly increases the opportunity to apply environmental proteomic analyses (also termed metaproteomics). Comparative metaproteomic analysis offers the opportunity to detect physiological and metabolic responses to changes in environmental conditions. Biological phosphorus removal in activated sludge is a process of high interest to the wastewater treatment industry. However, detailed understanding of the process is lacking, as the microbes responsible are presently uncultured. We have performed comparative metaproteomic investigations of mixed culture sludge operating at different levels of phosphorus removal. These investigations provide interesting insights into the metabolism of the microbiological phosphorus removal process. When combined with other recently developed culture-independent methods, metaproteomics can facilitate linking individual microbial species to function.

Linking functional analysis to the metagenome

J. Banfield

University of Berkeley, USA

Abstract not received

Metagenomics: the reality

Nucleic acid extraction and metagenome library constructed from soil

E.M.H. Wellington & **M. Krsek**

Biological Sciences, University of Warwick, Coventry CV4 7AL

One of the aims for metagenomic library preparation is to provide a stable and long term archive of molecular diversity encompassed in total community DNA extracted from a given environment at a certain time and type of sampling. In addition such an archive can be made in expression vectors to seek novel enzymes or useful metabolites. Two different approaches for DNA extraction from soil can be taken: an indirect technique based on preliminary isolation of microbial fraction followed by lytic steps and direct lysis of microbial population in soil slurry. Microbial fraction can be isolated using different methods combining chemical and mechanical treatments followed by centrifugation steps and/or using some gradient technique. Gentle release of DNA from microbial cells in low melting point agarose plugs is achieved by a combined chemical and enzymatical treatment followed by digestion of DNA by restriction enzymes and pulsed field fractionation. DNA fragments of desired size are then released from plugs, ligated into a suitable vector and incorporated into a competent cell. Resulting clones are then screened for desired features. A review of the preparation and analysis of metagenomic libraries will be presented and discussed in the context of molecular ecological studies of microbial communities.

Metagenomics is not the only fruit: integrating genomics and the environment

Jed Fuhrman

Dept of Biological Sciences, University of Southern California, Los Angeles CA 90089-0371, USA

Metagenomics and related genetic technologies have led to some remarkable discoveries about micro-organisms in nature, as reported by several presentations in this session. It is important to keep in mind that these exciting genomic techniques offer only one perspective on extremely complex systems. To take full advantage of this new information, the genomic approaches need to be integrated into overall research programs that include the 'classical' techniques (in microbiology, ecology, biogeochemistry) as well. The marine archaea present a good example. These were first discovered in my lab by 16S rRNA surveys of midwater marine plankton, where they are very abundant. Later they were shown to be autotrophic by isotope analysis of field-collected lipids and by experimental manipulations, then reported by Venter *et al.*'s metagenomic study to contain a gene for ammonium oxidation. Most recently, one was isolated in culture and shown to be an ammonium oxidizer, and field surveys show highly diverse archaeal ammonium oxidation genes. The need to integrate the studies presents a particular challenge for the next generation of microbial ecologists, who all understandably seem to want to learn genomics (i.e. to get a job these days), but who have only so much time available.

How far can we go: sampling the metagenome of hyperdiverse microbial communities

J. Dunbar

Los Alamos National Laboratory, New Mexico, USA

The effectiveness of sampling hyperdiverse metagenomes obviously depends on the type and structure of information that must be extracted. A metagenome represents many information categories. These categories include taxon diversity, guild (ecological functional group) diversity, COG diversity, and gene sequence diversity. In theory, the architecture of taxa and guilds within a community determines the architecture of COGs and gene sequence diversity.

Recently proposed species abundance distributions for hyperdiverse microbial communities provide useful, but rather discouraging guides for metagenome sequencing projects. These distributions indicate that only a tiny fraction of genetic diversity (obviously representing dominant species) will be sampled by conventional metagenome sequencing-a finding thus far corroborated by metagenome datasets. Given this limitation, the questions that metagenome samples can usefully address must be carefully considered. For example, current sample sizes (10-1000Mbp) are clearly adequate for bioprospecting, as new homologs & functional groups will continue to be found throughout the foreseeable future. On the other hand, existing data show that small samples provide extremely limited descriptions of the phylogenetic architecture of communities. In this talk, I will attempt to illustrate quantitative aspects of the link between the architecture of taxa and architecture of COGs

Making sense of the metagenome: novel approaches to bioinformatics for metagenome analysis

Frank Oliver Glöckner, Hanno Teeling & Thierry Lombardot (for the Microbial Genomics Group)

Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen and International University Bremen, 28759 Bremen, Germany

The possibility to sequence DNA samples from natural environments without prior cultivation has revealed new and unprecedented insights into the microbial community composition and function. A central problem with community sequencing approaches (metagenomics) is the classification of disjoint sequence fragments into bins that most probably resemble an organism or a set of closely related ones. Measures such as the average G+C content of the fragments, best BLAST hits, and the codon usage of the corresponding coding regions have often been often applied to solve this problem. Since the resolution of these approaches is limited, we developed methods based on the analysis of intrinsic DNA signatures e.g. using Markov model-based statistics on oligonucleotide

abundances or chaos game representations. When tested on real metagenomic data sets they clearly show an increased sensitivity and specificity, see www.megx.net/tetra. To address environmentally relevant questions like organism adaptations to oceanic provinces and regional differences in the microbial cycling of nutrients using ecological (meta)genomics, it is necessary to couple sequence data with geospatial information and supplement them with contextual information like physical, chemical and biological data. This is the task of Megx.net – a set of specialized databases resources and tools for marine ecological genomics see www.megx.net.

Identification of eukaryotic open reading frames in metagenomic cDNA libraries

Shaun Heaphy

University of Leicester, University Road, Leicester LE1 9HN

Established protocols can be used for isolation of DNA from prokaryotic environmental samples, to construct metagenomic libraries. These can be searched for novel proteins without the cultivation or identification of the source organism. 'Gene mining' of metagenomic eukaryotic microbial DNA, with a widespread presence of introns, requires the construction of cDNA libraries from mRNA in the sample- the objective of the work described here. RNA was stabilized in samples for at least three months using RNAlater (Ambion). Total or polyA enriched RNA was extracted from Chinese hot spring algal mats and activated sludge from an English sewage plant. Following reverse transcription, cDNA was cloned directionally making libraries permitting expression of ORFs in all reading-frames. ORFs up to 378 amino acids in size were identified. Some resembled known proteins over their full length. This methodology permits the (polyadenylated) transcriptome to be isolated from environmental samples with no knowledge of the microorganisms or necessity to culture them. As well as identifying novel proteins it can be used to measure temporal changes in the environmental transcriptome in response to external change.

Challenges for metagenomic data analysis and lessons from the viral metagenome

R. Edwards

San Diego State University, USA

Abstract not received

Strategies for sampling the metagenome

Strategies for directed sampling of the metagenome: SIP-enabled metagenomics

J.C. Murrell

University of Warwick

Abstract not received

Metagenomics of anaerobic methane oxidation

A. Meyerdierks & R. Amann

Max Planck Institute for Marine Microbiology, Celsiusstr 1, 28359 Bremen, Germany

The microbially mediated anaerobic oxidation of methane (AOM) coupled to sulfate reduction is a globally significant process, decreasing the flux of the greenhouse gas methane from marine sediments to the atmosphere. First indications for AOM have been found in the 1970s, but it took more than twenty years to find out that the process is catalysed by consortia of sulfate-reducing bacteria related to *Desulfosarcina* or *Desulfobulbus* and anaerobic methanotrophs (ANME) phylogenetically affiliated with methanogenic archaea.

As all attempts to cultivate the supposed key players of AOM have been unsuccessful, so far, metagenomic studies have been initiated to gain deeper insights into their genetic equipment. The complete sequencing of fosmids carrying a 16S rRNA gene affiliated with different ANME groups, gave first insights i) into rRNA operon structure as well as ii) into general sequence characteristics such as G+C content and oligonucleotide frequencies, and led iii) to the identification of as yet undescribed genes putatively involved in the unique metabolism of archaeal methanotrophs. Moreover, an integrated approach of protein biochemistry and metagenome analysis recently revealed that methanotrophic archaea might use a reversed methanogenesis pathway. The ongoing reconstruction of the ANME-1 genome based on metagenomic libraries together with proteome analysis will provide further valuable information on genetic equipment, and metabolic pathways in the future.

Testing fidelity of assembly, gene calling and binning using synthetic metagenomic datasets

K. Mavromatis, E. Goltsman, K. Barry, H. Shapiro, F. Korzeniewski, I. Rigoutsos, A. Salamov, **P. Hugenholtz** & N. Kyrpides

DoE Joint Genome Institute, USA

It is well known that draft isolate genomes contain misassemblies and incorrect gene calls that are identified and corrected during finishing and annotation QC respectively. Draft metagenomes also contain misassemblies and bad gene calls that are compounded to an unknown degree by the presence of multiple species and strains. Since it is currently unfeasible to progress metagenomes beyond draft assemblies (with the possible exception of dominant populations) quantifying the extent and character of misassemblies and miscalls is a useful exercise. We constructed synthetic metagenomic datasets to mimic a number of real metagenomic datasets by combining reads from a selection of 114 isolate genome sequencing projects available through the Joint Genome Institute. Isolate genomes were selected to represent populations in metagenomic datasets based on similar patterns of genome size, GC content and phylogenetic position. Reads were randomly sampled from the selected genomes to match the read depth of their corresponding populations in the metagenomic assemblies. Sampled reads were then assembled and annotated using the same programs and parameters used to assemble and annotate the real metagenomic data. The extent of chimeric assembly (assembly of multiple genomes into the same contig) could then be quantified since the source of each read in a given contig was known. The effect of multiple genomes vs single genomes on ab initio gene calling could also be assessed. An additional benefit of having synthetic metagenomic datasets for which the identity of all contigs is known is to provide benchmarks for binning methods. The results of this analysis will be presented and discussed in relation to the use of the data, e.g. metabolic reconstruction vs population structure.

Fermentation & Bioprocessing Group session

Cells as factories

Commercial fermentation of *Aspergillus oryzae* for the production of industrial enzymes

S.M. Stocks

Novozymes, Denmark

Novozymes A/S is the largest producer of food, feed or technical enzymes world wide. These competitive sectors demand robust high yielding fermentation process and drive constant optimization. Compared to pharmaceutical GMP, food/feed GMP often permits optimization, leading to a dynamic research–development–production environment. *A. oryzae* is used by Novozymes for production of a range of native & GM products; it is an excellent excretor of proteins and grows well in a range of media under a range of temperatures and pH's, giving robustness at scales up to at least 160m^3. From its historical use in the production of soy sauce, it is known to be food safe, and Novozymes has developed an extensive genetic tool box for the organism making it a good choice for expression of a range of native or engineered proteins from various donors. So it would appear to be an ideal host, but what challenges can this production organism throw up for the production engineer? This organism is filamentous and produces a viscous broth, giving rise to various challenges in mixing and mass transfer, which can be solved by creative application of chemical engineering principles; at the same time the complex biology of this micro organism leads to examples of counterintuitive phenomena which can also be exploited.

Therapeutic antibody fragments from *Escherichia coli*

David J. Glover

UCB Celltech, 216 Bath Road, Slough, Berkshire SL1 4EN
(Email David.Glover@ucb-group.com)

Monoclonal antibodies (mAbs) of the IgG format are becoming commonplace therapeutics in a wide range of disease indications. The mode of action (MoA) by which a mAb intervenes in disease biology may be antagonistic, agonistic, effector function mediated cell killing, or as a vehicle for delivery of agents, such as radioisotopes and cytotoxic drugs. Whilst IgG may be the optimal antibody format in some applications, for others, sub-fragments or engineered antibody domains may be preferred, particularly where IgG effector function and/or cross-linking events are undesirable. One approach to the design of antibody therapeutics is to use the monovalent Fab' unit as a flexible building block to tailor the therapeutic entity to match the required mode of action. Valency, cell killing and optimized pharmacokinetics (half-life) can be conferred using linker technology to attach multiple Fab', cytotoxin and PEG components respectively.

Conventionally, mammalian cell culture systems have been used for the manufacture of mAbs, which have complex requirements for protein folding and post-translational modification. Where the use of simpler antibody fragments is an option, alternative expression systems, such as bacteria and yeast, may be considered. Fab' antibody fragments are particularly amenable to high level expression in *Escherichia coli* offering advantages in, scale, cost of goods, availability of plant and process time when compared to mammalian cell culture systems.

Rapid cell line development for microbial strains, the use of generic manufacturing platform technology and parallel activities in process development make it possible to move from selection of the therapeutic antibody to release of clinical lots in <10 months. However, it is essential that robust, transferable processes are developed so that scale-up for the clinic is not compromised by development speed. Identification of key operating parameters at an early stage facilitates technology transfer, facility fit and process control. The ease with which microbial strains can be manipulated for improved manufacturing properties combined with the use of fermentation control strategies offers appealing advantages for production of antibody therapeutics.

Global transcriptional profiling of a pseudo-industrial bioprocess with *Bacillus* spp.

Britta Jürgen[1], Mogens Wümpelmann[2], Michael Hecker[3] & Thomas Schweder[1]

[1]Institute of Pharmacy, Pharmaceutical Biotechnology, Ernst-Moritz-Arndt-University Greifswald, F.-L.-Jahn-Straße 17, 17487 Greifswald, Germany; [2]Novozyme A/S, Novo Allé, 2880 Bagsværd, Denmark; 3Institute of Microbiology, Ernst-Moritz-Arndt-University Greifswald, F.-L.-Jahn-Straße 15, 17487 Greifswald, Germany

Transcriptome and proteome analyses allow a global investigation of gene expression of cells and thus a comprehensive view of the physiological state of microbial hosts under growth conditions. The analysis of global expression profiles of cells at different time points throughout a fermentation process renders it possible to explore physiological and genetic bottlenecks of a production strain (1). Furthermore, this is a suitable approach for the detection of genes, which are critically for the performance of selected bioprocesses. Thereby, potential targets for the optimization of the fermentation process can be identified. The knowledge of such process-relevant genes establishes the basis for a better understanding of the adaptation of microbial cells to the special bioprocess conditions (2).

In this study a detailed gene expression analysis of industrial-close Bacillus subtilis fed-batch fermentation processes with casamino acids as the only nitrogen source and with a reduced casamino acids concentration but supplemented by ammonia was carried out.

The obtained data give insights into the gene expression and adaptation of B. subtilis cells during fed-batch fermentation processes and thus under conditions, which are frequently applied for the overproduction of industrial proteins. Such a comprehensive genomic view on the cellular physiology represents one possibility for further optimization of industrial B. subtilis bioprocesses. The genomic approach allowed the identification of new 'marker genes', which could be suitable for a future monitoring of B. subtilis fermentation processes (3).

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Production of target proteins using automated fed-batch cultivations

M. Ganzlin, B. Gerwat & P. Garbers

AstraZeneca, 13S28 Mereside, Alderley Park, Macclesfield SK10 4TG

Background and objective of investigation Automation can improve efficiency for fast development of reproducible bioreactor processes required for drug discovery. Target protein production in bioreactors can also benefit from online biomass determination, since the problem of undefined maximum specific growth rates for a high through-put of newly-designed recombinant *E. coli* strains can be addressed.

Results A software tool was programmed in MATLAB® and linked to the SCADA system MFCS/win® via an OPC Client. Using this tool with user-friendly graphical interfaces, automated induction and exponential increasing mass feeding strategies were carried out successfully. A reproducible automated batch end detection was implemented and successfully validated for twenty cultivations. Target protein production was studied, using various feed forward strategies, aiming at a different constant growth rate.

Conclusions Automated fed-batch technology can significantly improve productivity of target proteins, while reducing requirements for out of hours working time. Using the online base consumption signal as an indicator for biomass production improved the quality of decision making during running time.

Scale-up of *Escherichia coli* recombinant fed-batch fermentation systems

Christopher J. Hewitt

Biochemical Engineering, Centre for Formulation Engineering, School of Engineering (Chemical Engineering), University of Birmingham, Edgbaston B15 2TT

Scale-up is usually the final step in a research and development programme leading to the large scale synthesis of biologically active products by fermentation. It is known that chemical gradients exist in large scale bio-reactors where additions of a concentrated, substrate at a single point mean that mixing times are high. In laboratory scale bioreactors where much development work is done, mixing times are low and essentially such gradients do not exist. The composition of a cells micro-environment is a product both of fluid dynamics and a cells physiological response to it. Cells circulating around a large scale bioreactor will experience rapidly changing micro-environments. Knowledge of how a cell reacts to such changes is essential if mathematical models are to be derived that accurately predict process times and product yields on scale-up. In this work, we report on the further development of scale-down, two compartment experimental simulation models. For the first time, the effect on high cell density *Escherichia coli* fed-batch fermentations of a changing microenvironment with respect to all three of the major spatial heterogeneities that may be associated with large-scale processing (pH, glucose and dissolved oxygen concentration) were studied simultaneously.

The National Biomanufacturing Centre

R. Dennett

Eden Biodesign Ltd, Ellesmere Port

Abstract not received

Near and mid infrared spectroscopy and fermentation monitoring

Linda Harvey & **Brian McNeil**

University of Strathclyde, Glasgow

Vibrational spectroscopy potentially offers the capability to measure the condition of a bioprocess very accurately every few minutes via non-destructive measurement of the concentrations of multiple analytes. There is therefore almost a perfect match between the capabilities of this technology and the analytical needs of the fermentation industry. In the last decade or so both near and mid infrared spectroscopy have been widely applied to fermentation processes ranging from complex fungal cultures to mammalian cell culture processes. Each technique has different strengths and drawbacks, and the relative advantages of each will be critically discussed in the context of individual bioprocess case studies.

Large scale factories for cells: specializations needed to provide turnkey large scale high quality fermentation facilities (and cGMP)

P. Bentley

Pierre Guerin UK Ltd, Sittingbourne

Pierre Guerin Technologies specializes in design and manufacture of aseptic process equipment for the pharmaceutical, food, cosmetic preparation and wine industries, and Biolafitte laboratory, to pilot to production fermenters for the global pharmaceutical and biotechnology markets.

The focus of the presentation will be on how this can be efficiently accomplished utilizing resources and expertise encompassing:

- Industrial resources
- Process expertise
- Specialization of turnkey engineering
- Process vessel manufacture
- Mixing / O_2 and thermal transfers
- Cleaning in place technology
- Design criteria
- Design examples
- Engineering organization and methodologies
- Automation possibilities
- Applications examples
- System examples 2l to ‘x’m3
- Documentation package (for cgmp)
- Integration of upstream and downstream systems
- Flavour of the region

Food & Beverages Group

Intestinal microbiota and health

Functional analysis of the gut flora

Functional overview of flora development from birth to old age

George T. Macfarlane

Microbiology & Gut Biology Group, University of Dundee, Ninewells Hospital Medical School, Dundee DD1 9SY

The human large bowel is sterile at birth, but microbial colonization of the gut occurs rapidly following exposure to the mother's vaginal and faecal microbiotas. Facultative anaerobes are the initial colonizing species, and they reduce pO_2 and E_h sufficiently to allow the establishment of anaerobic bacteria. Development of the infant gut microbiota is affected by whether the baby is breast or formula-fed, and significant differences are apparent in microbial community structure. However, they disappear after weaning, and by between 2-3 years of age, a recognizable adult-type microbiota has developed. The adult gut microflora is stable over time, but instabilities in some communities can occur over the short-term. Marked differences can occur in the composition and metabolic activities of the gut microbiota in elderly people. For example, numbers of bifidobacteria, which are viewed as being protective species in the gut, often decline, while potentially pathogenic organisms such as clostridia, enterococci and enterobacteria increase. In addition, functional differences are evident in some microbial hydrolytic/degradative functions and fermentation product (SCFA) excretion. These shifts can be attributed, in part, to changes in host digestive physiology, diet and exercise, as well as the increased use of antibiotics in older people.

Carbohydrate metabolism by the gut microbiota

Karen P. Scott

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB

The colonic microbiota comprises a large and diverse microbial community capable of growth on dietary substrates escaping digestion in the upper digestive tract. Such substrates include fibrous plant material, resistant starch, inulin and various oligosaccharides. Some bacteria are also able to use host-derived substrates (mucin and epithelial glyco-conjugates including fucose) as energy sources. The mainly anaerobic bacterial communities ferment these substrates releasing products that are important for human colonic health (e.g. butyrate) but are in some cases detrimental e.g. (hydrogen sulfide). The primary substrate degraders also release soluble sugars into the gut lumen that are scavenged by other bacteria and used for growth.

Cultured isolates of many of the numerically dominant groups of gut bacteria are now available. Detailed study of representative species has established how different bacteria vary in their substrate preferences and substrate degradation mechanisms. Certain *Butyrivibrio* and *Roseburia* isolates possess large cell-wall associated multidomain α-amylase enzymes for starch degradation. In contrast, cellulolytic *Ruminococcus* isolates assemble various enzymes into large extracellular multifunctional complexes, cellulosomes. Microarray analysis has been used to investigate switches in gene expression required to utilize specific carbohydrates, illustrating that fucose utilization enzymes are distributed on a large polycistronic operon in a *Roseburia* isolate.

Which bacteria ferment carbohydrates in the human colon?

M. Egert, P. Kovatcheva-Datchary, A.A. de Graaf, A. Maathuis, H. Smidt, W.M. de Vos & K. Venema

WCFS, P.O. Box 557, 6700 AN Wageningen, The Netherlands

From a microbiological perspective, the colon is the most important part of the human alimentary tract. Here, microbe-mediated fermentations are pivotal for human health. We use RNA-stable isotope probing (SIP) to identify bacteria involved in the colonic fermentation of carbohydrates. In a pilot study, the microbial community of an *in-vitro* model of the human colon (TIM-2) was probed with [U-^{13}C]-glucose. Within 2 h, the added glucose (40 mM) was completely fermented yielding mostly lactate, acetate and butyrate. Of the five microbial species dominating the model in this experiment, phylogenetic analysis of ^{13}C-labelled 16S rRNA indicated bacteria closely related to *Clostridium perfringens* and *Streptococcus bovis* as the most active glucose-fermenters in the system. In a second experiment, [U-^{13}C]-starch was added to the model. Preliminary fingerprint analysis of ^{13}C-labelled 16S rRNA indiactes a single group of bacteria to be the primary starch fermenters. In conclusion, RNA-SIP appears promising to reveal bacteria actually involved in colonic fermentations, not only in the course of *in-vitro* but also of *in-vivo* studies.

β-Glucuronidase and β-glucosidase activities in human colonic bacteria

Marta Dabek, Harry J. Flint & **Petra Louis**

Rowett Research Institute, Gut Health Division, Microbial Ecology Group, Greenburn Road, Bucksburn, Aberdeen AB21 9SB

Members of the human gut microbiota harbour enzymatic activities with potential impact on human health. β-glucuronidases counteract the detoxification of xenobiotics by glucuronidation, whereas β-glucosidases act on a range of plant glucosides and liberate aglycones that can exhibit either toxic or health-promoting effects. The aim of the present study was to screen a range of gut bacterial isolates for those activities, with special emphasis on low G+C Gram positives, a predominant but under-studied group of the microbiota.

β-glucuronidase and β-glucosidase activities were measured in cell extracts of isolates from the following groups: clostridial cluster IV, XIVa, and XVI low G+C Gram positives, *Bifidobacterium* sp. and *Bacteroides* sp. Measurable levels of β-glucuronidase activity were found only in several members of the genus *Roseburia* from within clostridial cluster XIVa and some *Faecalibacterium prausnitzii* strains (clostridial cluster IV), whereas β-glucosidase activity was more widespread, with the highest activities found in bifidobacteria. Further studies will examine the effect of environmental factors on those enzyme activities in candidate strains exhibiting high activity.

Dendritic cell and microbial interactions using functional genomics

M. Foti

Dept of Biotechnology and Bioscience, University of Milano-Bicocca, Milano, Italy

The immune system evolution is the result of the cross-talk between hosts and micro-organisms. Host recognition of bacterial pathogens is a critical component of the immune response. Therefore, understanding the host-pathogen interactions can lead to the

development of novel strategies for immune intervention. During host–parasite interactions, dendritic cells (DCs) play a central role, as they are located in close contact with the mucosal surfaces where they can sample incoming pathogens. If the amount of pathogen exceeds a certain threshold level for an extended period of time, DCs become activated and acquired a migratory capacity; this maturation process promotes gene transcription re-programming that might involve the differential expression of up to one thousand genes with the sequential acquisition of immune regulatory activities.

We carried out a systemic examination of the gene expression program in mouse Dendritic Cells in response to different micro-organisms using a genome-wide expression study.

Application of molecular and proteomics technologies in the detection and diversity of foodborne pathogens

H.N. Shah[1], G. Hallas[1], L. Molenaar[1], R. Culak[1], V. Encheva[1], V. Mithani[1], J. Mclauchlin[1], K.A. Grant[1], D. Dare[2], H. Sutton[2], M. Lunt[3] & M. Roberts[3]

[1]Molecular Identification Services Unit; Food Safety Microbiology Laboratory, Health Protection Agency, London; [2]Manchester Metropolitan University, Manchester; 3Waters Corporation, Manchester

Bacillus spp. represent a significant component of the isolates from contaminated foods and, as such, necessitates clear circumscription of species for accurate identification. Present criteria are inadequate; consequently the genus has accumulated a large number of diverse species, many of which remain *incertae sedis*. Despite the plasticity of microbial genomes and the frequency of horizontal gene transfer, species retain a large number of stable traits that should enable the assignment of wild type strains to a given taxon. Identifying these stable genes or proteins should be the goal of modern phylogenetics. 16S rDNA is now used widely as a molecular chronometer, hence its use in the application of any new methodology is indispensable. It is our view that the addition of proteomic data (termed here as proteosystematics), may now be superimposed upon the backbone of genomic data as high resolution technologies become available. Here, we describe such a model using *Bacillus* species which were analysed using 16S rDNA sequence analysis. Initially, a database of some 4,000 mass spectral profiles was constructed which spans most lineages of the microbial kingdom. This database was then interrogated with putative bacillus isolates. The results show good congruence between methods and supports the introduction and further advancement of these technologies.

Development of a DNA based microarray for detection of bacteria from the human GI tract

C.R. Harrington, S. Lucchini, T.J. Eaton, A. Narbad, J.C.D. Hinton & M.J. Gasson

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

The microbial community of the human gastrointestinal (GI) tract is extremely complex with diverse array of bacteria present at 1 x 10^{12} cells per gram of luminal content. Most gut bacteria are unculturable and the study of gut ecology requires the use of DNA based profiling methods. A high throughput microarray was developed to detect human gut bacteria using 16S rDNA probes. Arrays using 20, 40 and 50bp oligonucleotides were prepared and tested. The results indicated that 20 bp probes gave optimal signal specificity. A control using faecal samples spiked with *Thermus thermophilus* cells was developed to allow accurate data normalization. Spiking of the faecal samples with *Salmonella* Typhimurium SL1344 Δ*yciE-yciG* cells indicated that the detection limit was between 8.8 x 10^5 and 8.8 x 10^4 cells/g faecal sample. This is a low detection limit compared to the total bacterial population of 1 x 10^{12} cells/g. The microarray has subsequently been used to observe differences between bacterial populations in a gut model experiments.

Identification of prebiotic fructooligosaccharides metabolism in *Lactobacillus plantarum* WCFS1 using microarray

D.M.A. Saulnier[1], D. Molenaar[2], S. Kolida[1] & G.R. Gibson[1]

[1]Food Microbial Sciences Unit, School of Food Biosciences, University of Reading; [2]NIZO, Ede, The Netherlands

Short-chain fructo-oligosaccharides (scFOS) are recognized as prebiotics: 'non-digestible food ingredients that selectively stimulate the growth and/or activity of a limited number of bacteria in the colon that have the potential to improve host health'. However, little information is available about the metabolism of these oligosaccharides in purportedly beneficial bacteria like lactobacilli and therefore the reason why particular substrates behave as prebiotics. To study the genomic basis of scFOS metabolism, by *Lactobacillus plantarum* WCFS1, two-colour microarrays were used to screen differently expressed genes when grown on scFOS as compared to glucose, which is not selectively metabolized by the gut flora. A sucrose phosphotransferase system (PTS) and a β-fructofuranosidase were 60-fold up-regulated, a pyruvate dehydrogenase 4-fold, for the transport and the hydrolysis of scFOS. A mannose PTS was 50-fold repressed.

Here, microarrays were used to determine the genes responsible for prebiotic metabolism in a well characterized probiotic and has demonstrated the need for a particular PTS and specific enzymes for scFOS degradation in this strain of *L. plantarum*.

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Of microbes, mice and men: understanding the mammalian-microbial metabolic axis

J.K. Nicholson

Imperial College London

Abstract not received

The GI barrier to acute and chronic infections

R.K. Buddington

Dept of Biological Sciences, Mississippi State University, USA

The gastrointestinal tract (GIT) represents an interface between the external environment and the internal milieu of the host.

Corresponding with the vast amount of exposed surface area and the diversity and abundances of potential antigens and pathogens in the GIT, the enteric immune system plays a central role in host health. The a priori expectation is that the enteric immune system has co-evolved with members of the bacterial assemblages, similar to the co-evolution of the rumen and selected bacteria, and can discriminate between commensal organisms and potential health threats. This is corroborated by evidence of immunomodulation by components of the GIT bacteria and the ability to improve disease resistance by increasing the abundances of specific bacteria, notably some of the lactic acid producing bacteria (e.g. lactobacilli and bifidobacteria). This presentation describes how different 'management' strategies that increase the abundances of GIT lactobacilli and bifidobacteria (i.e. probiotics and prebiotics) modulate immune functions and increase resistance to enteric and systemic pathogens and other health challenges. A better understanding of the complex interactions between the GIT bacteria and host enteric and systemic immune functions will facilitate efforts to improve health in a world with growing concerns about the resistance of pathogens to antibiotics.

Probiotics – new developments in relation to colon cancer prevention

Chris Gill & Ian Rowland

Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA

Studies in cell cultures and animal models provide evidence that probiotics can beneficially influence various stages in the initiation and development of colon cancer including tumour initiation, promotion and metastasis. For example, using the Comet assay for assessing DNA damage in the colon after carcinogen treatment of rats, it has been shown that oral administration of *Lactobacillus* and *Bifidobacterium* strains can prevent genotoxic damage to the colonic epithelium. Heat-killed preparations of the bacteria were ineffective indicating the importance of viable organisms. Administration to rats of probiotics reduced the incidence of carcinogen-induced pre-cancerous lesions known as aberrant crypt foci in the colon. Furthermore a combination of *Bifidobacterium longum* and inulin (a growth substrate for bifidobacteria) was more effective than either treatments alone. In this latter study, the dietary treatments were given after exposure to the carcinogen, which suggests that the protective effects were being exerted at the promotional phase of the carcinogenic process. There is limited evidence from epidemiological studies for protective effects of products containing probiotics in humans. SYNCAN and CROWNALIFE are recent dietary intervention studies in healthy subjects and/or polyp and cancer patients that have yielded promising results on the basis of biomarkers of cancer risk.

Probiotics and health: from fads to facts and future potential

S. Blum

Nestle, Switzerland

Abstract not received

Microbes and intestinal homeostasis

J. Wells

TNO Quality of Life, Utrechseweg 48 P.O. Box 360, 3700 AJ, Zeist; University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands (Email jwells@science.uva.nl)

Shortly after birth our intestinal tract is colonized by a vast ecosystem of bacteria collectively known as the microbiome that thereafter maintains a life long reciprocally beneficial interaction with the host [1]. There is now a large body of evidence that nutrition and the activities of the microbiota may contribute to the aetiology of inflammatory disease, allergy and gastrointestinal cancer by exerting either harmful or protective effects [2]. Chronic inflammation and intestinal cancer are closely associated in the intestine as evidenced by the link between inflammatory bowel disease and cancer [3]. In inflammatory bowel disease patients the microbiota is known to drive an excessive inflammatory response leading to colitis whereas in healthy individuals this is controlled by a number of homeostatic regulatory mechanisms. In this presentation the beneficial role of commensal micro-organisms in maintaining homeostasis in the intestine will be discussed including the ability of certain bacteria to attenuate NF-kappa-B signal transduction pathways and induce regulatory T cells (Tregs) in the mucosa [4]. Tregs are now viewed by many as an integral component of mucosal homeostasis that appear to fine tune protective immunity and prevent harmful immune pathology. A better understanding of this field may open up new avenues for the use of nutrition and functional foods in balancing the microecology of the intestine and in preventing allergy inflammation and cancer.

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Dietary intervention

Probiotics for neonatal application

Anna M. Snelling

Dept of Biomedical Sciences, University of Bradford, Bradford BD7 1DP

Probiotics are well established dietary supplements for children and adults, and can be of benefit for various gastrointestinal disorders and allergies. Much less research has been done on administration of probiotics to neonates, which remains controversial from safety and ethical perspectives. In clinical studies certain strains of lactobacilli and bifidobacteria, have been shown to benefit neonates with regard to the incidence of sepsis, diarrhoea, weight gain and immunostimulatory effects. They may even offer protection against the extremely serious condition of necrotizing enterocolitis. In the Czech Republic a non-pathogenic *E. coli* has been administered to newborns in certain clinics for over 30 years. Evidence amassed from several thousand patients suggests efficacy in preventing nosocomial infections in both full and preterm babies, and may even lead to fewer illnesses and allergies in later life. The strains *Lactobacillus rhamnosus* GG and *Bifidobacterium* lactis BB12 can significantly reduce the incidence of atopic dermatitis, which affects upto 15 % of babies, if mothers take the probiotics during pregnancy or infants take them during the first few months of life. Nutritional benefits of fermented milks used as complementary feeds are being explored. To date, the safety profile of probiotics given to babies has been good, and this should encourage larger scale trials, which are needed if products are to gain regulatory approval for neonatal applications.

Gut flora intervention in the elderly

Kieran Tuohy

Food Microbial Sciences Unit, School of Food Biosciences, University of Reading, Whiteknights, Reading RG6 6AP (Email k.m.tuohy@reading.ac.uk)

World wide, the population is ageing, with declining birth rates (especially in developed countries) and increasing life expectancy, contributing to the growing aged population. The impact of this ageing population is likely to be felt most keenly in Europe, where the proportion of older people will increase from 20 % in 1998 to 35 % in 2050 and, where one in every three people will be over 60 years. With ageing comes a reduction in overall health and an increase in morbidity and mortality due to infectious disease, many associated with the gastrointestinal tract. The composition and ecology of the intestinal microflora changes with in old age and account, in part, for the increase in severity of gastrointestinal infections with age. Dietary interventions with functional foods may fortify gastrointestinal health and offer the possibility of reducing the burden of gastrointestinal illness. The gut microflora is amenable to modulation using probiotics, prebiotics and synbiotics, and the elderly represent a population group for which such strategies may be particularly suitable. The EU funded project CROWNALIFE has confirmed that the gut microflora within elderly people differs from younger adults, showing a much greater degree of species diversity.

Through generation of 16S rRNA based phylogenetic inventories of the faecal microflora of healthy elderly individuals (>65 years) the elderly 80 % of cloned 16S rDNA sequences corresponded to previously uncultured bacteria while 1/3 of these represented novel phylogenetic lineages. To affect beneficial modulation within the gut microflora CROWNALIFE and other studies, have employed the synbiotic concept, and investigated the suitability and efficacy of this approach by monitoring changes within relative bacterial numbers and microbial biomarkers important in gastrointestinal health and disease.

A *Lactobacillus* sp. degrades oxalate in a multi-stage human colon simulator

T.R. Lewanika[1,2], G.T. MacFarlane[2], S.J. Reid[1], V.R. Abratt[1] & S. MacFarlane[2]

[1]Dept of Molecular and Cell Biology, UCT, Rondebosch, 7701, Cape Town, South Africa; [2]Microbiology & Gut Biology Group, University of Dundee, Dundee DD1 9SY

Oxalate in humans is either degraded by gastrointestinal micro-organisms, or absorbed from the bowel and excreted in urine. Urinary oxalate concentration affects kidney stone formation, and colonic oxalate-degrading bacteria play an important role in oxalate homeostasis. This study was done to identify and characterize novel oxalate-degrading gut bacteria for use as probiotics in managing kidney stone disease. A three-stage continuous culture system (CCS), inoculated with faecal bacteria, was used to model environmental conditions in the proximal and distal colons, at retention times comparable to normal colonic transit rates (30 and 60 hours). A candidate *Lactobacillus* sp. was introduced into the CCS during steady-state growth conditions. The probiotic survived in all three culture vessels and initiated oxalate degradation. PCR amplification and sequencing demonstrated the existence of a cluster of novel genes involved in oxalate degradation in the probiotic, suggesting that this organism has potential therapeutic use in managing kidney stone disease.

Competitive exclusion of pathogens in animals

P.A. Barrow

Institute for Animal Health, Compton

Abstract not received

Microbial Infection Group / Clinical Microbiology Group joint symposium

Vaccines

Fundamental biology underpinning vaccine research

The current status of vaccines: where we are and where the world is going

Rino Rappuoli

Chiron Vaccines, Via Fiorentina 1, 53100 Siena, Italy

Since the beginning of vaccinology, more than two centuries ago, we have grown pathogens in animals (smallpox), in eggs (influenza), or in the laboratory using a variety of media and fermentation technologies. The grown pathogens have been used as live attenuated vaccines, as killed vaccines or as a source of material for the discovery and manufacturing of antigens to be used in subunit vaccines.

The availability of the genomic sequence of most pathogens allowed for the first time to discover vaccines without the need to grow pathogens. Vaccine discovery can be done in silico, starting from the computer analysis of the genomes (reverse vaccinology).

Genome-based vaccine discovery was applied for the first time to serogroup B meningococcus, a bacterium that is a major cause of sepsis and meningitis, which had been resistant to all conventional approaches to vaccine development. The sequence of the genome allowed the in silico prediction 600 potential antigens. 350 of them were expressed in *Escherichia coli*, then purified and used to immunize mice. 29 were found to induce bactericidal antibodies, a property which correlates with protective immunity. A subgroup of the genome-derived antigens is now being tested in clinical trials.

Today reverse vaccinology is a standard technology. No vaccine project is started without knowing the sequence of the pathogen. Successful examples of genome-based vaccine discovery are pneumococcus, group B streptococcus, *Staphylococcus aureus*, and a variety of viruses. Coupled with the advances in the understanding the molecular basis of the immune response, the advances in proteomics, and the development of new adjuvants, the new technologies represent a quantum jump in vaccine discovery.

Predictive features of the genome-wide host response to infectious diseases

D. Relman

Stanford University Medical Center, California, USA

Abstract not received

The immunological principles surrounding novel adjuvant and vaccine technologies

Nils Lycke

Dept of Clinical Immunology, University of Göteborg, S-413 46 Göteborg, Sweden

Novel adjuvant strategies must be based on a better understanding of where, how and which immunomodulating properties can be employed to potentiate the immunogenicity of vaccines. This is particularly true for mucosal vaccines. In this context a fundamental question is whether mucosal tolerance and IgA immunity are mutually exclusive or can co-exist and whether they represent priming of the local immune system through the same or different activation pathways. The target populations for immunomodulation have to be defined and the mechanisms by which these cells can enhance immune responses should be analyzed. We have explored two strategies to investigate these questions: Both were based on the ADP-ribosylating enterotoxin, cholera toxin, CT. The first model used whole CT or the enzymatically inactive receptor-binding B-subunit of CT (CTB). The second approach, was using the CTA1-enzyme or an inactive mutant CTA1R7K., linked, in a fusion protein, to the B-cell targeting moiety, DD, from *Staphylococcus areus* proteinA. Our studies provide compelling evidence that delivery of Ag in the absence of ADP-ribosylation can promote tolerance, whereas, ADP-ribosyltransferase-active conjugates, prevent tolerance but induce IgA immunity. Although CT-conjugates potentially could bind to all nucleated cells we found that CT targets dendritic cells (DC) in vivo. On the other hand, DD-conjugates distinctly targeted B cells and probably also follicular dendritic cells (FDC) in vivo. Our analysis revealed unique subsets of mucosal and systemic DC that appeared to be responsible for the ADP-ribosyltransferase sensitive dichotomy between tolerance and IgA immunity.

Bacterial ghosts for mucosal antigen delivery

Werner Lubitz

Dept of Medicinal Chemistry, University of Vienna, Austria
(Email werner.lubitz@univie.ac.at)

The Bacterial Ghost platform system is a novel vaccine delivery system endowed with intrinsic adjuvant properties. Bacterial Ghosts (BG) are nonliving Gram-negative bacterial cell envelopes which are devoid of their cytoplasmic contents yet maintain their cellular morphology and antigenic structures, including bioadhesive properties. BG are particles themselves and contain a surface make-up that appeals to primary antigen presenting cells (APC) enhancing the immune response to target antigens, including T-cell activation and mucosal immunity. The intrinsic adjuvant properties of BG preclude need for artificial adjuvants in BG vaccine formulations. In animal models (mice, rabbits, pigs, possums) BG of different origin have been applied oral, aerogenic, intraocular or intranasal for mucosal immunization. In all of these applications it was necessary that primary immunization was followed by booster to achieve protective immunity against lethal challenge except for BG produced from enterhemorrhagic Escherichia coli (EHEC). A single oral or rectal dose of EHEC BG protected mice against a 50 % lethal challenge with a heterologous EHEC strain. Since native and foreign antigens can be carried in the envelope complex of BG, combination vaccines with multiple antigens of diverse origin can be presented to the immune system simultaneously. Beside the capacity of BG to function as carriers of protein antigens, BG also have a high loading capacity for DNA. Loading BG with recombinant DNA takes advantage of the excellent bioavailability of the carrier for DNA-based vaccines and the high expression rates of the DNA encoded antigens in target cells types such as macrophages and dentritic cells. Using EHEC BG as carrier of target antigens it seems to be possible to develop efficacious single dose mucosal BG vaccines. Furthermore, the BG antigen carriers can be stored as freeze dried preparations at room temperature for extended periods without loss of efficacy. The potency, safety and relatively low production cost of BG offer a significant technical advantage over currently utilized vaccine technologies.

Polio eradication: finishing the job, guaranteeing the investment

David L. Heymann

World Health Organization, Geneva, Switzerland

The polio eradication initiative is in its final phase, with intensified vaccination activities using oral polio vaccine (OPV) in all countries where polio transmission has not yet been interrupted, and in those countries that were once polio-free and have recently become re-infected. New tools have been developed and are in use to ensure interruption of the last chains of human to human polio transmission, including a monovalent type 1 oral polio vaccine (mOPV1), and surveillance is being intensified in all countries that are endemic or that have become re-infected during the past two years.

Once eradication of wild poliovirus has been confirmed, the public health benefits of routine immunization with OPV will no longer outweigh the burden of disease either due to vaccine associated paralytic polio caused by OPV, or by outbreaks caused by circulating vaccine-derived polioviruses. The eventual cessation of OPV use in routine immunization programmes worldwide will become necessary to assure a lasting eradication of polio.

As the world moves towards polio eradication and its certification, preparations are therefore being intensified for OPV cessation, and the pre-requisites for safe OPV cessation are being put in place. Pre-requisites include bio-containment of all known poliovirus and potentially infected substances, development of an international stockpile of oral polio vaccine, ensuring a mechanism for continued global surveillance of polio after eradication has been certified, and national policies if countries decide to continue vaccinating with inactivated polio vaccine (IPV).

It is ironic that the vaccine on which the world has depended for polio eradication will itself become a risk to eradication once the transmission of wild poliovirus has been interrupted. Final preparations for the eventual global and simultaneous cessation of OPV will require the same level of international cooperation and coordination that has brought the world to the verge of polio eradication.

New malaria vaccines: clinical trials

Sarah C. Gilbert

University of Oxford

In recent years an increasing number of clinical trials of malaria vaccines have been carried out, in studies designed to determine the safety, immunogenicity and in some cases protective efficacy of these vaccines. The more successful of these vaccines have now been tested in a variety of populations (non-malarial areas v. malaria-endemic areas, adults v. children) with differing results. The results of some of these trials and prospects for the future will be discussed.

Immunizations during pregnancy

W.P. Glezen, F.M. Munoz & P.A. Piedra

MVM Dept., Baylor Col. of Med., 1 Baylor Plaza MS:bcm-280, Houston, TX 77030, USA

Passive immunity against pathogens that cause life-threatening illnesses in neonates and young infants can be provided by immunization of women during pregnancy. Immunization during pregnancy often will provide important protection for the women also. Tetanus toxoid and influenza vaccines are examples of vaccines that provide a double benefit – one dose of vaccine protects two persons at a vulnerable period in their lives. Other vaccines under investigation include those for respiratory syncytial virus (RSV), group B streptococci and pneumococci. IgG antibodies are actively transported across the placenta – mostly during the 3rd trimester. The process is time dependent dictating that immunization should be accomplished at least 4 weeks before delivery. IgG1 antibodies are transferred preferentially. Maternal immunization has not significantly interfered with active immunization of the infant. Inactivated vaccines administered in the third trimester pose no known risk to the woman or her fetus. Passive protection against RSV infection deserves special consideration because, in the US, hospitalization rates of infants < 6 months old have doubled in the past 20 years and safe vaccines for newborn infants are not available.

Molecular adjuvants improve cellular immune potency of DNA vaccines in non human primates

David Weiner

University of Pennsylvania, USA

Evidence suggests that a strong vaccine induced CD8 HIV-1 specific cell mediated immune response may be an important aspect of an HIV vaccine induced immune response. However, current DNA vaccines studied in humans induced low level CD8 T cell responses supporting a need for improving their immune potency. Several laboratories including ours have reported that the cellular immune responses to DNA vaccines can be enhanced in by co-delivering DNA plasmids expressing specific immune modulators. We report that we can extend this improvement into non human primates and improve vaccine induced immunity and drive protection and complete control of viral challenge in vivo. Current implications for clinical studies of these new adjuvant approaches will be discussed.

New approaches to vaccines

Regulatory requirements for GMO-based live oral vaccines

Jean-François Viret

EtnaVax Ltd, Berne, Switzerland

The development of tailor-made live attenuated vaccines, allowing for the safe and effective immunization at mucosal surfaces, is one of the strategies attracting a large interest among vaccinologists, in particular for the prevention of bacterial enteric diseases. The main advantage of this strategy is the induction of strong mucosal responses, in addition to systemic immune responses, allowing targeting of the pathogen at the initial point of contact with the host. Further advantages include the ease of administration, which does not request special medical expertise, the high acceptance by vaccinees, and the relatively low production costs. Finally, well-characterized, safe and immunogenic vaccine strains are well suited as vectors for the mucosal delivery of foreign antigens from other pathogens as well as DNA vaccines. However, in addition to regulations governing conventional vaccines, vaccines containing live genetically modified organisms (GMO) are facing new regulatory hurdles. Specific issues include the potential for genetic reversion to partial or full pathogenicity, gene transfer into and out of the vaccine cells and, in general terms, the potential risks for humans and the environment. In-depth risk assessment is an integral part of the extensive package required for the registration of such vaccines.

A single dose oral typhoid vaccine: can it be achieved?

Gordon Dougan

Principal Research Scientist / Member of the Board of Management, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA (Tel. 01223495381; Fax 01223 494919; Email gd1@sanger.ac.uk)

Typhoid is still a very common and life threatening disease in many parts of the world. There are an estimated 20 million cases a year,

many of these in South East Asia and Africa. Multi-drug resistance in Salmonella Typhi and Paratyphi (causes of the disease) are complicating treatment and there is a need to improve immmunoprophylactic treatment. Injected vaccines are available based upon the Vi antigen of Typhi but these vaccines do not work in children. A conjugate Vi vaccine is in development but a single dose oral vaccine would have the attraction of easy and safe (needle-free) delivery in developing countries. We have been working towards developing a live oral typhoid vaccine based upon attenuated S. Typhi for over twenty years. Progress towards the development of this vaccine has been inhibited by the lack of economic interest in typhoid vaccines themselves and more generally in vaccines for under privileged populations. In this talk I will outline the history of this vaccine development programme from a personal perspective. Hopefully, this story will prove to be scientifically interesting as well as a political education for young investigators interested in entering the vaccine field.

A novel *Staphylococcus aureus* vaccine: iron surface determinant B (IsdB) that induces rapid antibody responses in Rhesus Macaques and specific protection in a murine *Staphylococcus aureus* sepsis model

Annaliesa S. Anderson

Merck and Co. Inc., 440 Sumneytown Pike, WP16 100, Westpoint, PA, 19486, USA (Email annaliesa_anderson@merck.com)

Staphylococcus aureus is a major cause of nosocomial infections world wide, and the rate of resistance to clinically relevant antibiotics such as methicillin is increasing, in community acquired as well as hospital infections. Effective treatment and prevention strategies are urgently needed. We investigated the potential of the *S. aureus* surface protein IsdB as a prophylactic vaccine against *S. aureus* infection. IsdB is an iron sequestering protein that is conserved among diverse *S. aureus* clinical isolates, both methicillin resistant, and sensitive and is expressed on the surface of all isolates tested. The vaccine is highly immunogenic in mice when formulated on aluminum hydroxyphosphate adjuvant and the resulting antibody responses were associated with reproducible and significant protection in a murine sepsis model of infection. The specificity of the protective immune responses in mice was demonstrated by using *S. aureus* deficient for IsdB and HarA, a protein with high homology to IsdB. We also demonstrated that this protein is immunogenic in rhesus macaques after a single immunization. Based on available data, IsdB has potential to be a vaccine against *S. aureus* disease in humans.

Visualizing the antigenome of *Shigella flexneri*: screening for potential vaccine candidate antigens

Amy Jennison & Naresh Verma

Australian National University, School of Biochemistry and Molecular Biology, Canberra, ACT, Australia

Shigella flexneri causes shigellosis, the most infectious form of bacterial diarrhoea. The rapid emergence of antibiotic resistance in *Shigella* spp highlights the need for an effective vaccine to control shigellosis. However, currently there is no licensed vaccine against *S. flexneri* available and vaccine development is still ongoing. The characterization of novel antigens of *S. flexneri* could aid in the construction of a successful vaccine.

We have profiled the immunogenic proteins of *S. flexneri* 2457T expressed during human infection, to generate an antigenome, using sera from shigellosis patients, by two-dimensional gel electrophoresis immunoblotting. A number of immunoreactive proteins were successfully matched and identified by MALDI-TOF. The majority of these proteins have not been previously reported as antigenic in *S. flexneri*. Selected proteins are currently being screened in cell culture and animal studies for their potential as virulence factors or vaccine targets against shigellosis caused by *S. flexneri*.

Non-parenteral vaccines: from concept to clinical validation

A.L.W. Po

University of Aston, Birmingham

Abstract not received

Adjuvants and delivery

Microbial carbohydrates as adjuvants and vaccines

Simon Y.C. Wong

The Edward Jenner Institute for Vaccine Research, Compton, Berkshire

Capsular polysaccharide (CPS) and CPS-protein conjugate vaccines are invaluable in preventing serious human infections due to extracellular encapsulated bacteria such as *Streptococcus pneumoniae* (Pn), but the immunological mechanisms underlying the induction and modulation of protective anti-CPS antibodies have not been clearly defined. Our studies on the contribution of natural IgM, IgM cross-reactivity, complement dependency and C-type lectins in humoral responses to 12 Pn CPS in mice indicate important differences in humoral responses between Pn CPS and a prototype T-independent type II antigen, DNP-Ficoll. They also suggest the presence of B cell populations with broad anti-carbohydrate specificity, which are stimulated by Pn CPS independent of complement and in the apparent absence of adjuvant. Majority of Pn CPS were able to bind several C-type lectins including the macrophage mannose receptor and SIGN-R1, but did not induce macrophage or dendritic cell maturation. In contrast to Pn CPS, yeast β-glucans have reported potent anti-tumour and anti-microbial effects. Studies on Dectin-1, a major receptor on macrophages and dendritic cells for β-glucans provide a potential mechanism for their adjuvanticity. Dectin-1 and other cell surface lectins are thus potential targets for adjuvant development and vaccine delivery to dendritic cells to shape adaptive immunity.

Bacterial spores as vaccine vehicles

Le H. Duc[1], Helen S. Atkins[2], Richard W. Titball[2] & **Simon M. Cutting**[1]

[1]School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 OEX; [2]Defence Science and Technology Laboratory (Dstl), Porton Down, Salisbury SP4 OJQ

Endospores of *Bacillus subtilis* have been developed for the display and delivery of heterologous antigens by mucosal routes. The intrinsic robustness and heat stability of spores makes them attractive for use in developing countries and in emergency situations. We will outline studies where we have developed and optimized spores for delivery of a tetanus antigen, tetanus toxin fragment C (TTFC). Spores displaying the TTFC antigen can be used to immunize mice orally and protect them to a challenge dose of 20 LD_{50} of tetanus toxin. *B. subtilis* spores have also been developed as an anthrax vaccine and can be used to protect mice against challenge with *Bacillus anthracis* spores. Our work in developing *B. subtilis* spores as a potential nasal anthrax vaccine will be outlined.

Transcutaneous immunization

G.M. Glenn

IOMAI Corporation, Maryland, USA

Abstract not received

Computational vaccinology

Darren R. Flower

University of Oxford

Historically, vaccine discovery has focused on attenuated whole pathogen vaccines such as BCG for TB or Sabin's Polio vaccine. More recently, safety concerns have lead to the emergence of other targets for vaccine development, such as cell, antigen, and epitope -based vaccines. Extant microbial genomes represent an unprecedented opportunity for rational and mechanism-based vaccinology, allowing computational predictive methods to identify immunogenic candidate antigens or epitopes. Computational Vaccinology is a component of Immunoinformatics, a newly emergent sub-discipline within bioinformatics which deals with the unique problems of Immunology. In this talk, I will explore how Immunoinformatics complements, but not replaces, laboratory experimentation, allowing researchers to focus on key questions in vaccine discovery.

Physiology, Biochemistry and Molecular Genetics Group

Cyclic-di-GMP and the physiological control of intracellular signalling networks in diverse bacteria

Cyclic-di-GMP, bacterial physiology and development

The discovery of cyclic-di-GMP as a regulatory molecule

H. Weinhoiz[1], P. Ross[1], Y. Alony[1], D. Michaeli[1], P. Ohana[1], E. de Vroom[2], G.A. van der Marel[2], J.H. Van Boom[2] & M. Benziman[1]

[1]Dept of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel; [2]Gorlaus Laboratories, P.O.B. 9502, 2300 RA Leiden, The Netherlands

The *in vitro* synthesis of β-1,4-glucan from UDP-glucose, using a membrane preparation derived from extracts of *Acetobacter xylinum* was first reported by Glaser (1958). However, this membrane preparation synthesized the β-1,4- glucan product at the rate which are only a fraction of 1 % relative to the *in vivo* rate of cellulose synthesis from glucose.

25 years later, we succeeded to get very high rate of *in vitro* synthesis of β-1,4-glucan from UDP-glucose (about 80 % relative to the *in vivo* rate). This activity is subject to a complex multi-component regulatory system, in which the synthase is directly affected by an unusual cyclic nucleotide activator enzymatically formed from GTP, and indirectly by a Ca^{2+}-sensitive phosphodiesterase which degrades the activator. This activator has been identified as bis-(3'-5')-cyclic diguanilic acid on the basis of mass spectroscopic data, nuclear magnetic resonance analysis and comparison with chemically synthesized material.

The enzyme responsible for the synthesis of the c-di-GMP is the diguanylate cyclase which has been purified 3000 fold from the soluble protein fraction.

Cyclic-di-GMP signalling in *Caulobacter*

U. Jenal & **J. Malone**

University of Basel, Switzerland

Extensive research into the regulation of biofilm formation in diverse bacterial species has led to a greatly increased understanding of the underlying principles of cyclic-di-GMP synthesis and degradation. GGDEF and EAL domains have been shown to function as di-guanylate cyclases (DGC) and phosphodiesterases (PDE) respectively, and cyclic-di-GMP levels have been shown to correlate directly with the adoption of aggregative behavior. This seminar will first highlight several emerging concepts of cyclic-di-GMP signaling, including catalytic, structural and regulatory aspects of the 'making and breaking' of cyclic-di-GMP. The role of cyclic-di-GMP in pole development in *Caulobacter crescentus* will then be discussed. *C. crescentus* is an organism with an obligate switch between a planktonic and surface attached lifestyle, integrated in its replicative cycle. Initial evidence suggests that a cyclic-di-GMP signal transduction network coordinates pole differentiation events to facilitate *C. crescentus* surface colonization. The significance of these findings for our understanding of bacterial persistence and biofilm formation will be discussed.

c-diGMP-mediated signaling within the σ^S network of *Escherichia coli*

Harald Weber, Christina Pesavento & **Regine Hengge**

Institut für Biologie, Mikrobiologie, Freie Universität Berlin, 14195 Berlin, Germany

Cyclo-diGMP (c-diGMP) is a bacterial 'second messenger' molecule produced by diguanylate cyclases (carrying GGDEF domains) and specific phosphodiesterases (EAL domains). Neither its full physiological impact nor its effector mechanisms are currently understood. In microarray analyses, expression of eight of the 29 GGDEF/EAL genes in *E. coli* exhibited dependence on σ^S (RpoS). These genes show highly specific and distinct expression patterns suggesting an important role for c-diGMP in stationary phase. We demonstrate that a specific σ^S-controlled pair of a GGDEF-only protein and a GGDEF/EAL protein plays an antagonistic role in the expression of the biofilm-associated curli fimbriae. The target of this control is both the transcriptional and the post-transcriptional regulation of the curli and cellulose regulator CsgD. Another σ^S-controlled GGDEF protein is involved in the control of motility. We also demonstrate a GGDEF cascade in which one σ^S-dependent GGDEF protein controls the expression of another. Microarray analyses indicate that specific small sets of target genes are affected by knockout mutations in different σ^S-dependent GGDEF/EAL genes which suggests a certain microcompartimentation of c-diGMP synthesis and action.

Characterization of *Escherichia coli yfgF*: a cyclic-diGMP phosphodiesterase?

J. Partridge, M. Wragg & J. Green

Dept of Molecular Biology & Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN

Inspection of the *Escherichia coli* genome sequence revealed the presence of a consensus site (TTGATnnnnATCAA) for the oxygen-responsive transcription factor FNR located upstream of the *yfgF* open reading frame. *In vivo* transcription studies with a *yfgF-lacZ* fusion showed that expression is activated by FNR under anaerobic conditions. Transcript mapping, gel retardation and mutagenesis studies showed that *yfgF* expression is driven from a class II FNR-dependent promoter (FNR site located at –42.5). The amino acid sequence predicts that YfgF contains a membrane anchored N-terminal region linked to sequences resembling both GGDEF and EAL domains, suggesting a role in cyclic-di-GMP signalling. The amino acid sequence also suggests that the EAL domain (phosphodiesterase activity) is active, whereas the GGDEF domain (diguanylate cyclase) may be inactive. Phenotypic analysis of the *yfgF* mutant indicated a role in the *E. coli* oxidative stress response, with the mutant exhibiting sensitivity to peroxides. Transcript profiling experiments comparing parent and *yfgF* mutant strains suggest that YfgF influences the expression of genes linked to sugar transport and citrate metabolism in *E. coli*.

101 ways to skin a cat: di-guanylate cyclases and the repeated evolution of cooperation

Jacob G. Malone, Stefanie M. Gehrig, Hubertus J.E. Beaumont, Andrew J. Spiers & **Paul B. Rainey**

School of Biological Sciences, University of Auckland, New Zealand and Dept of Plant Sciences, University of Oxford

The repeatability of evolution has long fascinated biologists. When allowed to evolve in spatially structured microcosms experimental populations of *Pseudomonas fluorescens* rapidly diversify producing a range of niche specialist genotypes. One type that evolves repeatedly, in independent microcosms, is the wrinkly spreader (WS) – an adaptive mutant that forms a simple cooperating group. Careful examination of WS genotypes shows that they encompass a morphologically diverse collection, although all share a common ability to colonize the air-liquid interface of static broth microcosms. Analysis of the structural components of independent WS genotypes shows that each achieves its characteristic phenotype by over-production of an acetylated cellulose polymer and associated adhesive factors. Taken together these observations indicate that while WS genotypes evolve repeatedly and employ the same structural components there must be different genetic routes to achieving similar ends. Genetic analysis of WS has led to an understanding of the molecular causes of their evolution. Numerous independent mutations cause the evolution of WS and do so by affecting the activity of negative regulators of di-guanylate cyclases. I will describe the analysis of several such regulators, show how mutational changes affect the activity of di-guanylate cyclases and how changes in the activity of di-guanylate cyclases alters the form and fitness of WS genotypes. Repeatability of evolution (and capacity for reverse evolution) in this experimental system appears to owe much to the special features of the c-di-GMP network.

PigX, a GGDEF and EAL domain protein, regulates pleiotropically the biosynthesis of prodigiosin, swarming and virulence of *Serratia* sp. ATCC 39006

P.C. Fineran, N.R. Williamson & G.P.C. Salmond

Dept of Biochemistry, University of Cambridge

Gram-negative bacteria of the genus *Serratia* are opportunistic human, plant and insect pathogens. *Serratia* sp. ATCC 39006 secretes pectinases, cellulases and produces the secondary metabolites, carbapenem and prodigiosin. Prodigiosins are of interest because they have immunosuppressive and anticancer properties. We have identified a hyper-pigmented mutant with a transposon insertion in a gene (*pigX*) which encodes a protein containing putative GGDEF and EAL domains. Interestingly, the GGDEF and EAL motifs within these domains are poorly conserved. The *pigX* mutant is pleiotropically affected in the production of pectinases, prodigiosin, a surfactant-like compound, swarming and exhibits increased virulence in a potato tuber assay. We have mapped the transcriptional start site of *pigX* and shown that *pigX* is regulated by the pleiotropic 'master' regulator, PigP. Furthermore, *pigX* regulates the prodigiosin biosynthetic operon at the level of transcription. The nature of PigX and the hierarchical position of this regulator in secondary metabolite regulation will be discussed. In conclusion, a GGDEF and EAL domain protein, PigX, has an important role in regulating swarming, virulence and the production of exoenzymes, prodigiosin and a surfactant-like compound in *Serratia* 39006.

Cyclic-di-GMP signalling and bacterial pathogenesis

Cyclic di-GMP signalling in *Salmonella enterica* serovar Typhimurium

Ute Römling

Microbiology and Tumor Biology Center (MTC), Karolinska Institutet, MTC, Box 280, SE-171 77 Stockholm, Sweden

GGDEF and EAL domain proteins are involved in the turnover of the novel secondary messenger cyclic-di(3'→5')-guanylic acid (c-di-GMP) in many bacteria. In this way, cyclic di-GMP signaling determines the timing and amplitude of complex biological processes from photosynthesis to biofilm formation and virulence in many bacteria. To study the differential regulation of c-di-GMP signalling, a survey of the impact of 20 GGDEF and EAL domain proteins in *Salmonella* Typhimurium on cyclic di-GMP metabolism and multicellular behaviour and related phenotypes such as swimming and swarming was performed. Knockout studies revealed distinct task distribution of GGDEF/EAL domain proteins with respect to rdar morphotype expression as characterized by the expression of cellulose and curli fimbriae, biofilm formation and swimming and swarming motility. Western blot analysis showed that effects of GGDEF and EAL domain proteins on rdar morphotype expression are mediated by CsgD, thus confirming the role of CsgD as a major switchboard of biofilm formation in *S.* Typhimurium. Consequently, this work has shown that the c-di-GMP regulatory network of multicellular behaviour in *S.* Typhimurium is complex and acts even on several levels in the same pathway.

Cyclic-di-GMP signalling and virulence in the plant pathogen *Xanthomonas campestris*

Robert Ryan[1], Yvonne Fouhy[1], Jean Lucey[1], Yong-Qiang He[2], Jia-Xun Feng[2], Ji-Liang Tang[2] & J. Maxwell Dow[1]

[1]BIOMERIT Research Centre, Dept of Microbiology, National University of Ireland, Cork, Ireland; [2]Institute of Molecular Genetics, Guangxi University, Nanning, PRC

Xanthomonas campestris pathovar campestris (*Xcc*) is the causal agent of black rot disease of cruciferous plants. Recent work from our own laboratory has implicated the unusual nucleotide cyclic-di-GMP in regulation of virulence factor synthesis and biofilm formation in this organism. Three protein domains, GGDEF, EAL and HD-GYP have now been implicated in cyclic-di-GMP metabolism. Proteins with these domains may act in signal transduction systems that serve to link perception of different environmental cues to changes in cyclic-di-GMP levels. The aim of the work described was to examine the role of these elements of the proposed cyclic-di-GMP regulatory network in the virulence of *Xcc*. Our approach has been to test a library of defined mutants, created in Guangxi, for their ability to cause disease in Chinese radish and to produce virulence factors *in vitro*. The findings indicate that a subset of elements that may recognize different environmental cues are important for virulence.

Cyclic-di-GMP and cell-cell signalling in *Xanthomonas campestris*

Max Dow[1], Robert Ryan[1], Yvonne Fouhy[1], Jean Lucey[1], Lisa Crossman[2], Stephan Heeb[3] & Paul Williams[3]

[1]BIOMERIT Research Centre, National University of Ireland, Cork; [2]Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA; [3]Institute of Infection, Immunity & Inflammation, Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD

The *rpf* gene cluster of *Xanthomonas campestris* pv. *campestris* (*Xcc*) is required for the pathogenesis of this bacterium to plants. Several *rpf* genes are involved in the co-ordinate positive regulation of synthesis of extracellular enzymes and xanthan and in biofilm

dispersal, mediated by the small diffusible molecule DSF. A two-component sensory transduction system comprising RpfC and RpfG has been indirectly implicated in the perception of the DSF signal and signal transduction. RpfC is a complex sensory histidine kinase whereas the cognate regulator RpfG has a typical input domain of a two-component regulator attached to an HD-GYP domain. We have now shown that the HD-GYP domain of RpfG is a phosphodiesterase that functions in the turnover of cyclic di-GMP. A systematic mutational analysis of all the genes cognate to the cyclic di-GMP regulatory network in *Xcc* (encoding GGDEF, EAL and HD-GYP domain proteins) revealed a sub-set of proteins with significant roles in virulence to plants. These proteins contain additional sensory and signal transduction domains including a novel beta propeller structure. Our findings offer an insight into how *Xcc* may integrate cues from cell-cell signalling with other environmental inputs, that may include specific plant signals, in order to modulate virulence.

Cyclic-di-GMP as a regulatory signal in *Vibrio cholerae*

Andrew Camilli PhD

Tufts University School of Medicine, Dept of Molecular Biology and Microbiology, 136 Harrison Avenue, M&V 402, Boston, Massachusetts 02111, USA (Tel. +16176362144; Fax +16176362144; Email Andrew.Camilli@Tufts.edu)

Vibrio cholerae is a water-borne pathogen and the causative agent of cholera. Gene expression patterns in *V. cholerae* change upon entry into and exit from the host intestine, and insight into these changes may reveal much about the virulence and transmissibility of this organism. In a screen for infection-induced factors, we identified a dual function response regulator/c-di-GMP phosphodiesterase (PDEA) called VieA. VieA is one of 21 PDEAs in *V. cholerae*. We showed that VieA-mediated lowering of c-di-GMP concentration is required for virulence in classical biotype strains but not in El Tor biotype strains, the latter of which cause all cholera in the world today. By mutational analysis we show that multiple PDEAs are acting redundantly in El Tor strains to enhance virulence. By manipulating the c-di-GMP concentration in *V. cholerae* during infection through ectopic expression of PDEAs and/or diguanylate cyclases, we can alter virulence. These results support the model that *V. cholerae* uses c-di-GMP as an intracellular signaling molecule to regulate virulence. We are currently investigating which virulence factors and/or properties are regulated by c-di-GMP in El Tor biotype strains and how such regulation is mediated.

Task distribution between the EAL-domain proteins in the regulatory network controlling multicellular behaviour of *Salmonella* Typhimurium

Roger Simm, Astrid Lusch, Kader Abdul, Mats Andersson & Ute Römling

Microbiology and Tumorbiology Center (MTC), Karolinska Institutet, Box 280, SE-171 77 Stockholm, Sweden

GGDEF and EAL domain proteins are involved in the turnover of the novel secondary messenger cyclic-di(3'→5')-guanylic acid (c-di-GMP) in many bacteria. In this work the role of 20 GGDEF and EAL domain proteins in the multicellular behaviour of *Salmonella* Typhimurium was investigated. Surprisingly, the activity of the GGDEF domain proteins is not the only determinant of the multicellular behaviour of *S.* Typhimurium. Instead, the knock-out of EAL domain proteins turned out to have an equally significant impact on multicellular behaviour, biofilm formation and motility. Knock-out of specific EAL domain proteins dramatically increased the multicellular behaviour as visualized by the rdar morphotype on Congo Red plates and biofilm formation in liquid medium. Western blot analysis showed that the effects of GGDEF and EAL domain proteins on rdar morphotype expression are CsgD mediated. On the other hand, the knock out of other EAL domain proteins strongly influenced the flagella mediated motility of the bacteria showing that different chromosomally encoded EAL domain proteins have different tasks. This work has shown that the c-di-GMP regulatory network of multicellular behaviour in *S.* Typhimurium is complex and acts on several levels, even in the same regulatory pathway. However, the expression of CsgD is a central determinant in the regulation of multicellular behaviour in *S.* Typhimurium.

Integration of cyclic di-GMP signalling with the posttranscriptional regulation of multicellular behaviour in *Pseudomonas aeruginosa* via the small RNA-binding protein, RsmA

Stephan Heeb, Miguel Cámara & **Paul Williams**

Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD

Pseudomonas aeruginosa is an ubiquitous Gram-negative bacterium with a a 6.3MB genome, some 10 % of which is devoted to regulatory genes. Although many of these are of unknown function, they highlight the existence of sophisticated gene regulatory networks which facilitate the extra-ordinary adaptability of *P. aeruginosa*. Among these, the small RNA-binding protein RsmA is a post-transcriptional control element that mediates extensive changes in secondary metabolite synthesis, exoenzyme production and swarming motility. *P. aeruginosa rsmA* mutants are unable to swarm, and we discovered a gene, *cdgA*, which restores swarming motility and is one of numerous *P. aeruginosa* proteins predicted to be involved in cyclic-di-GMP-dependent signalling. Indeed, the *P. aeruginosa* genome sequence contains 17 predicted proteins with a GGDEF domain, 5 containing an EAL domain and 16 proteins contain both. In addition there are 3 proteins containing an HD-GYP domain also predicted to be involved in cyclic diGMP metabolism. *P. aeruginosa* therefore possess extensive intra-cellular signalling network(s) employing c-di-GMP as the cognate signal molecule, the contribution of which to the lifestyle of *P. aeruginosa* is only just beginning to be unravelled.

Systematics & Evolution Group

Eukaryotic evolution

Introduction

This meeting will bring SGM members up to date on developments in eukaryotic evolution and theories for eukaryogenesis that have occurred since the highly successful meeting 'Evolution of Microbial Life' in Warwick in March 1996. Since then the ribosomal RNA tree has come under severe criticism for its picture of early eukaryotic evolution and the concept of primitively amitochondriate eukaryotes has suffered devastating setbacks. New ideas for eukaryotic origins and diversification have resulted. Darwin's idea of a 'tree linking all the great kingdoms of nature' has come under scrutiny with widespread HGT revealed by genomics suggesting a net may be a more appropriate description than a tree. Methodological progress in probing the deep past of the eukaryotic cell has even suggested that genome fusion played a key role in eukaryogenesis. Secondary endosymbioses leading to photosynthetic lineages is more widespread than previously appreciated and in some cases may lead to novel therapies for major killers like Malaria. In his 1973 essay 'Nothing makes sense except in the light of evolution' Dobzhansky claimed that 'evolution is a light which illuminates all facts' – does this hold up for a key eukaryotic system – the eukaryotic cell cycle?

Horizontal gene transfer and the evolution of eukaryotes

W.F. Doolittle

Dept of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada

This talk will have two parts. In the first, I will discuss the extent to which horizontal gene transfer within and between lif'e's three domains renders meaningless any attempt to represent the relationships between them as a single tree. In the second I will revisit a proposal (the 'You Are What You Eat Hypothesis') in which I suggested that a gene transfer ratchet might account for the presence of bacterial genes in eukaryotic nuclear genomes, reviewing evidence since accumulated bearing on the plausibility of that notion.

The ring of life: evidence for a genome fusion origin of eukaryotes

James Lake

Molecular Biology Institute, MCD Biology, Astrobiology Institute, and Human Genetics, UCLA, Los Angeles, CA 90095

Genomes hold within them the record of the evolution of life on Earth. But genome fusions and horizontal gene transfer appear to have sufficiently obscured the gene sequence record such that it is difficult to reconstruct the tree of life. Here we determine the general outline of the tree using complete genome data from representative prokaryotes and eukaryotes and a new genome analysis method that makes it possible to reconstruct ancient genome fusions and phylogenetic trees. Our analyses indicate that the eukaryotic genome resulted from a fusion of two diverse prokaryotic genomes, and therefore at the deepest levels linking prokaryotes and eukaryotes, the tree of life is actually a ring of life. One fusion partner branches from deep within an ancient photosynthetic clade, and the other is related to the archaeal prokaryotes. The eubacterial organism is either a *Proteobacterium,* or a member of a larger photosynthetic clade that includes the *Cyanobacteria* and the *Proteobacteria*.

Early eukaryotic evolution – the key roles of redox chemistry and endosymbiosis

W. Martin

Heinrich-Heine-Universität, Dusseldorf, Germany

The contribution of endosymbiosis to eukaryote evolution is usually seen as a small add-on to a more general process of evolutionary invention that entailed a more general process known as the prokaryote-to-eukaryote transition, which entailed the origin of eukaryotic specific features such as the nucleus. However, the newly recognized ubiquity of mitochondria call into question the order of events concerning the origin of mitochondria, the nucleus, and other eukaryotic specific traits. It is an opportune time to rethink the nature of evolutionary processes that gave rise to contemporary eukaryote lineages and to pursue the notion that the host that aquired the mitochondriaon might have been an archaebacterium outright, in which case the origin of eukaryotic novelties would follow in the wake of mitochondrial (and hydrogenosomal) origins.

Eukaryote origins and early diversification before and after snowball earth

Thomas Cavalier-Smith

Dept of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS

Eukaryotes and archaebacteria are phylogenetically sisters, collectively called neomura to contrast them with the more primitive eubacteria that have murein peptidoglycan walls. Their common ancestor arose from an actinobacterium during the neomuran revolution, probably only ~0.9 Gy ago, by profound alterations in the cell envelope in the most radical quantum evolution ever in bacteria. N-linked surface glycoproteins replaced murein peptidoglycan and lipoproteins, with substantial coadaptive changes in ribosomes and signal recognition particles; simultaneously, core histones evolved, stimulating marked modifications of DNA-handling enzymes. The resulting ancestral neomuran diverged rapidly to produce hyperthermophilic archaebacteria, by evolving novel acid/heat-resistant flagella and isoprenoid ether lipids, and the first eukaryotes, by evolving phagotrophy, endoskeleton, endomembrane system, novel molecular motors, nuclear pore complexes, mitosis, cyclins, sex, mitochondria (by α-proteobacterial enslavement), peroxisomes, centrioles and cilia. Recent advances in cell biology help explain all these eukaryotic innovations as natural coevolutionary consequences of the origin of phagotrophy, the adaptive zone of protozoa and animals. Eukaryotes were ancestrally aerobic, with a primary bifurcation between unikonts (animals, fungi, Choanozoa, Amoebozoa) and bikonts (plants, chromalveolates, excavates, Rhizaria). The origin of methanogenic archaebacteria may have indirectly caused the Neoproterozoic snowball-earth episodes of global freezing soon after eukaryotes evolved. Cyanobacterial enslavement by an early bikont probably created chloroplasts before the snowball, whereas red-algal enslavement to yield chromalveolates, and most other early eukaryote diversification, probably rapidly followed its melting.

Mitochondria and early eukaryotic evolution

T.M. Embley

University of Newcastle

Abstract not received

The relict PLASTID of *Plasmodium* parasites: can we treat malaria with herbicides?

Geoffrey Ian McFadden

Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Victoria, 3010, Australia

The apicoplast (a relict plastid of malaria parasites) has emerged as a promising target for new antimalarials. Apicoplasts are indispensable but their exact function remains uncertain. To understand more about the apicoplast we assembled a predicted organelle proteome. The apicoplast synthesizes 23 proteins but also imports numerous nuclear encoded proteins. Targeting of these proteins to the apicoplast requires a unique N-terminal extension. After scrutinizing a large collection of these N-terminal extensions from the *Plasmodium falciparum* genome, we were able to extract a simple set of rules that could predict targeting of proteins to the apicoplast from primary sequence. Strategic mutagenesis of apicoplast targeting peptides demonstrated that a net basic charge and a chaperone-binding site are critical to accurate targeting. Our current estimates identify more that 500 apicoplast proteins, which represents about 10 % of the parasite genome, engaged in this compartment. These apicoplast proteins comprise complete pathways for fatty acid and isoprenoid biosynthesis plus a partial set of haem synthesis enzymes. We believe these anabolic pathways are essential to parasite survival because end products are exported from the apicoplast for use elsewhere in the parasite cell. Our current focus is to verify the biochemical activities for these pathways and to understand how this anabolism is fuelled. We have identified and characterized apicoplast membrane transporters that likely import triose phosphates plus apicoplast modifying enzymes that convert these sugars into substrates for fatty acid and isoprenoid biosynthesis. Numerous apicoplast enzymes for these pathways, and additional apicoplast housekeeping activities, are excellent drug targets.

Predators and prey: primary, secondary and tertiary events in plastid evolution

C.J. Howe, A. Barbrook, E. Nash & E. Nisbet

Dept of Biochemistry, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QW

It is widely accepted that plastids first arose from a photosynthetic prokaryote that was engulfed by a non-photosynthetic host. However, given the extent to which genes from the original prokaryote have pervaded the host, it may be more appropriate to regard this as an invasion by the prokaryote, rather than a capture of it. There has long been discussion as to whether all plastids are descended from a single primary endosymbiosis, or whether more than one primary endosymbiosis happened. We discuss the evidence for or against each proposal. It is clear that serial endosymbioses have also occurred, with photosynthetic eukaryotes being engulfed, giving rise to secondary and tertiary plastids. The dinoflagellate algae have a particularly complex pattern of plastid evolution, and this may be linked to the anomalous organization of the plastid genome found in peridinin-containing dinoflagellates. In these organisms the chloroplast genome has become highly reduced and fragmented into a number of small circular molecules most of which contain a single gene.

Dating the tree: can we trust the molecular clock?

Lindell Bromham

Centre for the Study of Evolution, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG

Molecular data are ideal for exploring deep evolutionary history because of their universality, stochasticity and abundance. These features provide a means of exploring the evolutionary history of all organisms (including those that do not tend to leave fossils), independently of morphological evolution, and within a statistical framework that allows testing of evolutionary hypotheses. But if the rate of molecular evolution can vary between species, can we trust molecular date estimates? Molecular dating methods have been developed that allow for rate change over phylogenies, but without an understanding of the tempo and mode of molecular evolution, we cannot judge whether the assumptions that these 'relaxed clock' methods make reflect real genomic evolution. Understanding the way a species biology, ecology or evolution might influence rates of molecular evolution may help to identify cases where molecular dates might be consistently misleading. I use a comparative approach, comparing a range of DNA sequences from a wide array of taxa, to ask which species characteristics, or modes of evolution, influence the rate of molecular evolution, and to examine what effect these factors may have on our ability to infer ecological and evolutionary patterns and processes from DNA sequences.

Virus Group

Virus interactions with subcellular structures

Spatial and functional interactions between HSV-1 and ND10 nuclear sub-structures

Roger D. Everett

MRC Virology Unit, Institute of Virology, Church Street, Glasgow G11 5JR

A decade ago, Gerd Maul and colleagues observed that the parental genomes of herpes simplex virus type 1, and many other DNA viruses, are frequently associated with small nuclear sub-structures known as ND10 or PML nuclear bodies. It has also been known for several years that HSV-1 regulatory protein ICP0 brings about the disruption of ND10 through its ability to induce degradation of the PML protein, the key constituent of ND10. Several important questions arise from these observations: how do HSV-1 genomes become associated with ND10, where are HSV-1 genomes in quiescently-infected cells, and what are the functional implications of ND10-association and PML degradation? This talk will describe some recent advances in each of these topics. The common association of HSV-1 genomes with ND10 can be attributed to the deposition of PML and other ND10 proteins at sites that are closely associated with the viral genomes, rather than the genomes moving towards pre-existing ND10. HSV-1 genomes in quiescently-infected cells are enveloped by what appears to be a shell of ND10 proteins. Finally, recent evidence indicates that PML plays a role in the efficiency by which HSV-1 genomes are repressed in quiescently infected cells. These data indicate that ND10 play fundamental roles in the biology of HSV-1 infections.

Coronaviruses and the nucleolus

J.A. Hiscox

University of Leeds

Abstract not received

Trafficking and metabolism of mRNA in influenza virus infected cells

P.E. Digard

University of Cambridge

It is over 20 years since the publication of experiments that showed that influenza A virus RNA synthesis takes place in the cell nucleus and that here, the virus parasitises the cellular RNA polymerase II transcription machinery to express and replicate its own single-strand RNA genome. In the years since, our understanding of the organization of the nucleus has increased enormously, particularly with regards to the functional integration of the RNA polymerase II transcriptosome. Recent research suggests an intimate interplay between viral and cellular RNA polymerases that goes beyond a simple dependence on a source of mRNA cap structures to prime viral mRNA synthesis. Data will be presented that suggests a second requirement for RNA polymerase II in the influenza virus life cycle.

Vaccinia virus liduced cell motility requires F11L-mediated inhibition of RhoA signalling

Ferran Valderrama[1], João V. Cordeiro[1], Sibylle Schleich[1], Friedrich Frischknecht[2] & **Michael Way**[1]

[1]Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3PX; [2]Dept of Parasitology, Heidelberg University School of Medicine, Germany

The spatial and temporal regulation of cell adhesion and motility is essential during development and throughout the lifetime of multi-cellular organisms. Deregulation of these two fundamental cellular processes frequently occurs during pathological situations and is an important factor contributing to tumour cell metastasis. Dramatic changes in cell migration and adhesion, as well as loss of contact inhibition, are also observed during viral infections including that of vaccinia virus. Using a combination of approaches we have found that the vaccinia F11L protein interacts directly with RhoA, inhibiting its signalling by blocking the interaction with its downstream effectors ROCK and mDia. RNAi-mediated depletion of F11L during infection resulted in an absence of vaccinia-induced cell motility and inhibition of viral morphogenesis. Disruption of the RhoA binding site in F11L, which resembles that of ROCK, led to an identical phenotype. Thus inhibition of RhoA signalling is required for both vaccinia morphogenesis and virus induced cell motility.

Extreme machines: structures of the metastable pre-fusion and stable post-fusion forms of the parainfluenza virus F protein

Robert A. Lamb[1,2], Hsien-Sheng Yin[1,2], Reay G. Paterson[2], Xiaolin Wen[2] & Theodore S. Jardetzky[2]

[1]Howard Hughes Medical Institute; [2]Dept of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208-3500, USA

Enveloped viruses have evolved complex glycoprotein machinery that drives the fusion of viral and cellular membranes, permitting entry of the viral genome into the cell. For the paramyxoviruses, the fusion (F) protein catalyzes this membrane merger and entry step and it has been postulated that the F protein undergoes complex refolding during this process. The crystal structure of the parainfluenza virus 5 F protein in its pre-fusion conformation, stabilized by the addition of a C-terminal trimerization domain has been determined. The F structure reveals profound conformational differences between the pre- and post-fusion states, involving transformations in secondary and tertiary structure. The positions and structural transitions of key parts of the fusion machinery, including the hydrophobic fusion peptide and two helical heptad repeat regions, clarify the mechanism of F protein mediated membrane fusion.

How infectious prions meet cellular prion protein on the neuronal surface

Roger Morris, Celia Parkyn, Esmeralda Vermeulen, Roy Mootoosamy & Angela Jen

Wolfson Centre for Age-related Disease, King's College London, Guy's Campus, London SE1 1UL

Cellular prion protein (called PrP^C) is a natural GPI-anchored protein of the neuronal surface that recycles within minutes (1) via coated pits, through recycling endosomes and back to the surface. Prion protein present as the major component of infectious fibrils differs from PrP^C only in conformation. The defining event in prion infection is thought to be binding of these two forms, with infectious PrP^{Sc} (Sc for scrapie-associated) imposing its pathogenic conformation upon PrP^C.

I will show that infectious fibrils of PrP^{Sc} bind to neurons in primary culture and are very rapidly endocytosed, just like PrP^C. However, PrP^C is not the receptor for prion fibrils on neurons. Instead, both

forms of prion protein bind to a common transmembrane receptor of the LDL-receptor superfamily. Inhibition of this receptor proportionately down-regulates the internalization of PrP^{C} and the binding of PrP^{Sc}, indicating a central role for the receptor in prion disease.

Reference Sunyach, C. *et al.* (2003). The mechanism of internalization of GPI anchored prion protein. *EMBO J* 22, 3591–3601.

Involvement of cellular actin on virus release of *Autographa californica* nucleopolyhedrovirus

Felicity J. Haines[1], Caroline Griffiths[1], Katja Graumann[1], Chris Hawes[1], Robert D. Possee[2] & Linda A. King[1]

[1]School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Oxford OX3 0BP; [2]NERC Institute of Virology and Environmental Microbiology (CEH, Oxford), Mansfield Road, Oxford OX1 3SR

Budded viruses (BV) of the baculovirus *Autographa californica* nucleopolyhedrovirus (AcNPV) are produced as nucleocapsids (NCs) that exit from infected insect cells, acquiring cellular membranes enriched with GP64, the BV attachment and fusion glycoprotein. Actin is thought to be critical for transport of NCs to the nucleus during the early stages of infection, and an association with microtubules is thought to be involved in the egress of progeny virus. In this study, observations of GP64 localization confirmed a role for F-actin in GP64 membrane targeting and BV release from infected insect cells. Results demonstrated inhibition of actin polymerization had no effect on virus entry or on virus gene expression. Treatment of cells with Latrunculin B (LB) significantly reduced trafficking of GP64 to the PM and yield of BV. However, production of infectious occluded virus was not affected, suggesting nucleocapsid assembly within the nucleus was not affected by abrogation of F-actin polymerization. It is proposed that the reduction of BV release in LB-treated cells may be a consequence of reduced trafficking of BV-associated proteins on exit from the Golgi or of NCs to the PM, with F-actin having multiple roles during BV formation.

Prototypic foamy virus entry into host cells via clathrin-mediated endocytosis

Stephen A. Morris, Saba Abdul Hussein, Dev C. Menon, Jan Domin & Myra O. McClure

Division of Medicine, Imperial College London

Foamy viruses constitute a distinct family of complex retroviruses. Despite having a broad tropism, no cellular receptor has been identified and little is known of the mechanism of virus entry. Prototypic foamy virus (PFV) entry has been shown to be pH-dependent and, thus, is likely to require access to endosomal compartments.

The phosphoinositide 3-kinase inhibitor, wortmannin, was used to inhibit endosomal trafficking. We found that both wild type PFV and PFV vector entry were significantly inhibited, demonstrating that an intact endosomal system is required for PFV replication. Since a number of endocytotic mechanisms are operational in gaining entry to the endosomal pathway, this was further investigated. Inhibition of clathrin-mediated endocytosis by siRNAs to the clathrin heavy chain and the clathrin-associated plasma membrane AP2 adaptor complex inhibited PFV entry. PFV entry was also inhibited by expression of a dominant negative form of AP180 which prevents coated pit formation.

In conclusion, we have demonstrated that PFV infection proceeds via clathrin-mediated endocytosis and entry to the acidic pH of the endosomal system.

Characterization of the structural determinants that target the HCV core protein to lipid droplets

Steeve Boulant[1], Roland Montserret[2], Graham Hope[1], Maxime Ratinier[2], Paul Targett-Adams[1], Jean-Pierre Lavergne[2], Francois Penin[2] & John McLauchlan[1]

[1]MRC Virology Unit, Institute of Virology, Glasgow G11 5JR; [2]IBCP, CNRS-UMR 5086, Lyon, Cedex 07, France

HCV core protein is targeted to lipid droplets (LDs) by a C-terminal domain, termed D2. Our study aimed to characterize in detail the determinants that enable attachment of core to LDs. We have established that the major structural elements within D2 consist of two amphipathic α-helices (Helix I and Helix II) separated by a hydrophobic loop (HL). Both helices require a hydrophobic environment for folding, suggesting that lipid interactions contribute to their structural integrity. A combination of Helix I, HL and Helix II is essential for efficient LD association, with hydrophobic amino acids in each element playing a critical role. By contrast, hydrophilic residues do not contribute to targeting. These studies identify critical determinants within a targeting domain that enable trafficking and attachment of core to LDs and serve as a unique model for elucidating the specificity of protein-lipid interactions. Moreover, they serve as a basis for examining the role of lipid droplet association by core in a novel infectious system for HCV.

Nucleolar targeting of the herpesvirus ORF 57 protein is required for viral mRNA export

Jim Boyne & Adrian Whitehouse

Institute of Molecular and Cellular Biology, University of Leeds, Leeds LS2 9JT

ORF 57 is a RNA-binding, nucleocytoplasmic shutting protein that functions to export intronless viral mRNAs during herpesvirus lytic-replication. ORF 57 localizes to splicosomes and the nucleolus. In order to determine the functional significance of ORF 57's nucleolar targeting a series of ORF 57 deletion mutants were constructed. Our data demonstrate that ORF 57 contains two distinct nuclear localization signals (NLS) and that either of these NLS was sufficient for nuclear localization of ORF 57, however, loss of either NLS was enough to prevent localization of ORF 57 to the nucleolus. In order to determine if a loss of nucleolar localization affected ORF 57-mediated mRNA export, a viral mRNA export assay was performed. Results demonstrated that ORF 57 mutants, which localized to the nucleus but not the nucleolus were unable export viral mRNA. This suggests a possible role for the nucleolus in viral mRNA export.

Molecular organization of Gag protein shells in immature retroviruses

M.V. Nermut

National Institute for Biological Standards and Control, South Mimms, Herts

One of the specific features of retroviruses is the process of maturation. The nascent virus particles formed by a precursor protein (Gag) are soon after release from infected cells transformed into mature virus particles – the virions. This maturation process is initiated by cleavage of Gag by a specific viral protease into four domains: MA, CA, NC and a small C terminal peptide. Our study by electron microscopy of *in vitro* assembled immature HIV VLP (1) revealed a hexagonal (fullerene-like) network of small rings with CTC spacing of 6.75 nm (STD 0.65 nm, N = 240) and holes about 4 nm in diameter. A very similar 'cage-like' organization was also observed in

immature Gag shells of M-PMV (2) produced in *E. coli*. The size of holes was about 4 nm and the CTC spacing 7.05 nm (STD 0.65 nm, N = 220). The number of molecules in HIV VLP 141 nm in diameter was 2700 substantially less than published by Briggs *et al.* (3).

References (1) Gross *et al.* (2000). *EMBO J* 19, 103–113. (2) Nermut *et al.*, (2002). *J Virol* 76, 4321–4330. (3) Briggs *et al.* (2004). *Nat Struct Mol Biol* 11, 672–675.

Structure and function of the nidovirus replication complex

Eric Snijder[1], Martijn van Hemert[1], Kevin Knoops[2], Sjoerd van den Worm[1], Jessika Zevenhoven-Dobbe[1], Yvonne van der Meer[1], Alexander Gorbalenya[1], Clara Posthuma[1], Ketil Pedersen[3] & Mieke Mommaas[2]

[1]Dept of Medical Microbiology; [2]Dept of Cell Biology, PO Box 9600, 2300 RC, Leiden, Leiden University Medical Center, The Netherlands; [3]Dept of Biology, University of Oslo, Norway

The replicase polyproteins of Nidoviruses (Coronaviruses/Arteriviruses) are proteolytically processed into 12-16 nonstructural proteins (nsps) that localize to unusual virus-induced double membrane vesicles (DMVs) in the perinuclear region of the cell, which are the site of viral RNA synthesis. The arterivirus EAV, SARS-coronavirus and MHV coronavirus were used to study DMV origin and (ultra)structure, and the composition and activity of the viral replication complex that is associated with it. From studies using modern (immuno-)EM techniques, like freeze-substitution and electron tomography, the endoplasmic reticulum was concluded to be the most likely source of DMVs. EAV and SARS-CoV replication complexes were isolated to analyze their composition and were found to retain RNA synthesis activity in vitro. Using EAV reverse genetics, viral transmembrane nsps were targeted and in particular the tetra-spanning nsp3 was found to be crucial for DMV formation.

Herpesvirus interactions with the secretory pathway in immune evasion

H. Ploegh

Harvard Medical School, Boston, USA

Abstract not received

Viral interactions with the cellular DNA damage machinery

Matthew D. Weitzman

Salk Institute, La Jolla, California, USA

Virus replication presents the host cell with large amounts of exogenous genetic material and unusual DNA structures. Mammalian cells are equipped with complex machinery to monitor and repair damaged DNA, and we have discovered that the DNA repair machinery also recognizes viral genetic material. In the case of adenovirus, the linear double-stranded DNA genome can be joined into long concatemers too large to be packaged by the virus. This is accompanied by accumulation of DNA repair factors into foci at viral replication centers, and activation of DNA damage signaling pathways. Wild-type adenovirus blocks signaling and concatemer formation through targeting the DNA repair complex of Mre11/Rad50/NBS1 for degradation and mislocalization. While adenovirus dismantles the host machinery to evade viral genome processing, other viruses may utilize DNA repair to their own advantage. For example, we have found that herpes simplex virus (HSV-1) infection can activate and exploit a cellular DNA damage response, which aids viral replication in non-neuronal cells. Early in HSV-1 infection, the cellular DNA damage sensing machinery is activated and accumulates at sites of viral DNA replication. Exploring interactions between viruses and the host DNA repair machinery has revealed novel host responses and insights into cellular pathways of DNA repair.

Picornavirus polymerase lattices

K. Kirkegaard

Stanford University, USA

Abstract not received

Plant virus movement proteins

Jamie Ashby[1,2], Vitaly Boyko[1,2], C. Laporte[3], Emmanuel Boutant[3], Anna Groner[2], Monika Fasler[1,2], Mark Seemanpillai[3], Christophe Ritzenthaler[3] & **Manfred Heinlein**[1,2,3]

[1]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; [2]Dept of Plant Physiology, Botanical Institute, University of Basel, Basel, Switzerland; [3]Institut de Biologie Moléculaire des Plantes (IBMP-CNRS), Strasbourg, France (Email manfred.heinlein@ibmp-ulp.u-strasbg.fr)

Studies in higher plants have revealed the existence of proteins and RNA species that travel cell-to-cell and through the vasculature to serve as signaling molecules in plant development and gene silencing, thus confirming the role of plasmodesmata (Pd) in the mediation and control of intercellular and systemic communication via macromolecules. Compelling evidence for macromolecular trafficking through Pd comes from RNA viruses, which encode movement proteins (MP) to interact with Pd and other components of the macromolecular transport machinery in order to spread their genomes from cell-to-cell. The MP of *Tobacco mosaic virus* (TMV) is believed to form a ribonucleoprotein complex (RNP) with viral RNA (vRNA) and to represent the core of the infectious particle that spreads between cells. The MP of *Grapevine fanleaf virus* (GFLV), in contrast, belongs to a class of MPs that form tubules within plasmodesmata to facilitate viral transport in the form of whole virions. Our research is aimed at elucidating the cellular mechanisms involved in the targeting of vRNA, MPs, virions, and other macromolecules, to Pd.

Herpes simplex virus envelopment

Tony Minson

Division of Virology, Dept of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP

Herpesvirus nucleocapsids assemble in the nucleus and bud through the inner nuclear membrane releasing an enveloped particle into the perinuclear space. Several studies have shown that the composition of this particle is distinct from that of the mature virus particle found on the cell surface, and the current view is that the perinuclear particle fuses with the outer nuclear membrane to release a naked nucleocapsid into the cytoplasm. Acquisition of a full complement of tegument proteins and final (secondary) envelopment then occurs in a late cytoplasmic compartment, probably the TGN. While this model fits most of the available evidence it leaves many questions unanswered, notably the mechanism of fusion with the outer nuclear membrane, the order of addition of tegument proteins, the identification of proteins required for anterograde transport, and the mechanism by which multiple envelope proteins are targeted to the envelopment site. Recent evidence shows that both intracellular and mature extracellular particles exhibit a marked compositional heterogeneity, raising the possibility that envelopment and secretion may occur by more than one route.

HIV-1 budding and maturation

Eric O. Freed

Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD 21702-1201, USA

HIV-1 particle production is a multistep process that begins with the transport of the Gag polyprotein precursor to the site of assembly.

We have observed that the localization of HIV-1 assembly is regulated by the phosphoinositide PI(4,5)P_2. Membrane binding and virus assembly are promoted by the association of Gag with cholesterol-rich lipid raft microdomains; virus budding and release are stimulated by interactions between the 'late' domain in the p6 domain of Gag and components of the cellular endosomal sorting machinery, e.g., Tsg101. Following release from the cell, the Gag and GagPol polyprotein precursors are proteolytically processed in a highly ordered fashion by the viral protease. We have shown that depletion of cholesterol from virus-producing cells inhibits virus release, and that the cholesterol-binding compound amphotericin B methyl ester (AME) disrupts both virus production and particle infectivity. Furthermore, blocking the interaction between Gag and Tsg101 potently and specifically inhibits virus release from the cell surface. Finally, we have demonstrated that the betulinic acid derivative PA-457 inhibits a late step in Gag processing, thereby blocking particle maturation and infectivity. These studies demonstrate that HIV-1 assembly/release and Gag processing offer a variety of new targets for antiviral intervention.

HSV infection induces phosphorylation and delocalization of Emerin, a key inner nuclear membrane protein

James Morris, Helmut Hofemeister & Peter O'Hare

Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL

The inner nuclear membrane (INM) is composed of specialized membrane proteins which selectively interact with nuclear components including the lamina. Alterations in INM/lamina organization are likely to be a critical step in herpesvirus exit from the nucleus. Emerin is a member of the LEM domain class of INM proteins shown to bind Lamin, the DNA bridging protein BAF and, more recently, F-actin. We report that Emerin is quantitatively modified during HSV infection. Modification begins early in infection, involves multiple steps and is reversed by phosphatase treatment. Modification is not inhibited by inhibitors of cellular kinases but is dependent upon the US3 viral kinase, a protein whose function is known to be involved in HSV nuclear egress. We show that the detergent-resistant nuclear anchored population of Emerin is depleted in virus infected cells. We propose that Emerin modification by US3, is involved in uncoupling its interactions with targets such as the Lamina and chromatin components. Defects in this pathway may account for defects in US3 –ve mutant egress.

Cell type specific trafficking of measles virus matrix protein

Christine Lehmann[1], Andreas Salditt[1], Paul Duprex[2], Bert Rima[2] & Sibylle Schneider-Schaulies[1]

1Institute for Virology and Immunobiology, University of Würzburg, Germany; 2School of Biomedical Sciences, Queen's University of Belfast

Maturing dendritic cells (DCs), restrict the production of measles virus (MV). This restriction could occur at the level of particle release therefore we comparatively analysed intracellular trafficking of MV proteins in fibroblasts and DCs. We specifically focussed on the MV matrix (M) protein as this is the driving force in the budding of negative-stranded RNA viruses. In fibroblasts the M protein associates with intracellular membranes, LAMP-1 positive compartments, multivesicular body complex components and lipid rafts, and promotes formation of virus-like particles at the plasma membrane. In infected, immature and mature DCs the M protein associates with LAMP1 negative compartments. However, in mature DCs it fails to reach the cell surface. This suggests trafficking of proteins essential for particle morphogenesis is altered in these cells and that a late step of virus production is restricted. Such a restriction may be a general mechanism, which prevents the infection of T cells scanning DCs for antigen.

Regulation of foot-and-mouth disease virus infection by cellular Rab proteins

Helen Johns, Graham J. Belsham & Terry Jackson

Institute for Animal Health, Pirbright, Woking, Surrey GU24 0NF

Foot-and-mouth disease virus (FMDV) is the type species of the genus *Aphthovirus* within the *Picornaviridae*. These viruses contain a single-stranded, positive-sense RNA within an icosahedral capsid formed from 60 copies of four different virus encoded proteins. FMDV infection is initiated by virus binding to a specific integrin receptor at the cell surface. This interaction triggers internalization of the virus-receptor complex via clathrin-dependent endocytosis. Within endosomes, the prevailing low pH triggers capsid disassembly and the concomitant translocation of the viral RNA (vRNA) across the endosomal membrane into the cytosol. However, the precise identity of the endocytic compartment required for infection by FMDV is currently not known.

The endocytic system is a complex network of membrane compartments each fulfilling specific tasks such as ligand- and receptor-sorting, and catabolism. Rab GTPases have been identified as central regulators of endocytosis. Rab proteins are enriched within specific membrane compartments and are required for virtually every membrane trafficking reaction. We have used dominant negative versions of a number of Rab proteins to investigate the early events in FMDV infection of pig kidney cells (IBRS-2). These studies have shown that infection is inhibited by expression of a dominant negative Rab5 (which suppresses fusion between internalized endocytic vesicles and early endosomes) but not by dominant negative versions of Rab4 or Rab11 (which regulate vesicular trafficking through recycling endosomes), or by a dominant negative Rab7 (which inhibits trafficking from early- to late-endosomes). These studies strongly suggest that the early endosomes are the critical endocytic compartment required for FMDV infection.

Ablation of the interaction between the Borna disease virus phosphoprotein and HMGB1 restricts viral replication in interferone-competent cells

Urs Schneider, Marion Pönisch, Daniel Mayer & Martin Schwemmle

Dept of Virology, University of Freiburg, 79104 Freiburg, Germany

The interaction between the Borna disease virus (BDV) phosphoprotein (P) and the high-mobility group box 1 protein (HMGB1) was previously demonstrated. We used the recently established BDV reverse genetics system to determine the function of this interaction in BDV propagation. We were able to define the HMGB1 interaction site on BDV-P by mutational analysis and to ablate the interaction by a single amino acid exchange. The mutant P protein supported efficient reporter gene expression from the BDV minireplicon and rescue of recombinant BDV encoding the mutant P protein (rBDVPmut) was possible. In Vero cells, which lack a functional interferon (IFN) system, wild-type and mutant rBDV's propagated with similar efficiency. However, in co-culture experiments of persistently infected Vero cells and uninfected target cells, rBDVPmut was unable to infect IFN-competent human oligodendroglioma cells. Infection of rat astroglioma C6 cells with rBDVPmut resulted in expression of the IFN-stimulated gene Mx1, which was strongly enhanced by superinfection with the IFN-inducer rift valley fewer virus clone 13 (RVFV13). In contrast, induction of Mx1 expression by RVFV13 was completely suppressed in C6 cells persistently infected with wild type rBDV. Remarkably, ablation of the HMGB1 interaction did not interfere with the inhibition of IFN-beta induction by binding of BDV-P to TBK1. This suggests that persistent BDV infection results also in TBK1-independent induction of the IFN response, which might be suppressed by sequestration of HMGB1.

Virus Group

Virus persistence and latency

Virus persistence in the face of innate and adaptive immune responses (overview)

A.A. Nash

University of Edinburgh

Abstract not received

Modeling HIV latency

Jerome A. Zack

David Geffen School of Medicine at UCLA, USA

One of the main obstacles inhibiting HIV eradication is a stable population of latently infected T cells. Upon withdrawal of antiretroviral therapy, the latent reservoir is able to rekindle infection and renew progression to AIDS. In order to develop strategies to eliminate this reservoir, improved model systems that would allow greater understanding of factors that control latency are needed. We have developed the SCID-hu mouse as a model for HIV pathogenesis in human lymphoid tissues. During human T cell differentiation in this model, immature transcriptionally active thymocytes become mature cells that are transcriptionally quiescent. When these tissues are infected by HIV, this decrease in cellular gene transcription results in the efficient formation of integrated latent virus. More recently, we have developed an in vitro human thymocyte culture model that mimics differentiation of thymocytes in vivo. This system can be adapted to employ HIV-based reporter viruses, which remain transcriptionally silent for greater than a week. These latent reporter viruses can be activated to express viral genes if the cells are activated by co-stimulation one week following infection. Experiments designed to dissect factors controlling HIV latency using these two models will be presented.

Cell death control by cytomegalovirus

E.S. Mocarski

Stanford University, California, USA

Abstract not received

Viral sequence evolution, genome structure and virus persistence

Peter Simmonds[1] & David J. Evans[2]

[1]Centre for Infectious Diseases, University of Edinburgh, Summerhall, Edinburgh EH9 1QH; [2]Division of Virology, Faculty of Biomedical and Life Sciences, University of Glasgow, Church Street, Glasgow G11 5JR

The ability of many RNA viruses to persist in their hosts is essential for their survival and ongoing transmission, although mechanistically the phenomenon generally remains poorly understood, and is clearly multi-factorial. Through new developments in bioinformatic analysis, we have discovered that naturally persistent and non-persistent viruses differ fundamentally in their genome configurations, with highly evolutionarily conserved, extensive RNA secondary structure (we have called genome-scale ordered RNA structure [GORS]) detectable in many genera and families of positive-strand animal and plant RNA viruses.

There is remarkable variability between genera that possess this characteristic; for example hepatitis C virus (HCV) in the *Flaviviridae* shows evidence for extensive, evolutionarily conserved internal base-pairing throughout the genome that was absent in both pestivirus and flavivirus genera within this virus family. Similar genus-associated variability was observed in the *Picornaviridae*, the *Caliciviridae* and many plant virus families. We have developed of automated methods for very large scale analysis of conserved base-pairings predicted by MFOLD, it has been possible to characterize the nature of the RNA structure found in viruses with GORS. RNA structures comprise tandemly repeated short stem-loops (generally less than 100 bases) throughout the viral genome, and with duplex lengths almost invariably limited to a maximum of 10 consecutively paired bases (median 5), systematically distinct from the configuration of cellular RNA structures, such as formed in ribosomes.

The association of GORS with persistence raises the intriguing possibility of an interaction between RNA structures and cellular components, such as dsRNA recognition pathways that modulate activation of innate intracellular defence mechanisms, and secondarily, the acquired immune system in vertebrates. To obtain a better functional understanding of the role of GORS in persistence, ongoing work is aimed at characterizing the cellular components and nature of the interaction with structured RNA viral genomes during infection of a cell.

The biology of Epstein–Barr virus: B cell interactions

Alan Rickinson, Claire Shannon-Lowe, Rose Tierney, Gemma Kelly & Andy Bell

CRUK Institute for Cancer Studies, University of Birmingham

Epstein-Barr virus (EBV) is a human gamma-herpesvirus that needs to access two cell types *in vivo*, oropharyngeal epithelium in which infections are predominantly lytic and the B lymphoid system in which infections are predominantly latent and where the virus persists. Recent work addresses three issues:-

(i) *how does a virus that binds preferentially to B cells through the complement receptor CR2 achieve efficient entry to epithelium?*

We show that only a minority of surface-bound virions enter B cells, the rest are sequestered into a cap on the B cell surface in a complex with CR2 and its associated proteins. Surface-capped virions are then capable of rapid transfer into epithelium through a lineage-specific inter-cellular synapse. We infer that, by exploiting this (presumably physiologic) B cell-epithelial interaction, orally-transmitted EBV can gain simultaneous access to both its B cell and epithelial reservoirs *in vivo*.

(ii) *if EBV can transfer efficiently into non-B cell types, why are growth transforming infections restricted to B cells?*

EBV's growth-transforming programme is initiated through sequential activation of two adjacent promoters, Wp and Cp, that drive expression of the six nuclear antigens (EBNAs 1,2,3A,3B,3C,LP) required for transformation. Regulatory sequences governing Wp/Cp activity were found to contain multiple binding motifs for the B cell–specific activator protein Pax5. We show that mutating these sites in the context of a recombinant EBV genome leaves infectivity for epithelium intact but completely abrogates B cell transformation. Thus EBV appears to exploit Pax5 to achieve the B cell-specificity of its transforming function.

(iii) *how do individual EBNAs influence B cell behaviour?*

EBV-positive Burkitt's lymphoma (BL) exhibits alternative forms of virus latency, more limited than the full transforming programme,

that may mirror physiologic virus:B cell interactions. We have recently identified endemic BLs presenting with three different profiles of restricted antigen expression (EBNA1 only, EBNAs 1,3A,3B,3C,LP and EBNAs1,2,3A,3B,3C,LP). Work on isogenic clones from one such tumour shows how the EBNA profile influences both cellular differentiation status and apoptosis sensitivity of the infected cells. This may reflect viral functions that are important not just for BL pathogenesis but also for EBV's normal strategy of persistence in the B cell system.

Role of the hepatitis C virus non-structural proteins in viral persistence

Mark Harris

Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT

The WHO estimate that 123 million individuals are infected with hepatitis C virus (HCV). The virus establishes a persistent infection in >80 % of patients, leading to the development of chronic liver disease – fibrosis, cirrhosis and ultimately hepatocellular carcinoma. The mechanisms by which the virus establishes and maintains a persistent infection remain to be defined, however recent evidence points to critical roles of the non-structural proteins, particularly the NS3 and NS5A proteins, in this process. NS3 has been shown to block the innate immune response via both TLRs and intracellular dsRNA detectors (eg RIG-I). NS5A interacts with a number of cellular proteins to perturb MAP kinase and phosphatidylinositol 3-kinase signalling pathways, and has also been reported to block the interferon induced kinase, PKR. I will review the latest developments in these areas, putting them in the context of the recent development of a tissue culture infectious system for the study of HCV biology. This is an important advance that will at last allow us to analyse effects of HCV on cellular physiology in the context of the complete viral replication cycle.

Evasion of the interferon response in the persistence of pestiviruses

John W. McCauley[1], Gang Zhang[1], Munir Iqbal[1], Michelle Hill[1], Louise Hilton[2], Kartykayan Moganeradj[2] & Steve Goodbourn[2]

[1]Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN; [2]Division of Basic Medical Sciences, St. George's, University of London, London SW17 0RE

The ability of pestiviruses to sustain a persistent infection in the absence of an immune response is unusual. The virus initiates its persistence by infection of the developing foetus prior to immune competence. For Bovine viral diarrhoea virus (BVDV) infection of a pregnant cow in the first trimester of pregnancy by the non-cytopathogenic form of BVDV can result in a persistently infected viraemic calf which will remain viraemic for its lifetime. It is this persistently infected calf that may succumb to super-infection by a cytopathogenic form of the virus and develop mucosal disease, which has an invariably fatal outcome.

Although infection of the foetus prior to immune competence will result in immune tolerance to the virus, the innate immune response would be expected to recognize the virus and resolve the infection. However, infection of the foetus with non-cytopathogenic BVDV fails to result in the production of type I-IFN, the main mediator of innate anti-viral immunity. The evasion of interferon induction can also be observed in tissue culture in which interferon induction in BVDV infected cells by varied stimuli are blocked. The mechanisms that this RNA virus uses to block and evade the induction of an interferon response in infected cells and achieve the persistent infection will be discussed. Why the virus might have evolved such an unusual life-style will also be discussed.

The molecular basis of herpes simplex virus latency and reactivation

Stacey Efstathiou

Division of Virology, Dept of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP

Following the establishment of herpes simplex virus (HSV-1) latency, virus DNA is retained in a transcriptionally repressed state with the exception of the region encoding the latency associated transcripts (LATs). Understanding the mechanisms responsible for the repression of lytic cycle gene expression during latency and the processes underlying the resumption of gene expression to facilitate reactivation are central questions, which underpin the virus survival strategy. During latency the viral genome exists in an endless state consistent with circularization and adopts a nucleosomal organization. Studies using chromatin immunoprecipitation assays have shown that the HSV latency associated promoter is enriched with acetylated Histone H3 whilst representative immediate early promoters show a decreased association with this modified histone during latency. These data support the view that histone modifications play a key role in the regulation of virus gene expression during latency. Our current studies are focused on evaluating histone modifications associated with a variety of HSV promoters following the induction of reactivation and examining the role of the virus encoded, immediate early, ICP0 gene product in genome de-repression.

Interferon as a modulator of paramyxovirus persistence

R.E. Randall

School of Biology, University of St Andrews, Fife

As well as causing acute infections many viruses, including paramyxoviruses, are able to establish prolonged/persistent infections *in vivo* that may, or may not, lead to chronic disease. However, the molecular basis and host cell factors which influence the establishment of persistent infections for RNA viruses are very poorly understood. One factor that will influence the ability of a virus to establish and maintain a persistent infection is how the virus interacts with the interferon system. Viruses that inhibit cellular processes in a gross way, for example by blocking host cell transcription and/or translation, will kill infected cells. In contrast viruses, like SV5, which specifically block the interferon response may do so without necessarily inducing cell death, thus potentially allowing such virus to establish persistent infections. Furthermore, if a virus establishes a persistent infection whilst interfering with important cellular functions, such as the IFN response, the resulting infection may lead to chronic disease. Although the role of paramyxoviruses in chronic human disease often remains highly controversial, a greater understanding of the interplay between these viruses and the IFN response, as well as the role of IFN in the control of normal cellular function, may lead to more rational approachs in studying their possible involvement in chronic disease.

Special Lectures

Marjory Stephenson Prize Lecture

Invasion by influenza viruses

Sir John Skehel

MRC National Institute for Medical Research, London

John Skehel graduated in Agricultural Biochemistry in Aberystwyth in 1962 and received his PhD in Biochemistry in Manchester in 1965. He began his research in virology with Derek Burke in Aberdeen in 1966 on the induction of interferon by viruses, and then as a Helen Hay Whitney Foundation Fellow worked with Bill Joklik at Duke on reovirus transcription and with Helio Pereira, Geoffrey Schild and Willie Russell in NIMR, Mill Hill on influenza and adenoviruses. He has remained at Mill Hill becoming Head of Virology in 1985, Head of Infections and Immunity in 1985 and Director since 1987. His research has concerned influenza structure and replication and since 1975 mainly the haemagglutinin membrane glcyoprotein with the objective of understanding its roles in receptor binding and membrane fusion and its antigenicity.

Fleming Lecture

Constructing the wonders of the bacterial world: biosynthesis of complex enzymes

Dr Frank Sargent

University of East Anglia

Having completed a degree in Biochemistry at the University of Edinburgh in 1992 Frank Sargent was fortunate to be offered a place in David Boxer's group at the University of Dundee studying hydrogen and nickel metabolism in *Escherichia coli*. His PhD was awarded in 1996 and he took a postdoctoral position studying *E. coli* protein export systems with Tracy Palmer at the John Innes Centre, Norwich. A second postdoc analysing the *E. coli* twin-arginine protein translocase followed in 1998 with Ben Berks (then at the University of East Anglia) before he won a University Research Fellowship from The Royal Society in 2000. Dr Sargent currently runs a research team of 7 people which studies, still primarily using *E. coli* as the model system, various physiological and biochemical aspects of the biosynthesis of complex multi-subunit respiratory enzymes. It is essential that the assembly of multi-subunit, multi-cofactor enzymes is tightly coordinated in the cell. In many cases these enzymes must also be integrated into, or transported across, the cytoplasmic membrane. The coordination process ('proofreading') is mediated by small multi-functional chaperones that are widespread in bacteria.

Posters

Cells & Cell Surfaces Group

CCS 01 Investigating the molecular basis of cold temperature and pressure-adapted growth using *Photobacterium profundum* as a model system

D. Allcock[1], F. Lauro[2], D. Bartlett[2], L. Sawyer[1] & G.P. Ferguson[1]

[1]Institute of Structural and Molecular Biology, School of Biological Sciences, University of Edinburgh, King's Buildings, Edinburgh EH9 3JR; [2]Marine Biology Research Division, 0202, Scripps Institution of Oceanography, University of California, San Diego, USA

Photobacterium profundum is a 'pressure loving' (piezophilic) γ-proteobacterium which grows optimally at 28MPa (15ºC). Its ability to grow over a range of pressures (0.1-70MPa) and temperatures (2-20ºC) and its genetic tractability has made it an ideal organism to understand the molecular basis of pressure-adapted growth. Two random mini-Tn*5* mutants were selected by screening for growth defects at cold temperature (4°C); the mutants Q74-34 and Q39-83 have Tn*5* insertions in genes encoding a putative o-antigen ligase and a tyrosine protein kinase, respectively. We have found that Q74-34 has a reduced smooth LPS profile by SDS and DOC-PAGE silver staining, and Q39-83 has a reduced capsular polysaccharide, demonstrated by transmission electron microscopy. We are also purifying the *P. profundum* D-Lactate dehydrogenase (Ldh) in order to investigate whether piezophile proteins contain novel modifications. D-Ldh has been well studied in other bacteria and the activity can be measured by a simple colorimetric assay. This work will improve our understanding of the nature of piezophily and the role that surface polysaccharides play in this adaptation.

CCS 02 Isolating novel exopolysaccharides and cell envelope components from marine and deep-sea bacteria

Ziad El-Hajj[1], Theodora Tryfona[1], Federico Lauro[2], Douglas Bartlett[2] & Gail P. Ferguson[1]

[1]Institute of Structural and Molecular Biology, School of Biological Sciences, University of Edinburgh, King's Buildings, Edinburgh EH9 3JR; [2]Marine Biology Research Division, 0202, Scripps Institution of Oceanography, University of California, San Diego, USA

The deep sea is characterized by an average temperature of 3 °C and an average pressure of 38MPa, which can reach 110MPa. High pressure disrupts a variety of essential cellular processes, but pressure-loving bacteria, or piezophiles, have adapted to grow optimally at high pressure. The majority of marine bacteria that may possess novel and unique genes of interest, including piezophiles, are not culturable under laboratory conditions. Constructing a metagenomic library makes all genomes in the environmental sample directly available for genetic screening. We have constructed a fosmid library from seawater bacterial DNA, and are screening this library for resistance to detergents and production of Calcofluor-binding exopolysaccharides. Additionally, piezophiles can be studied by using *Photobacterium profundum* SS9, which grows optimally at 28MPa and 15 °C but can grow over a range of temperatures (2-20 °C) and pressures (0.1-70MPa). The ability of SS9 to grow under laboratory conditions makes it an ideal model system to study the molecular basis of pressure-adapted growth. We have found that SS9 produces Calcofluor-binding exopolysaccharides, and are characterizing a pressure and cold-sensitive mutant in a putative isomerase involved in polysaccharide biosynthesis.

CCS 03 Exopolysaccharides and membrane vesicles in biofilm formation and maintenance

Gary Dorken[1], Victoria L. Marlow[1], Wilson K. Poon[2], Stuart A. West[1] & Gail P. Ferguson[1]

[1]School of Biological Sciences, University of Edinburgh, EH9 3JR; [2]School of Physics, University of Edinburgh, EH9 3JZ

Biofilms are aggregates of bacteria attached to a surface, often encased in an extracellular matrix. Despite recent research interest, little is understood about the molecular and biochemical mechanisms underlying biofilm biology. The aims of this project are to look at the formation and biology of biofilms in two novel species (i) α-proteobacteria *Sinorhizobium meliloti*, a symbiotic bacterium that can reside within leguminous plant hosts but can also be free-living in the soil and (ii) pressure-loving marine bacterium *Photobacterium profundum* SS9. We have exopolysaccharide mutants in both of these species and we are now hoping to investigate how this may affect biofilm formation and maintenance relative to respective parent strains. We have shown that *S. meliloti* can form biofilms on abiotic surfaces at the air-liquid interface and we are currently characterizing membrane vesicles from the parent strain and defined mutants to investigate their role in biofilm formation and maintenance. We are also developing methods using confocal microscopy to image the structure of biofilms and gain a 3D view of the biofilm world.

CCS 04 Role of the BacA protein and lipid A very-long-chain fatty acids in the *Sinorhizobium meliloti*-legume symbiosis

Victoria L. Marlow[1], Carole Rougier[1], Sebastian Beck[1], Graham C. Walker[2] & Gail P. Ferguson[1]

[1]Institute of Structural and Molecular Biology, University of Edinburgh, EH9 3JR; [2]Dept of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

Sinorhizobium meliloti, a legume symbiont, requires the inner membrane BacA protein for persistence within plant cells. Although the precise function of BacA is unknown, *S. meliloti bacA* mutants display an array of phenotypes including low-level resistance to the glycopeptide bleomycin and an 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 BacA may result in further cell envelope alterations and that 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. Our finding that the sensitivity of the *bacA* mutant to crystal violet appears to be independent of the altered lipid A provides evidence for additional cell envelope changes such as a peptidoglycan alteration. We recently discovered that the bleomycin resistance phenotype of the *bacA* mutant is independent of the unusual lipid A. Using a *recA* mutant we have shown bleomycin is able to enter *S. meliloti*, even in the absence of BacA, suggesting a BacA-independent mechanism of uptake, which is blocked by the polyamine spermine. We present a model illustrating potential functions of the BacA protein.

CCS 05 Structure-function analysis of the unusual extended signal peptide of the autotransporter Pet

Mickaël Desvaux, Anthony Scott-Tucker, Sue M. Turner, Nina A. Filenko, **Lisa M. Cooper**, Jeffrey A. Cole & Ian R. Henderson

University of Birmingham, Edgbaston, Birmingham B15 2TT

Plasmid encoded toxin (Pet), a prototypical member of the serine protease autotransporters of the *Enterobacteriaceae* family, mediates enterotoxic and cytotoxic activities of Enteroaggregative *Escherichia coli* strain 042. In addition to the typical autotransporter passenger and β-domain, Pet possesses an extended N-terminal signal sequence; comprised of five regions termed N1, H1, N2, H2 and C domains. N1 and H1 appear conserved with other extended signal peptides. N2, H2 and C show sequence variability typical of Sec-dependent signal peptides. Here we demonstrate the extended signal peptide sequence (ESPR) is present only in proteins secreted via the Type V secretion pathway in the β- and γ- classes of *Proteobacteria*. *In vitro* approaches demonstrate that the DNA region encoding the ESPR is transcribed and translated. The ability of the extended Pet signal peptide to mediate translocation across the inner membrane was assayed using an alkaline phosphatase reporter system which revealed that translocation efficiency was severely impaired by the ESPR.

CCS 06 Topology modelling of the Pet autotransporter translocator domain

A. Scott-Tucker & I.R. Henderson

Medical School, University of Birmingham

Autotransporters are arguably the simplest of the five major recognized Gram-negative bacterial secretion systems, whereby all the information necessary for the secretion of the effector protein is encoded within a single transcript. Their conserved domain architecture consists of an N-terminal signal sequence, which directs export across the inner membrane, the effector domain, which is translocated through the C-terminal translocator domain. This translocation event is the subject of much controversy. Homology modelling showed that the C-terminal 299 amino acids of the Enteroaggregative *Escherichia coli* Plasmid encoded toxin (Pet) autotransporter has a high degree of similarity to the known structure of NalP, despite a lack of sequence identity. The effects of random and directed peptide linker insertions in-frame within the C-terminal domain were used to test the model. Insertion sites of secretion competent and incompetent clones were identified on the model, which then helped to discern the topology of the β-barrel. This work provides further evidence for a monomeric translocator domain and the hairpin model of translocation, and also provides a framework for research into translocator domain structure and function.

CCS 07 *Streptomyces coelicolor*: glycosylation at the cell surface

Anpu Varghese & Maggie C.M. Smith

University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD

Streptomyces coelicolor is a medically important organism, producing two-thirds of the world's antibiotics. The nature of the *Streptomyces* cell wall is poorly understood. Two genes whose products are predicted to be required for protein glycosylation have been found to be essential for phage infection. We therefore propose that *S. coelicolor* contains within its cell wall a glycoprotein that can act as a phage receptor. The pathway is predicted to be similar to the O-glycosylation pathway in yeasts, humans and other Actinomycetes, in particular *Mycobacterium tuberculosis*. Polyprenol phosphate mannose synthase (*ppm1*) synthesizes polyprenol phosphate mannose, which donates mannose to secreted proteins by protein mannosyl transferase (*pmt)*. The phage may then interact with the glycan moiety on the glycoprotein. The *ppm1* and *pmt* mutants have a small colony phenotype in addition to resistance to phage phiC31. Complemented mutants behave like the wildtype, producing large colonies and showing phage sensitivity.Growth morphology was studied by both light and electron microscopy but no major differences were observed between the wildtype, the mutants and the complemented mutants. A reproducible difference was observed between one of the *ppm1* mutants, DT3017, and the wildtype; DT3017 hyphae were more likely to accumulate propidium iodide, a stain that is normally pumped out of the cell in actively respiring cells. Further tests with antibiotic sensitivity indicated that all the mutants were more sensitive than the wildtype to the antibiotic monensin, although this sensitivity could not be restored in the complemented mutants. Phenotypic characterization of the glycosylation mutants continues along side the generation and study of knock out mutants in other glycosyl transferases.

CCS 08 Characterization of novel fibrillar surface appendages of the opportunistic pathogen *Staphylococcus epidermidis*

Miriam A. Banner[1], Emmy Hoyes[2], Mathew Upton[3] & Pauline S. Handley[1]

[1]Faculty of Life Sciences, 1.800 Stopford Building, University of Manchester, Oxford Road, M13 9PT, [2]NeuTec Pharma PLC; [3]Division of Laboratory and Regenerative Medicine, Clinical Sciences Building 1, Manchester Royal Infirmary, Manchester M13 9WL

Staphylococcus epidermidis is an opportunistic pathogen responsible for a high proportion of catheter-related infections. The main virulence factor of this organism is its ability to adhere to surfaces and form biofilms. We have detected fibrillar appendages on a sub-population of cells in *S. epidermidis* NCTC 11047. Fibrillar (Fib+) and non-fibrillar (Fib-) subpopulations were enriched by 34 sequential partitions of wild type (WT) cells between a buffer phase and a hexadecane phase. Cell surface protein analysis of the sub-populations identified two polypeptides (280 & 230 kDa) present on Fib+ cells and absent from Fib- cells which were identified as putative fibril proteins. The polypeptides were sequenced and both found to be the accumulation associated protein (Aap), a polypeptide involved with both adhesion and biofilm formation of *S. epidermidis*. Therefore, it is suggested that the fibrillar appendages of *S. epidermidis* NCTC 11047 are associated with the presence of Aap and may play an important role in both adhesion and biofilm formation of this organism.

CCS 09 Characterization of the effects of mutations within the lipoprotein processing enzymes of *Streptococcus uberis*

Emma Denham[1], Phil Ward[1], Lucas Bowler[2], Andrew Gill[1], Tracey Coffey[1], Peter Andrew[3] & Jamie Leigh[1]

[1]Institute for Animal Health, Compton, Berkshire, RG20 7NN; [2]University of Sussex, Falmer, Brighton BN1 9RY; [3]University of Leicester, University Road, Leicester LE1 7RH

Streptococcus uberis is a common cause of bovine mastitis worldwide. The *S. uberis* genome contains over 400 proteins containing signal peptide sequences, of which 31 contain classical lipoprotein processing signals.

Biosynthesis of the bacterial lipoproteins occurs via a highly conserved pathway unique to prokaryotes. Lipoprotein diacylglyceryl transferase (Lgt) and type II Signal peptidase (SPase II) are responsible for the anchoring of lipoproteins to the membrane and subsequent cleavage of the signal peptide. Mutants of *S. uberis* lacking Lgt and SPase II have been characterized.

The *lgt* mutant is unable to anchor lipoproteins correctly to the membrane. Western blot, ELISA and N-terminal sequencing showed the lipoprotein MtuA to be released into the extracellular space and cleaved atypically within the signal peptide. The form of MtuA in the *SPase II* mutant is approximately 2kDa larger than the processed form that appears in the wild type strain.

When analysed on SDS PAGE the extracellular profiles produced by the wild type strain, *lgt* and *SPase II* mutants vary greatly; these differences are being resolved by proteomics to help define the role of lipoprotein processing in the pathogenesis of the bacterium.

CCS 10 ORF15, a cell wall protein of *Clostridium difficile*

Jenny Emerson, Anne Wright & Neil Fairweather

Centre for Molecular Microbiology and Infection, Division of Cellular and Molecular Biology, Imperial College London, London SW7 2AZ

Clostridium difficile is an emerging nosocomial threat, and incidence in hospitals is increasing, both in frequency and severity. The surface of the bacterium is covered in a paracrystalline array of two surface layer proteins derived from a single gene, but there are also other proteins present on the cell surface layer. ORF15 is a gene with homology to one of the surface layer proteins and also contains nine 13kDa almost perfect repeats. We have shown that the nucleotide sequence of the gene is highly conserved between strains, where present, although the number of repeat domains varies from four to nine. ORF15 is transcribed and expressed throughout growth, as demonstrated by reverse-transcription PCR and Western immunoblotting of total cell lysates. The presence of the protein at the cell surface has been shown by subcellular fractionation and immunoblotting; immunostaining and FACS (fluorescence activated cell scanner) analysis; immunogold transmission electron microscopy; and proteomic analysis of the cell wall. Although implicated by the observed repetitive structure, no adhesion or haemagglutination properties have yet been found and the function of this protein is still to be elucidated.

CCS 11 Inhibition of infection of short tailed Stx-phage through changes at the *Escherichia coli* cell surface

D.L. Smith, J.R. Saunders, H.E. Allison & A.J. McCarthy

School of Biological Sciences, University of Liverpool

Shigatoxigenic *Escherichia coli* (STEC) *e.g.* O157:H7 are a global health concern and infection is characterized by severe bloody diarrhoea and can lead to downstream sequalae such as haemolytic ureamic syndrome (HUS). The major virulence determinant in STEC is the Stx toxin, which is encoded by a bacteriophage (Stx-phage). We have identified an outer membrane protein, Vpr, which is utilized as the ligand for infection by a short tailed Stx-phage ($\Phi 24_B$) originally induced from a clinical strain of *E.coli* 0157. The *vpr* gene (also annotated as *yaeT*) is conserved throughout the *Enterobacteriaceae*, and is now known to be involved in the biogenesis of the outer membrane. We have used the selective pressure of $\Phi 24_B$ infection to isolate spontaneous bacteriophage-resistant *E. coli K12* mutants, reasoning that if the essential gene undergoes spontaneous mutation it will produce functional Vpr that exhibits epitopal changes such that phage binding is obviated. The mutants generated in this way were all characterized by a heavy mucoid colony morphology suggesting overproduction of polysaccharide. When *vpr* was re-introduced into the mutants, the colony morphology reverted to that of wild-type *E. coli* K12, but this was unstable. Spontaneous phage resistant mutants in native *E. coli* populations will impact on the epidemiology of Stx phage, but their generation in the laboratory also provides a potential tool for studying cell envelope biogenesis.

CCS 12 The biosynthesis and export of *Escherichia coli* group 2 capsular polysaccharides involves a multi-protein complex at the poles of the cell

James Thompson, Clodagh McNulty, Liz Lord & **Ian S. Roberts**

Faculty of Life Sciences, University of Manchester, Manchester M13 9PT

The export of large negatively charged capsular polysaccharides across the outer membrane represents a significant challenge to Gram-negative bacteria. In the case of *Escherichia coli* group 2 capsular polysaccharides, the mechanism of export across the outer-membrane was unknown, with no identified candidate outer-membrane proteins. Here we demonstrate that the KpsD protein, previously believed to be a periplasmic protein, is an outer-membrane protein involved in the export of group 2 capsular polysaccharides across the outer-membrane. Also we demonstrate that KpsD and KpsE, an inner membrane polysaccharide export protein, are both located at the poles of the cell and that polysaccharide biosynthesis and export occurs at these polar sites. By *in vivo* chemical cross linking and MALDI-TOF-MS analysis we demonstrate the presence of a multi-protein biosynthetic/export complex in which cytoplasmic proteins involved in polysaccharide biosynthesis could be cross-linked to proteins involved in export across the inner and outer membranes. In addition, we show that the RhsA protein, previously of unknown function, could be cross-linked to the complex and that a *rhsA* mutation destabilizes the polar location of the polysaccharide biosynthetic/export complex reducing K5 biosynthesis. This suggests a role for RhsA in coupling biosynthesis and export.

Posters

Clinical Microbiology Group

CM 01 16S rDNA PCR and sequencing for the identification of culturable isolates in the diagnostic clinical microbiology laboratory

L. Berry, S. Warwick, B. Duke, M. Wilks & M. Millar

Barts and the London NHS Trust, Dept of Medical Microbiology, St Bartholomew's Hospital, London EC1A 7BE

A two month prospective audit carried out in a large London Teaching Hospital laboratory revealed that the overall success rate for API kits was 88 %, with 62.5 % of isolates being attributed to species level and 25.5 % to genus level. API strips failed to give an acceptable result in 12 % of cases, even after repetition. API-Strep and API-20NE kits had the highest failure rates with 23.6 % and 23 % respectively. Clinically relevant isolates that were not initially identified by the API system were analysed by 16S rDNA PCR and sequencing. Of these, 93.6 % were identified with 80.8 % designated to species level and 12.8 % to genus level. Analysis of 16S rDNA sequence was able to identify species such as *Helcococcus kunzii*, *Brevibacillus agri* and *Paenibacillus lautus* which are not recognized by API kits. The API system is not designed to identify more uncommon isolates and so is unlikely to capture rare, unusual or emerging pathogens, this may result in failure to recognize possible epidemiological links. Sequence based identification is an important adjunct to conventional techniques which will result in improved diagnosis and treatment of patients, and detection of emerging and unusual pathogens.

CM 02 Real-time PCR assay for the detection of *Mycoplasma amphoriforme*, a potential human pathogen

C.L. Ling[1], S.H. Gillespie[2], D. Webster[1] & T.D. McHugh[2]

[1]Royal Free Hospital NHS Trust, Pond Street, NW3 2QG; [2]Royal Free and University College, Medical School, Rowland Hill, London NW3 2PF

Mycoplasma amphoriforme has been detected in both immunocompromised and immunocompetent patients with respiratory tract symptoms, but not in healthy controls. As it is a fastidious *Mycoplasma* culture can be difficult and therefore molecular based methods for its detection are important. Targeting a gene identified by our group, we have successfully designed and evaluated a RT-PCR assay for the detection and identification of *M. amphoriforme* from respiratory samples. This assay is more specific than an existing 16S rRNA PCR and is equally sensitive (10 organisms per reaction). The assay was found to be 100 times more sensitive when performed using Invitrogen Platinum® QPCR SuperMix-UDG compared with the ABgene Absolute™ QPCR mix. The RT-PCR assay is currently being converted into a quantitative assay and should prove to be invaluable in determining the true significance of *M. amphoriforme* in respiratory disease.

CM 03 Analysis of DNA repair responses of *Mycobacterium tuberculosis* exposed to sub-lethal doses of quinolone

Denise O' Sullivan[1], Timothy D. McHugh[1], Philip D. Butcher[2] & Stephen H. Gillespie[1]

[1]Centre for Medical Microbiology, Dept of Infection, Royal Free & University College London Medical School, Rowland Hill Street, London NW3 2PF; [2]Medical Microbiology, St George's Hospital Medical School, Cranmer Terrace, London SW17 ORE

Quinolones induce the SOS response with maximal induction occurring at about ten times the minimum inhibitory concentration (MIC). The correlation between SOS error prone repair and mutagenic effects indicate that quinolone-induced mutagenic effects in bacteria are almost entirely due to SOS-processed DNA damage. We have previously shown that exposure to sub-inhibitory concentrations of quinolones can increase the mutation rate of mycobacteria by up to 120 fold. Therefore quinolone therapy could lead to an increase in the development of antibiotic resistance. Genome wide expression profiling was applied to sub-inhibitory ciprofloxacin treatment of *M. tuberculosis* to investigate the DNA repair mechanisms involved in protecting the bacilli. Whole genome arrays were provided by the BuG@S microarray group to be used in the study. The biggest change in gene expression was observed after 4hr. Genes involved in the SOS response (*recA*), base excision repair (*xthA, tagA*) and in the removal of nucleotides (*dut*) were induced. General defensive pathways for *M. tuberculosis* under stress reported in other studies were also observed in this study.

CM 04 Analysis of mouse immune response to vaccination with pneumococcal and meningococcal conjugate vaccines using an adapted multiplex assay

Claire L. Mattick, Ian M. Feavers & Rory S. Care

Divisions of Bacteriology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG

The development of new paediatric vaccines and the need to rationalize immunization programmes to maximize vaccine uptake has resulted in an increasing number of vaccine components being either combined or co-administered. The effects on the immune response caused by increasing the number and combination of antigens delivered simultaneously during vaccination are complex. Animal models provide one way of identifying and characterizing interactions between individual vaccine components affecting the immune response. Serum IgG levels specific for *Streptococcus pneumoniae* are currently quantified using a separate ELISA for each capsule serotype-specific antibody. As a result the evaluation of immunological response to multi valent pneumococcal capsular polysaccharide-based vaccines can be time-consuming and requires large volumes of serum. Lal *et al.* have recently describe a method for the simultaneous quantification of IgG to nine pneumococcal serotypes in which specific polysaccharides were covalently attached to fluorescent microspheres and pooled beads were used to assay human serum. Adaptation of this method for use with mouse serum has given similar levels of specificity, sensitivity and variability when compared with the human assay and the results correlated with ELISA data. This assay has been successfully employed for the rapid analysis of immune serum of mice concomitantly vaccinated with combinations of different pneumococcal and meningococcal conjugate vaccines.

CM 05 Development of assays for the evaluation of protein-based meningococcal vaccines

H.E.W. Chan[1], J. Findlow[2], K.J. Clow[1], C.L. Mattick[1], M.C.J. Maiden[3], R. Borrow[2] & I.M. Feavers[1]

[1]National Institute for Biological Standards and Control, South Mimms, Potters Bar EN6 3QG; [2]Health Protection Agency North West, Manchester Royal Infirmary, Mancheste M13 9WZ; [3]Peter Medawar Building for Pathogen Research and Dept of Zoology, University of Oxford, Oxford OX1 3SY

Vaccines based on capsular polysaccharide are in use to prevent meningococcal disease caused by serogroups A, C, W135 and Y. As a result of the poor immunogenicity of group B capsular polysaccharide and its similarity to glycoslylated neuronal cell adhesion molecules, it is unlikely to be used as a vaccine component. Protein-based vaccines (either recombinant or proteins in outer membrane vesicles) have the potential to provide comprehensive protection against meningococcal disease. To aid the evaluation of vaccines that contain mixtures of several components, molecular and genetic tools are being developed to be used in serological assays. Isogenic strain panels of mutants based on six isolates of *Neisseria meningitidis* have been made by systematically mutating one of five key antigen genes (*porA*, *porB*, *opcA*, *nspA* and *fetA*) in each isolate. The strain panel based on H44/76 has been used in whole-cell ELISAs and serum bactericidal antibody (SBA) assays and may be used to assess the contribution of these antigenic components in immune sera. In addition, antigenic variants of the immunogenic PorA and FetA proteins have been expressed in, and purified from *Escherichia coli*. The use of these proteins with xMap Technology will facilitate the evaluation of the immune response to different antigenic variants.

CM 06 Evaluation of FTA paper™ for the rapid extraction of bacterial DNA for use in PCR

Abeer A.A. Barhameen & Mathew Upton

Division of Laboratory and Regenerative Medicine, Faculty of Medical and Human Sciences, University of Manchester, Oxford Road, Manchester M13 9WL

Many recent advances in methods for detection of bacterial pathogens include PCR of target DNA sequences. The rate limiting step in such approaches is often extraction of DNA.

FTA paper™ was developed for DNA storage and is widely used in forensic science. The paper cards stabilize DNA contained in samples. Following a simple wash procedure, DNA can be stored at room temperature for prolonged periods.

We have assessed the utility of FTA paper™ cards for extraction of DNA from cultures of eleven different species of pathogenic bacteria. DNA was suitable for amplification by PCR and the approach killed all organisms that were present. This has significant implications for making safe samples that contain highly pathogenic organisms. The sensitivity of PCR was as low as 1 x 10^3 but some organisms may require additional lysis steps prior to application to the cards. We suggest that the cards represent a significant advance in DNA extraction and storage methods for PCR detection of pathogenic bacteria.

CM 07 Uropathogenic ESBL producing *Escherichia coli* in North West England are clonal, but differ in their resistance genotype

S.H. Lau[1], S.B. Mannan[1], M. Armstrong[2], F.J. Bolton[3], J. Cheesbrough[4], A. Fox[3], S. Reddy[5], G. Smith[4], C. Thornhill[6] & M. Upton[1]

[1]University of Manchester; [2]Manchester Royal Infirmary; [3]HPA North West; [4]Centre for Infections; [5]Preston Royal Hospital; [6]Wythenshawe Hospital, Manchester

Escherichia coli isolates with extended-spectrum beta lactamase (ESBL) phenotypes are increasingly causing infections. We have identified a clone (ST 131) of ESBL *E. coli* by Multilocus Sequence Typing (MLST) and shown that it is widely distributed in NW England and is strongly associated with urinary tract infections.

Antibiotic resistant genotypes were determined for 24 isolates from Central and South Manchester and the Preston District. There was notable variation in the carriage of target genes and no correlation between MLST clonal lineage and carriage of specific resistance determinants.

Clearly, a particularly problematic clone of uropathogenic, ESBL *E. coli* with CTX-M, TEM and AmpC determinants has become widely disseminated in NW England. The acquisition of resistance determinants by a clone that is well adapted to cause urinary tract infection, is particularly worrying. We propose that MLST and resistance genotyping should be used for surveillance of ESBL *E. coli*.

CM 08 Identification of OprF from *Pseudomonas aeruginosa* as the major antigen against human patient sera

Zhiyi Zhu, Peter Williamson, Emmy Hoyes & James P. Burnie

Dept of Medical Microbiology, University of Manchester, Manchester Royal Infirmary, 2nd Floor, Clinical Microbiology building, Oxford Road, Manchester M13 9WL

P. aeruginosa contributes to be a major cause of opportunistic nosocomial infections in immunocompromised patients, and the death of patients with chronic cystic fibrosis. Clinical isolates resistant to virtually all anti-pseudomonal agents are increasingly being reported. Knowledge of the predominant immunogenic components of an infectious agent is essential for the analysis of the molecular mechanisms of virulence, the study of the route of infection, the serological diagnosis of the disease, and the development of strategies for efficient immune protection and eradication of the disease. It is especially important in the production of human recombinant antibody which represents a new approach to the development of therapies against these difficulties.

In the present project, Outer membrane protein porin F (OprF) was identified as the major antigen against human patient sera. There were 6 clinical isolates of *P. aeruginosa* included in the research in which they were grown in normal Muller-Hinton Broth (MHB) and MHB under antibiotics conditions imipenem (8 μg/ml), amoxicillin (1024 μg/ml) and ceftazidime (2 μg/ml) respectively. Outer membrane proteins and crude lysates of the 6 *P. aeruginosa* isolates were prepared by sonication. In one isolate profile, a protein at approximate 37 kDa was first identified as the major antigen using 2-D gel electrophoresis analysis, which was displayed by Silver stain and immunoblotting against human patient sera. All of the 6 profiles were further analyzed by SDS-PAGE, and subsequently displayed by Silver stain and immunoblotting. The results demonstrated that all the protein profiles of the cells grown in antibiotics had a significant identical protein band with the size of approximate 37 kDa which was very weak or not contained in that of the cells grown without antibiotic. This indicated that the protein with this molecular weight has been over expressed when the bacteria were exposed to these antibiotics. Identification by Mass Spectrometry, OprF (37.6 kDa) demonstrated to be the major antigen of *P. aeruginosa*, which also displayed significant change after the micro-organism grown in antibiotic environments.

CM 09 Heterogeneous resistance amongst Belgian MRSA causing vancomycin treatment failure

Constantinos Souliotis, Urmi Davda, James P. Burnie & Issam Alshami

Dept of Microbiology, University of Manchester, 2nd Floor Clinical Science Building, Oxford Road, Manchester

Over the years the excessive and improper use of antibiotics has resulted in the acquisition of resistance among strains of S. *aureus*. The first case of methicillin resistant strains or MRSA was isolated in the United Kingdom in 1961, and since then the prevalence of MRSA has increased rapidly world wide. Vancomycin which is the last therapeutic option for treating MRSA, have been documented, posing a potentially serious threat.

Different methods were applied on all Belgian samples in order to assess their tolerance to vancomycin and detect the presence of any

heterogeneous subpopulations. Time-Kill kinetics, MIC/MBC testing used by which the antibiotic resistance and tolerance of the strains was determined. The presence of heterogeneous subpopulations was detected by Population Analysis Profiling. None of the isolates showed any tolerance to vancomycin although some were able to survive at higher antibiotic concentrations than others. Additionally, Population Analysis showed that three samples out of the thirteen had heterogeneous subpopulations that were able to grow at higher concentrations of vancomycin than the parent cells. All the data and information accrued gave an insight on the occurrence of heterogeneity as well as the degree of resistance and tolerance of the Belgian MRSA strains.

CM 10 Heterogeneous resistance to vancomycin amongst MRSA a major clinical issue to combat

Urmi Davda, Constantinos Souliotis, Issam Alshami & James P. Burnie

Dept of Microbiology, University of Manchester, 2nd Floor Clinical Science Building, Oxford Road, Manchester

Methicillin resistant *S. aureus* is a major cause of hospital and community acquired infections that are increasing and are extremely difficult to combat because of emerging resistance to various antibiotics. Glycopeptide antibiotic vancomycin was introduced to treat gram positive bacteria. Usage of vancomycin has increased in last 20 years leading to a situation today where decreased susceptibility to vancomycin in *S. aureus* is seen with intermediate resistance pattern. The phenomenon of heterogeneous vancomycin resistance in *S. aureus* being the major problem screening methods plays a major role.

In our study Hungarian MRSA samples assessed using various methods such as MIC, MBC, time kill and population analysis to look for vancomycin susceptibility and degree of tolerance. None of the samples were found to be totally resistant to vancomycin even though different degrees of susceptibility pattern existed. Out of all the samples two strains of MRSA demonstrated the phenomenon of heterogeneous resistance producing subpopulations which were resistant to vancomycin at various levels compared to the parent which were totally susceptible. Development of better understanding of these mechanisms and organisms will help in preventing and development of treatment for these infections in the future.

CM 11 Synergism between biofilm producing and non-biofilm producing strains of *Pseudomonas aeruginosa* from cystic fibrosis patients

Elena Deligianni[1], **Panayiotis Panas**[1], G. McMullan[1], R.W. Haylock[1], J. Moore[2] & J.S.G. Dooley[1]

[1]Microbial Biotechnology Group, University of Ulster; [2]Dept of Microbiology, Belfast City Hospital, Belfast

Cystic Fibrosis (CF) is a genetic disease derived from a mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Colonization of the lungs in CF patients by the opportunistic bacterial pathogen *Pseudomonas aeruginosa* is the principal cause of mortality in the CF population. Bacteria bind to the epithelium of the lower respiratory tract forming an antibiotic resistant biofilm. In this study, RAPD typing of 100 clinical *P. aeruginosa* isolates was performed. 20 RAPD genotypes were defined and patient's were found to be colonized with up to 5 different *P. aeruginosa* genotypes. Furthermore isolates of the same RAPD genotype were found among patients attending the same clinic, indicating possible cross infection. Representatives of each genotype were tested for their ability to form biofilm along with phenotypic characteristics such as mucoidy, motility and production of quorum sensing signal molecules. Isolates were found to comprise both biofilm producing strains and strains that appeared to produce no biofilm.

CM 12 Laser capture microscopy: an emerging tool in the genomics and proteomics of *Cryptosporidium*

O. Sunnotel[1], W.J. Snelling[1], J.E. Moore[2], F. Kenny[3], V. Nicholson[3], B.C. Millar[2], P.J. Rooney[2], J.S.G. Dooley[1] & C.J. Lowery[1]

[1]Centre for Molecular Biosciences, University of Ulster Coleraine BT52 1SA; [2]Northern Ireland Public Health Laboratory, Dept of Bacteriology, Belfast City Hospital, Belfast BT9 7AD; 3Sligo Public Health Laboratory, Sligo General Hospital, Enniskillen Road, Sligo, Ireland (Email cj.lowery@ulster.ac.uk)

Human tissues are composed of complex admixtures of different cell types and their biologically meaningful analysis necessitates the procurement of pure samples of the cells of interest. Microdissection techniques enable selection and recovery of cells from fixed or embedded samples. It is therefore widely used in cancer research to separate effected cells from non-effected cells from tissue samples. This provides the opportunity to analyse differences in gene expression between different cell populations from the same tissue section. Further applications abound from defining new molecular targets, developing new molecular diagnostics, studying progression of disease, identifying the cell/cell interactions between normal and diseased tissue, understanding developmental biology, to in vitro fertilization. Although this technique has emerged as a powerful tool in cancer research and developmental biology, its potential in (medical) microbiology has yet to be defined. This oral presentation concerns a recent advance in microdissection techniques, namely laser capture microdissection (LCM) and focuses on the application of this technique in the field of medical microbiology. The principle underlying this technique is outlined, and practical issues concerning to LCM are considered. This study was partly funded through a Laboratory Links Network Project grant, with Northern Ireland Public Health Laboratory, Sligo Public Health Laboratory, University of Ulster (Coleraine) and the Centers for Disease Control & Prevention (CDC)(USA).

CM 13 *In vitro* susceptibility of staphylococcal species, including methicillin-resistant *Staphylococcus aureus*, to tea-tree oil

A.J. Brady[1], P.F. Kearney[2] & M.M. Tunney[1]

[1]Clinical and Practice Research Group, School of Pharmacy, Queen's University of Belfast, Belfast; [2]Microbiology Dept, United Hospitals Trust, Antrim

Despite conventional decolonization measures staphylococcal species are still a common cause of surgical site infections, accounting for 49 % of all isolates in a Surveillance of Surgical Site Infections in English Hospitals. The essential oil of *Melaleuca alternifolia* [tea-tree oil (TTO)], has demonstrated promising efficacy against several bacterial pathogens, including methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).

This study was designed to investigate the *in vitro* susceptibility of a range of staphylococcal species to TTO. A total of 30 MRSA, 25 MSSA and 29 coagulase-negative staphylococcal (CoNS) isolates cultured from patient samples were tested. The Minimum Inhibitory Concentration (MIC) was determined by the broth microdilution method according to the British Society for Antimicrobial Chemotherapy guidelines. The Minimum Bactericidal Concentration (MBC) was also determined for each isolate. All 84 isolates tested had an MIC within the range 0.25–2 % TTO. MRSA and MSSA isolates demonstrated a higher MIC_{90} than CoNS isolates although the difference was not significant ($P>0.05$). In addition, CoNS isolates demonstrated a wider range of MBC values (0.5–8 %) than *S. aureus* isolates (2–8 %) as well as a lower MBC_{50} value. The results of this study suggest that TTO possesses significant antibacterial activity against a range of common skin pathogens at a concentration which has previously been demonstrated to be well tolerated.

CM 14 Antimicrobial susceptibility of clinical *Pseudomonas aeruginosa* isolates in comparison with antibiotic concentrations found at the site of cystic fibrosis pulmonary infection

T.F. Moriarty[1], J.S. Elborn[2] & M.M. Tunney[1]

[1]Clinical and Practice Research Group, School of Pharmacy; [2]Respiratory Medicine Research Group, Queen's University of Belfast, Belfast BT9 7BL

Treatment of pulmonary infections in cystic fibrosis (CF) patients routinely involves combination antibiotic therapy, the efficacy of which is dependent on the concentration of antibiotic(s) at the site of infection rather than in the bloodstream.

In this study sputum samples were collected from CF patients receiving intravenous ceftazidime (3g three times daily) and tobramycin (8mg/kg once daily) therapy. The concentration of both antibiotics in sputum was determined by high performance liquid chromatography. Infecting bacteria were also isolated and the susceptibility of the isolates to ceftazidime and tobramycin determined by measurement of the minimum inhibitory concentration (MIC).

In total 22 *P. aeruginosa* isolates were cultured from 14 patients. The sputum ceftazidime concentration exceeded the MIC in 55 % of samples taken from patients infected with isolates with an MIC of ≤16 µg/ml. However, in those patients infected with an isolate with an MIC for ceftazidime of >16 µg/ml sputum ceftazidime concentrations did not exceed the MIC at any time throughout the dosing interval. The sputum tobramycin concentration did not reach or exceed the MIC of any of the isolates.

This study has shown that intravenous administration of ceftazidime or tobramycin at the recommended doses for the treatment of CF lung infection does not achieve adequate sputum concentrations of either antibiotic.

CM 15 Effect of decolonization with chlorhexidine on the susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) to antiseptics

D.F. Gilpin[1], R. Loughlin[1], S. Small[2], P. Kearney[2], D. Gardiner[2], S. Patrick[3] & M.M. Tunney[1]

[1]School of Pharmacy, Queen's University of Belfast, 97 Lisburn Rd, Belfast BT9 7BL; [2]United Hospitals Trust, 45 Bush Rd, Antrim BT41 2RL; [3]School of Medicine, Queen's University of Belfast, Grosvenor Rd, Belfast BT12 6BN

Eradication of colonization in MRSA colonized individuals is considered to be a vital step in MRSA control measures. However, studies have shown that commonly used decolonization protocols are not effective in every patient and the factors contributing to this failure have not been fully explored. This study aimed to examine and characterize isolates from MRSA colonized individuals before and after undergoing decolonization (washing with 4 % chlorhexidine and application of mupirocin nasal ointment daily for one week). In cases where decolonization failed, MRSA isolates were collected by swabbing and confirmed as MRSA by multiplex PCR using primers to detect *mecA*, *nuc* and 16S rRNA genes. Isolates were typed by pulsed field gel electrophoresis: no difference was found in isolates cultured from an individual patient before and after decolonization. The susceptibility of the isolates to antiseptics (chlorhexidine, povidine-iodine and Tea-tree oil) was compared by measuring the minimum inhibitory concentration. The MIC for chorhexidine, povidine-iodine and Tea-tree oil were determined as 7.8 $x10^{-4}$ mg/ml, 0.1875 mg/ml and 0.5 % v/v respectively for all isolates, indicating that there was no difference in antiseptic susceptibilities between isolates obtained before and after decolonization. These initial results confirm previous work, which show that MRSA isolates are much less susceptible to povidone-iodine than to chlorhexidine. Future work will focus on assessing the susceptibility of the different isolates grown as biofilms in the presence or absence of human serum, in order to more closely mimic the *in vivo* situation.

CM 16 Population structure of a haemophilus antibiotic resistance ICE

Lucille Mansfield, Abdel R.E. Elamin, Ioanna D. Dimopoulou, Tim E. Peto, Angela B. Brueggemann, Rosalind M. Harding & **Derrick W. Crook**

University of Oxford

Antibiotic resistance emerged worldwide in *Haemophilus influenzae* starting in the 1970s. A family of genomic islands represented by the exemplar, ICE*Hin1056*, encodes for most of this resistance. How this ICE has spread worldwide is not known. The aim was to use sequence analysis of core ICE genes to infer how these ICEs have evolved and spread.

Five evenly distributed core genes were selected from the ICE for comparative sequence analysis analogous to multi-locus sequence analysis. 206 strains of haemophili from different regions of the world were studied containing 103 epidemiologically unlinked ICEs. There were two lineages of integrase gene with independent phylogenies diverging by 15 % in sequence identity. The remaining 4 core genes diverged by 2 %. There was no structure to the diversity. On frequency analysis, one lineage of integrase was associated with *H. influenzae* while the other with *H. parainfluenzae*.

This family of ICEs is globally panmixic. Data also suggest frequent recombination between ICEs. This is consistent with a global pandemic of this ICE.

CM 17 Functional analysis of ICE*Hin1056*: a genomic island

Mario Juhas, Ioanna Dimopoulou, Zaini Mohd-Zain, Derek W. Hood & Derrick W. Crook

University of Oxford

The horizontal gene pool contributes to the diversification and adaptation of bacteria. Genomic islands are a major component of this gene pool. A family of syntenic genomic islands with deep evolutionary origins has been shown by comparative genomic analysis to be related to an ICE found in *Haemophilus* spp., which accounts for a pandemic of antibiotic resistance. Analysis of the *Haemophilus* ICE*Hin1056* offers an opportunity to identify how these genomic islands propagate.

Forty-seven putative core genes of ICE*Hin1056* were clustered into modules predicted to encode the three properties: conjugation, replication and site-specific-recombination with $tRNA^{leu}$. Twenty of these core genes have been inactivated with a kanamycin cassette. Analysis of these mutants provides strong functional evidence that ICE*Hin1056* encodes genes involved in conjugation (including pilus biosynthesis and DNA processing), replication and site-specific-recombination.

These results provide a model for how one family of genomic island propagates, generates bacterial phenotypic evolution and diversification and may offer an approach for investigating genomic islands in general.

CM 18 Characterization of aerobic pathogenic streptomycetes

Mayami Sengupta, Nicholas E.E. Allenby, Alan C. Ward & Michael Goodfellow

School of Biology, King George VI Building, University of Newcastle, Newcastle-upon-Tyne NE1 7RU

Thirty representative pathogenic *Streptomyces* strains, isolated from patients with mycetoma and donkeys suffering from fistula withers, were characterized using both phenotypic and genotypic methods.

16S rRNA gene sequencing showed that the strains were distributed across several clades within the zone of evolutionary radiation occupied by the genus *Streptomyces*. The strains were examined for 143 unit characters and fell into two major numerically defined groups. Suppressive subtractive hybridization (SSH), used to identify potential pathogenicity determinants between closely related pathogenic and non-pathogenic strains, gave low yields of differential DNA. Results from the application of novel subtractive hybridization method using magnetic capture beads of biotinylated DNA is being developed and compared to SSH data. The initial experiment based on the magnetic bead capture procedure showed that the beads can bind biotinylated genomic DNA, confirmed by PCR of captured 16S rDNA. Additional experiments are being carried out using this method in order to identify differential DNA.

CM 19 Molecular screening of tick-borne pathogens in dogs and horses from Trinidad by PCR and macro-arraying

Karla Georges[1,2], Aweeda Newaj- Fyzul[1], Mervyn Campbell[1], Natasha Mootoo[1], Olivier Sparagano[2] & Chukwudozi D. Ezeokoli[1]

[1]University of the West Indies, School of Veterinary Medicine, Faculty of Medical Sciences, St Augustine Campus, Trinidad and Tobago; [2]University of Newcastle, School of Agriculture Food and Rural Development, Agriculture Building, Newcastle upon Tyne NE1 7RU

Tick infestation and tick borne pathogens are widespread throughout out Trinidad causing high morbidity and mortality in livestock, companion animals and equines. Diagnosis is often based on clinical signs, response to treatment and the identification of the pathogen on blood smears. Diagnosis of these diseases is a challenge to the veterinarian due to a lack of sensitivity of traditional diagnostic methods and variable clinical signs in the host. To improve the quality of patient care, the application of more sensitive and specific methods of characterizing tick- borne haemopathogens are therefore being investigated.

Two PCR reactions based on the 16S rRNA (*Ehrlichia* / *Anaplasma* group) or 18S rRNA (*Babesia* / *Theileria*) genes followed by a macro-arraying technique (RLB) using seven DNA probes (*Babesia/ Theileria* genera, *E. canis*, *E. chaffeensis*, *A. platys*, *A. phagocytophila* group, *Erhlichia* genera, *A. phagocytophila sp.*,) successfully detected *Ehrlichia canis* and *Anaplasma platys*, unknown species within the *Ehrlichia* / *Anaplasma* group and some unknown species within the *Babesia* / *Theileria* group.

Blood samples were collected from 182 dogs, 16 horses and 4 cats between June and August 2004 on the island of Trinidad. Overall tick transmitted haemopathogen DNA was detected in 42 (23.1 %) dogs and 2 (12.5 %) horses. All 4 cats were negative. *E. canis* was most frequently detected in dogs and *Babesia* / *Theileria* spp. as the only haemoparasite detected in horses. A mixed infection of *E. canis* and a member of the Piroplasmidae family was detected in one dog.

The majority of dogs (61.5 %) in which tick-borne haemopathogen DNA was detected presented with at least one of the following clinical signs: bleeding, oedema of limbs, anorexia, pyrexia and lethargy. These characteristic clinical signs in dogs, were statistically significantly associated with a positive reverse line blot (RLB) result ($P = 0.0$, χ^2 1 *df*). The RLB positive horses appeared normal on clinical examination. The reverse line blot proved to be a powerful tool in screening multiple species to detect specific blood pathogens. Further investigations are in progress to identify the new species identified by the Piroplasmidae family probe but not by the species-specific probes so far.

CM 20 Effect of gaseous atmosphere on growth and nutrient consumption in batch cultures of *Campylobacter jejuni* NCTC 11351

Kwang-Kuk Cho, Linda M. Harvey & Brian McNeil

Dept of Bioscience, University of Strathclyde, Royal College, Building, 204 George Street, Glasgow G1 1XW

The influence of atmosphere composition on the metabolism of *Campylobacter jejuni* in a batch culture system was studied by analysing the consumption of amino acids and the production of acetate under atmospheres containing different combinations of oxygen and carbon dioxide. Growth studies also introduced different modes of culture gassing, sparging through liquid, and headspace gassing. The growth studies indicated that *C. jejuni* can grow in complex medium under atmospheres containing 5 % to 20 % oxygen and 5 % to 10 % carbon dioxide. The typical fluctuating growth was not observed under sparged aeration. Acetate only accumulated during stationary phase. The total amount of acetate produced was greater at low concentrations of O_2 and higher concentration of CO_2. The bacteria consumed L-aspartic acid, L-serine, and L-asparagine under both aeration methods. However, L-glutamic acid was only consumed under sparged aeration. We concluded that the cell growth and acetate production pattern were dependent on gassing mode, and that consumption of four amino acids supported cell growth.

CM 21 Invasive pneumococcal disease in children: serotypes and sequence types prior to the introduction of pneumococcal conjugate vaccines in the UK

S.C. Clarke[1,2,3], **J.M. Jefferies**[1], A.J. Smith[4], T.J. Mitchell[2] & G.F.S. Edwards[1]

[1]Scottish Meningococcus and Pneumococcus Reference Laboratory, Glasgow; [2]Division of Infection and Immunity, Institute of Biomedical and Life Sciences, Glasgow University; [3]Portsmouth City Primary Care Trust, Portsmouth; [4]Infection Research Group, Glasgow Dental Hospital, Glasgow

Aims To identify serotypes and sequence types (ST's) of pneumococci causing invasive disease in children prior to the introduction of pneumococcal conjugate (Pnc) vaccines.

Methods Invasive pneumococci from children aged less than 5 years between 2000 and 2004 in Scotland were used. Only pneumococci that were fully serotypeable were included. These were characterized by serotyping and multi-locus sequence typing.

Results 217 pneumococci were isolated during the study period, 195 of which were from blood and 23 were from CSF. 122 were from males, 89 from females and the sex was unknown in 6. 14 isolates were from children aged less than 2 months, 22 were 2-5 months old, 44 were 6-11 months old, 99 were 1-2 years old and 38 were 2-4 years old. There were 22 different serotypes, serotype 14 being the most common (80 isolates (37 %)), followed by serotypes 19F and 6B (both 10 %), 18C (6 %), 23F (5.1 %), 9V (4.6 %) and 4 (3.7 %). These are all represented in the 7-valent Pnc vaccine. Overall, 7-valent Pnc coverage was therefore 76 %. However, serotypes 1 and 19A also each accounted for 3.7 % of isolates. There were 77 different ST's, of which ST9 was the most common (25.3 %), followed ST's 162 and 176 (8.3 % and 6.9 % respectively). A total of 16 new ST's were described during the study. All isolates grouped into 16 lineages although there were an additional 28 singletons.

Conclusions The 7-valent Pnc vaccine includes the most common paediatric serotypes in Scotland but may benefit from the addition of serotypes 1 and 19A. Although 22 serotypes were identified, these possess considerable heterogeneity which may have implications for the introduction of Pnc vaccines as a particular serotype is not always limited to a single genotype. Further analyses are therefore required in order to understand better the relationship between serotype and sequence type on a genomic level.

CM 22 Development of a recombinant salmonella subunit vaccine

L.J. Caproni[1], M.M. Mogensen[1], J.E. Allen[2] & M.P. Gallagher[1]

ISMB[1]; IIIR[2], School of Biological Sciences, King's Buildings, University of Edinburgh, EH9 3JT

Salmonella is an important human and animal pathogen. Symptoms of infection range from gastroenteritis to systemic disease or death. Infection mainly occurs via consumption of contaminated foods. Current live vaccines offer a degree of protection but are inappropriate for use with immunocompromised individuals. Development of an efficacious subunit vaccine would circumvent this but requires knowledge of which antigens are important and immunodominant in the immune response to *Salmonella*. Few protective antigens have been reported for *Salmonella enterica sv. Typhimurium*, although two proteins in combination (SseB and Mig14, Rollenhagen *et al*., 2004) were shown to be protective in hypersusceptible BALB/c mice when immunizing with CFA. In previous work in this lab serum from hyperimmunized mice was used to identify immunodominant antigens seen during natural infection of mice with *Salmonella*. In the present study we examine the effectiveness of different adjuvant/antigen combinations in eliciting protection following immunization with SseB and/or other proteins which we have identified as in-vivo antigens, and the outcomes of these are reported.

Posters

Environmental Microbiology Group

EM 01 Immunomagnetic capture of *Mycobacterium bovis* from environmental samples

Frank P. Sweeney, Orin Courtenay, Atiya Ul-Hassan, Vicky Hibberd, Lucy A. Reilly & Elizabeth M.H. Wellington

Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* is a serious and growing problem for the agricultural community in the UK. The European badger (*Meles Meles*) is generally considered to be a wildlife reservoir for the organism although the method of transfer between species has yet to be elucidated. Badgers chronically infected with *M.bovis* can shed large numbers of the organism *via* faeces, urine and respiratory aerosols into the environment. Controversy exists as to the longevity and infectivity of these environmental bacteria, although studies with *Salmonella* and *E.coli.* 0157:H7 have provided evidence that pathogenic organisms can persist in environmental samples such as soil and faeces. The presence of hotspots of bTB occurrence which persist even after wildlife sources are removed also implicates an environmental source of infection. Slow-growing mycobacteria are notoriously difficult to culture using traditional techniques and often require harsh decontaminating procedures, for example acid washes, to reduce growth of other organisms. They also require highly selective media with both antibiotics and antifungals. Isolation from soil and faeces is particularly difficult due to the large number of other bacteria in the sample. To avoid the need for culturing the organism, PCR screening using MPB70 and RD4 primers was used to detect the presence of *M.bovis*. Badger setts and latrines were sampled from a well documented badger population in Woodchester Park, Gloucestershire, UK. 63 % of setts and 56 % of latrine samples were positive for *M.bovis*. A correlation was found between positive samples and the number of badgers actively excreting *M.bovis*. There was no correlation between PCR positive samples and badger density. In addition, to prove the link between PCR screening and the presence of *M.bovis* we have developed a method to retrieve *Mycobacterium bovis* cells from soil and other environmental samples using immuno-magnetic capture techniques (IMC). Cells of *M.bovis* BCG (Pasteur) have been successfully isolated and cultured from seeded soil and *M.bovis* has been captured from environmental soil and faeces collected from badger setts and latrines.

EM 02 The phage growth limitation system (pgl): a comparison of ϕC31-*Streptomyces coelicolor* in liquid and soil environments

D.S. Lymperopoulou[1], **L.A. Calvo-Bado**[2], S. Griffiths[2] & E.M.H. Wellington[2]

[1]Dept of Botany, Faculty of Biology, National and Kapodistrian University of Athens, 157 81 Athens, Greece; [2]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

The Phage Growth Limitation (pgl) system is a resistance mechanism possessed by a limited number of species within the *Streptomyces* genus against to ϕC31 and some homoimmune relatives. In *S. coelicolor*, pgl prevents plaque formation on lawns of growing bacteria. Phage produced from *S. lividans* (without pgl system) or *S. coelicolor* mutants (pgl-) can infect pgl+ host and produce a normal burst of progeny phage, however when these phage are used to reinfect a pgl+ host, the number of second or successive generation, the phage produced is greatly attenuated-no replication (no burst). Four genes (*pgl*W, *pgl*X, *pgl*Y, *pgl*Z) constitute the whole pgl systems, however, this resistance mechanism has not fully elucidated and even less is known about the mechanism and dynamics of the phage-host interaction in soil. The objectives of this work are to understand the phage-bacteria interaction in liquid and soil environments. In order to understand the biology of this interaction, phage and host population dynamics were monitored during the first, second and third infection cycle. Effect of variables such as resistance and sensitive host, host and phage density (multiplicities of infection, moi), phage-host competition among others were evaluated. The results showed that in liquid environment (continuous culture, dynamic system), the phage population disappeared from the system during the 48 h of growth at < 0.1 moi and after 72 h of growth at > 0.1 moi at 10^5-10^6 cfu/ml for host-phage density effect assays. For soil environment (static culture), the phage population dynamics attenuation was slower (over 15 days), and the phage population vanished from the system after 3 continuous transfers at < 0.1 moi. Host competition experiments showed that after a second infection cycle phage had preferential affinity for the sensitive host. Mathematical modelling of the infection process in both systems showed that the host-phage interaction is affected by host-phage density, residual infection and host resistance.

EM 03 Metabolism of DMS by methylotrophic bacteria from the marine environment

Hendrik Schäfer, J. Colin Murrell & Sanjeev Kumar

Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

Dimethyl sulfide (DMS) is an organic sulfur trace gas believed to have a cooling role in global climate regulation. DMS is mainly produced in seawater by enzymatic cleavage of dimethylsulfoniopropionate. Being volatile, it is emitted from the ocean to the atmosphere, where it forms aerosols that backscatter heat radiation into space and that affect cloud formation. However, up to 90 % of the marine DMS production is rapidly oxidized by bacteria in the surface ocean, making bacterial DMS oxidation an important biogeochemical process. Despite this, little is known about the identity of marine DMS degrading bacteria and the enzymes and genes of bacterial DMS oxidation pathways. The aim of this study was to enrich and isolate marine DMS degrading bacteria and to gain insight into the bacterial metabolism of DMS in the marine environment. *Methylophaga* strains were enriched and isolated from several UK coastal water samples. These strains appear to degrade DMS using a methyltransferase pathway and are candidate model organisms for studying enzymes and genes underpinning pathways of marine DMS oxidation.

EM 04 Functional diversity of methanotroph in an acid peatbog

Y. Chen[1], M. Dumont[1], N. McNamara[2], L. Bodrossy[3] & J.C. Murrell[1]

[1]Dept of Biological Sciences, University of Warwick; [2]Soil Ecology Section, CEH-Lancaster, Lancaster Environment Centre, Lancaster; [3]Dept of Biotechnology, ARC Seibersdorf research GmbH, Austria

90 % of the methane produced in peatbogs is consumed by methanotrophic bacteria before it is released to the atmosphere. The activity and diversity of methanotrophs in an acid peatbog with different vegetation covers was investigated. We analyzed 16S rRNA, *pmoA* and *mmoX* genes in peat samples by clone library analysis,

denaturing gradient gel electrophoresis, microarrays and T-RFLP. Several methanotroph genera were detected, including *Methylomonas*, *Methylocella* and *Methylocystis*, but results indicated that an uncultured type II methanotroph was the most abundant methanotroph in this acid wetland. Transcripts of *pmoA* and *mmoX* encoding subunits of the particulate methane monooxygenase (pMMO) and the soluble methane monooxygenase (sMMO), respectively, were targeted by the direct extraction of mRNA from environmental samples and reverse transcriptase PCR. Transcripts of *pmoA*, but not *mmoX*, were detected by RT-PCR, suggesting the methanotrophs express the pMMO enzyme in this environment. Our results provide further insight into functional diversity of methanotrophs in acidic wetlands.

EM 05 Diversity of marine dimethyl sulfide oxidizing bacteria revealed by stable-isotope probing

Rich Boden, Josh Neufeld, Colin Murrell & Hendrik Schäfer

Dept of Biological Sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL

Dimethyl sulfide (DMS) is a volatile gas released into the oceans during the degradation of various algae and phytoplankton. Around 10 % of DMS produced is released into atmosphere where it plays key roles in the biogeochemical cycling of sulfur and in climate control, acting as a cloud condensation nucleus and negative greenhouse gas. Around 85 % of DMS produced in the oceans is bacterially degraded. The phylogenetic and functional diversity of DMS oxidizing bacteria in the marine environment have not been previously investigated in any depth. In this study, stable-isotope probing (SIP) with $^{13}C_2$-DMS was used to identify bacterial populations in seawater that actively oxidized DMS and assimilated its carbon as cell material. ^{13}C-DNA isolated by gradient centrifugation from SIP experiments was characterized by denaturing gradient gel electrophoresis, cloning and sequencing of PCR amplified 16S rRNA encoding genes in order to evaluate the diversity of DMS oxidizing bacteria in the marine environment.

EM 06 Regulation of methane oxidation in the facultative methanotroph Methylocella silvestris BL2

Andreas R. Theisen[1], M. Hanif Ali[1], Stefan Radajewski[1], Marc G. Dumont[1], Peter F. Dunfield[2], Ian R. McDonald[1]*, Svetlana N. Dedysh[3], Carlos B. Miguez[4] & J. Colin Murrell[1]

[1]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL; [2]Institute of Geological and Nuclear Sciences, Wairakei Research Station, Private Bag 2000, Taupo, New Zealand; [3]S.N. Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow 117312, Russia; [4]Biotechnology Research Institute, NRC Montreal, QC, H4P 2R2, Canada; **Present address:* Dept of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand

The molecular regulation of methane oxidation in the first fully authenticated facultative methanotroph *Methylocella silvestris* BL2 was assessed during growth on methane and acetate in fermentor culture. The genes encoding soluble methane monooxygenase (sMMO) were cloned and sequenced. Transcriptional analysis of sMMO genes revealed that they formed an operon and expression studies on fermentor grown cultures showed that acetate repressed transcription of sMMO in *M. silvestris* BL2. The presence of a particulate, membrane-bound methane monooxygenase enzyme in *M. silvestris* BL2 and the copper-mediated regulation of sMMO was investigated. Both were shown to be absent. A promoter probe vector was constructed and used to assay transcription of the promoter of the sMMO operon of *M. silvestris* BL2 grown under various conditions and with different substrates. These data represent the first insights into the molecular physiology of a facultative methanotroph.

EM 07 Stable isotope probing of marine methanol degradation: implication of *Methylophaga* spp.

Josh D. Neufeld, Hendrik Schäfer & J. Colin Murrell

Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

The ocean is an important environment for cycling of one-carbon compounds, but the organisms that degrade them are poorly characterized. Stable isotope probing (SIP) is a powerful tool for identifying organisms that assimilate stable-isotope labelled substrates. However, seawater presents a unique set of challenges for SIP due to the low biomass and quick response of seawater micro-organisms to carbon substrates. Presenting data from initial SIP experiments we identify technical considerations required for the application of SIP to coastal seawater samples. These include incorporation rates of substrates, effect of nutrients, and enrichment caused by the addition of excess carbon source. Labelled DNA from initial DNA-SIP experiments conducted with methanol was characterized by DGGE and clone libraries of genes encoding 16S rRNA and methanol dehydrogenase (*mxaF*). Predominant DGGE phylotypes and 16S rRNA gene libraries agreed with enrichment isolations, implicating the moderately halophilic *Methylophaga* species in methanol degradation. Methanol dehydrogenase sequences from SIP heavy bands and *Methylophaga* isolates formed a novel clade, representing the first *mxaF* sequences obtained from these ecologically-relevant marine methylotrophs.

EM 08 Horizontal transfer of 2,4-D degradative genes within biofilms

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

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

In natural and anthropogenic environments bacteria often form biofilms. The role of biofilms in horizontal gene flow for degradative plasmid transfer is little understood. Within complex bacterial communities catabolism of 2,4-dichlorophenoxy-acetic acid (2,4-D) is encoded by 2,4-D degradation genes (*tfdA-F+K*), frequently located on plasmids. The study aims were the isolation and identification of 2,4-D degrading bacteria from soil and subsequent horizontal gene transfer between bacteria within biofilms. Bacterial enrichments were performed from agricultural soils. Community analyses were undertaken using denaturant gradient gel electrophoresis (DGGE) after subculture with 2,4-D. PCR amplification of the 16S rRNA and *tfd* genes enabled identification of the isolates. A high homology to *Burkholderia hospita* was determined. DGGE analysis demonstrated bacterial community changes during successive subcultures. DNA sequence analysis identified species within the degradative communities, which proved undetectable in successive subculture. This suggests these organisms are not significant in initial 2,4-D degradation. Plasmid profiling indicated the presence of *tfd* genes. *B. hospita* formed thick biofilms which were observed via flow cell microscopy. We have developed a system to study *tfd* horizontal gene flow within degradative biofilms using *B. hospita* (pMM172::*gfp*) to allow visualization of degradative gene flow and expression within biofilms *in situ*.

EM 09 Biodegradation of undefined complex mixtures

M. Frenzel[1,2], S.J. Rowland[2], S.K.Burton[1] & H.M. Lappin-Scott[1]

[1]School of Biosciences, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4PS; [2]Petroleum & Environmental Geochemistry Group, SEOES, University of Plymouth, Plymouth PL4 8AA

Cyclohexylalkyltetralins are proposed to be appropriate model compounds for components of aromatic undefined complex mixtures (UCMs). UCMs are dominant features of environmental samples

contaminated with weathered crude oils and are considered to be resistant to microbial degradation. A recent estimate has suggested that whole oil UCMs may contain around 250,000 mostly unidentified compounds. 6-cyclohexyltetralin was synthesized to high purity for use in biodegradation studies. Optimization of methods to monitor the biodegradation of cyclohexylalkyltetralins has been successfully undertaken. Sediment samples were collected from sites around the UK with a known history of long-term hydrocarbon pollution, including industrial sites and natural sources of hydrocarbons. Enrichment cultures were established in minimal salts medium containing 6-cyclohexyltetralin. Degradation was monitored over time and quantified using gas chromatography (GC-FID). DNA extraction, PCR and DGGE of the 16S rRNA gene was used to monitor changes in the microbial community over the enrichment period. Preliminary observations suggest degradation of the compound and DNA has successfully been extracted from enrichments from four different sample sites. This holds promise for future remediation of sites contaminated with such chemicals.

EM 10 Molecular characterization of the Mecoprop degrading bacterium *Burkholderia glathei*

D. Zakaria, S.K. Burton & H.M. Lappin-Scott

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

Chlorophenoxy alkanoic acid herbicides are a group of structurally related compounds and extensively used to kill broad leaved weeds in cereal crops. Accumulation in the environment presents a serious threat due to their carcinogenic and tetratogenic effects. Degradation of these herbicides occurs by natural microbial communities. However mecoprop [2-(2-methyl-4-chlorophenoxy) propionic acid] persists in the environment due to its resistance to microbial degradation. *tfdA* and *tfdB* are the genes encoding enzymes to catalyse the first two steps of mecoprop degradation. These genes are normally plasmid borne in the chlorophenoxyalkanoic acid herbicide degrading bacteria. A pure isolate was cultured from soil, which had the ability to degrade mecoprop. This was identified as *Burkholderia glathei* from which a large plasmid approximately 250 Kb was isolated. Plasmid transfer from *Burkholderia glathei* into soil bacteria or cured *Burkholderia hospita* was not detected by filter mating experiment. The cured strain could not degrade mecoprop and did not contain the functional genes *(tfdA,* and *tfdB*) implying that this plasmid plays an essential role in mecoprop degradation. Experiments carried out on the cured and parent strain proved that the parent is resistant to both mercuric chloride and ampicillin and contained the functional genes *tfdA* and *tfdB*. Interestingly, the phylogenetic trees of *tfdA* and the corresponding 16S rRNA genes of were incongruent, indicating the evolution of *tfdA* gene in *Burkholderia glathei* due to horizontal gene transfer. Further investigation is required to study other functional genes within this isolate and to characterize the isolated plasmid.

EM 11 The effects of nitrate addition and pressure on the microbial population of sea water

M.D. Jones[1], J.D. Porter[1], B.C. Dunsmore[2], S.K. Burton[1] & H.M. Lappin-Scott[1]

[1]University of Exeter, Hatherly Laboratories, Prince of Wales Rd, Exeter EX4 4PS; [2]Oil Plus Ltd, Hambridge Road, Newbury, Berkshire RG14 5TR

The presence of sulfate reducing bacteria (SRB) in oil production systems and their hydrogen sulfide production causes significant problems including; corrosion, and loss of reservoir porosity. The financial implications of SRB activity are therefore substantial. The use of nitrate addition has been investigated as an alternative to biocidal control. However the effect of nitrate on the inate microbial population is little understood.

The inability to replicate *in situ* reservoir conditions is a major problem when working in this field. Sea waters collected from the Gulf of Mexico(from 20 m and 2400 m depths) were cultivated with and with out nitrate addition. Enrichments were maintained at three pressures, room pressure, 20684 KPa (reservoir pressure) and 61368 KPa (sea water injection pressure). 16S rRNA PCR and denaturing gradient gel electrophoresis (DGGE) were used to determine the effects of pressure and nitrate addition on the microbial populations of the samples.

Total populations from the pure water samples, collected as pressurized and non-pressurized samples, were cloned and analysed to show how depth and pressure influenced the microbial population. Results indicate a diverse population devoid of SRB, where *Erythrobacter* are dominant throughout. Implications of this work suggest that for this site reservoir injection water would be equally beneficial whether taken from the top of the water column, or the bottom.

EM 12 An exploration of the genes responsible for the first stage of chlorophenoxyalkanoic acid herbicide degradation

N.J. Senior & H.M. Lappin-Scott

Dept of Biological Sciences, University of Exeter, Exeter EX4 4PS

The well-characterized degradation pathway for the chlorophenoxyalkanoic acid herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in *Ralstonia eutropha* JMP134 is catalysed by the *tfd* family of genes. The degradation pathway for the stereoisomeric herbicide 2-(2-methyl-4-chlorophenoxy)propionic acid (mecoprop) is analogous with that of 2,4-D. Mecoprop-degrading bacteria are likely to possess *tfd* genes, including *tfdA*, the gene responsible for the initial stage of degradation. However, recent research has suggested that the *rdpA* gene may be responsible for the initial stage of mecoprop degradation. The aim of our research was to look for the gene responsible for the first stage of degradation in bacteria that degrades both herbicides. Seven isolates were isolated from soil by enrichment on both 2,4-D and mecoprop and sequenced; they were identified as *Burkholderia glathei*. Molecular techniques revealed that both *tfdA* and *rdpA* genes could be PCR amplified from these isolates. RNA extraction and RT-PCR will be carried out to identify the functional gene(s) involved, and help to elucidate which are responsible for the first stage of degradation.

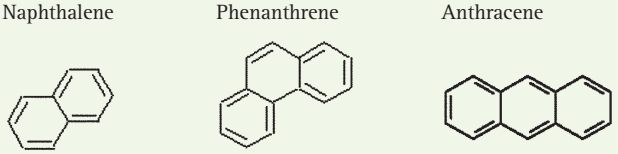
EM 13 A molecular analysis of polycyclic aromatic hydrocarbon-degrading bacterial communities

R.M. Long[1], C. Whitby[1,2], G. Tustin[3] & H.M. Lappin-Scott[1]

[1]School of Biosciences, University of Exeter, Exeter EX4 4PS; [2]Dept of Biological Sciences, University of Essex, Essex CO4 3SQ; [3]Sclumberger Cambridge Research, Cambridge CB3 0EL

Polycyclic aromatic hydrocarbons (PAHs; Fig. 1) are ubiquitous environmental pollutants and are generated by the incomplete combustion of organic matter e.g. home heating, traffic and waste incineration. The fate of PAHs in nature is of great environmental concern due to their toxic, mutagenic and carcinogenic properties.

Fig. 1. PAHs



Microcosm experiments were established and enriched with media containing PAHs and the degradation was monitored over time. Denaturing gradient gel electrophoresis (DGGE) of the 16S rRNA genes demonstrate the predominance of key microbial groups involved in PAH degradation. Targeting functional genes, such as the *nah*-like genes, provides a method for assessing the degradative capacity of bacterial populations. *NahAc* clone libraries were generated from PCR products obtained from naphthalene enrichments. Although sequence quality was high, sequence analysis revealed only 81-89 % similarity to *Pseudomonas* spp., *Bordetella* sp. and *Norcardia* sp. this may imply the presence of novel *nahAc* genes.

EM 14 Molecular identification of equine strongyles by a Reverse Line Blot hybridization assay

Donato Traversa[1,2], **Olivier A.E. Sparagano**[2], Narut Thanantong[2], Raffaella Iorio[1] & Annunziata Giangaspero[3]

[1]Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy; [2]School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne NE1 7RU; [3]Faculty of Agriculture, University of Foggia, 71100 Foggia, Italy

Small and large strongyles (Nematoda, Strongylidae) are horse parasites of primary importance worldwide. More that 50 species are recognized, which can be microscopically identified on the basis of tails and heads of the adult stages. However, their identification can be challenging and the differentiation of the immature stages is almost impossible. The characterization of the intergenic spacers (IGS) of the ribosomal DNA (rDNA) recently provided powerful genetic markers for the molecular identification of six small strongyles. In this work the IGS of eight species of small strongyles and of three species of large strongyles from different geographical areas were characterized. Species-specific probes designed in the strongyle consensus IGS regions were evaluated in a Reverse Line Blot (RLB) Hybridization for their ability to simultaneously identify the 11 species of strongyles. The probes hybridized specifically with the IGS of the strongyles included in the assay, whose conditions were validated by varying incubation time and temperatures, and probes concentration. The RLB herein presented allows the simultaneous molecular differentiation of 11 equine strongyles species irrespective of their life cycle stage, thus providing a powerful tool for faecal diagnosis of horse strongylosis and biological and epidemiological studies of this infection.

EM 15 The prokaryotic diversity associated with a marine worm *Arenicola marina*

Elizabeth Ashforth, Thanya Duangchinda, Alan Ward & Peter Olive

School of Biology and Psychology with School of Marine Science and Technology, University of Newcastle, Newcastle upon Tyne NE1 7RU

Extreme environments- sea ice, deep sea and hydrothermal vents, have been sampled for polyunsaturated fatty acid (PUFA) producing prokaryotes. However there must be prokaryote pathways in intertidal marine sediments leading to accumulation of PUFA but this microbial loop has been overlooked. Biosynthesis of PUFA (e.g. eicosapentaenoic acid [EPA – 20:5ω3]; docasahexaenoic acid [DHA – 22:6ω3]; and arachidonic acid [AA – 20:4ω6]) occurs in lugworm (*Arenicola marina*) culture, a sustainable food source for farmed fish.

In a closed sea water recirculation aquarium, in light and dark conditions, the prokaryotic microflora of an *A.marina* culture system is being determined, using conventional culture techniques and cloning of the environmental meta genome. The prokaryotic biodiversity is being determined by using eubacterial and taxon specific PCR primers, revealing δ-proteobacteria as a major component. Lipid biosynthesis is quantified using GC and GCMS, at different points in the sand-worm loop. The results unequivocally demonstrate net accumulation of PUFA in the dark and light.

EM 16 Are rhizobacteria able to support plant hosts with sulfur by desulfurization of aromatic sulfonates?

Achim Schmalenberger, **Sarah Hodge** & Michael Kertesz

Faculty of Life Sciences, University of Manchester, Manchester

Crop yields are often limited by the availability of sulfate, despite the presence of adequate sulfur in the soil as sulfonates or sulfate esters. This suggests that plants may benefit from associations with bacteria that can desulfurize sulfonates. *Pseudomonas putida* S-313 is known for such a plant growth-promoting effect.

Arylsulfonate-degraders were isolated from barley rhizospheres and screened for growth with toluenesulfonate. Four strains were isolated which desulfurized toluenesulfonate to *p*-cresol. These belonged either to the *Variovorax paradoxus* group or to the *Acidovorax* genus. The *V. paradoxus* type strain showed the same toluenesulfonate degrading ability as the *Variovorax* rhizosphere isolates. Homologues of the key gene for desulfurization of arylsulfonates (*asfA*) were identified in the *Variovorax* strains, and displayed high sequence identity to *asfA* of *P. putida* S-313. qRT-PCR of *asfA* in *V. paradoxus* showed >100-fold increase in gene expression when the strains were cultivated with toluenesulfonate as sole sulfur source. Future experiments will reveal if these strains are able to promote plant growth like *P. putida* S-313.

EM 17 High diversity of the desulfonation gene *asfA* in barley rhizosphere bacteria suggests an important role of rhizobacteria in plant sulfur supply

Achim Schmalenberger & Michael Kertesz

Faculty of Life Sciences, University of Manchester, Manchester

Previous work has shown that the plant growth-promoting effect of *Pseudomonas putida* S-313 is associated with its ability to desulfurize arylsulfonates. A key enzyme in this process is the oxidoreductase AsfA, and our studies have confirmed that many bacteria that use arylsulfonates as sole sulfur souce harbour *asfA* homologues.

Specific primers were designed to amplify the *asf* cluster, and used to generate clone libraries from barley rhizosphere DNA. 76 distinct RFLP genotypes were identified within 140 screened clones, and comparison of AsfA sequence fragments of 16 clones revealed a broad diversity. The majority of the sequences clustered together with AsfA from bacteria which are able to utilize toluenesulfonate as sulfur source, such as *Variovorax paradoxus* and *P. putida* S-313. Few sequences clustered with AsfA orthologues from *Sinorhizobium meliloti*, *Azotobacter vinelandii*, *Anabaena variabilis* and *Nostoc punctiforme*, which could not desulfurize toluenesulfonate under laboratory conditions.

The diversity of *asfA* in barley rhizosphere suggests that plants may benefit from associations with bacteria that can desulfurize arylsulfonates.

EM 18 The role of *Pseudomonas putida* sulfonatase and sulfatase genes in the rhizosphere

E. Fellows & M.A. Kertesz

Faculty of Life Sciences, University of Manchester, Oxford Rd, Manchester M13 9PT (Email e.fellows@postgrad.manchester.ac.uk)

Pseudomonas putida S-313 is a plant growth promoting bacterium and has been shown to promote the growth of *Arabidopsis thaliana*, barley and tomato. This plant growth promotion property has been

linked to organosulfur metabolism of the bacterium and is hypothesized to release plant available sulfur. Mutants in the organosulfur genes show reduced soil survival and do not promote tomato plant grown as the wild-type strain does. Reporter strains of *P. putida* S-313 were generated in which the promoters for known sulfonate and sulfate ester utilization genes (*asf*, *ats*, *ssu* and *sft*) were linked to the *gfp*mut3* gene as transcriptional fusions. The fusion constructs were introduced into the rhizosphere on a medium-copy vector (pBBRM1CS-3) in *P. putida* S-313. Levels of expression of the *gfp* fusions varied with sulfur supply and time post-transplantation. Different levels of *gfp* expression were displayed along the length of the root as shown by confocal microscopy and quantitative-PCR.

EM 19 Contribution of nitrifier denitrification to N_2O and N_2 emissions from soils

P. Garbeva, E.M. Baggs & J.I. Prosser

Dept of Plant and Soil Science, Cruikshank Building, St Machar Drive, Aberdeen AB24 3UU

Nitrous oxide (N_2O) emission from soil is a major contribution to the atmospheric loading of this potent greenhouse gas. N_2O may be produced in soils during aerobic nitrification, while both N_2O and N_2 are produced during denitrification and nitrifier denitrification. The process of nitrifier denitrification is undertaken completely by ammonia oxidizing bacteria (AOB). Autotrophic ammonia oxidizing bacteria detected in the soil, by both cultivation based and molecular techniques, belong to two genera, *Nitrosomonas* and *Nitrosospira,* within the Betaproteobacteria. Resent research revealed that the nitrifier denitrification is a universal trait in the betaproteobacterial AOB (Shaw *et al.*, 2005).

In the present work, sequence analysis of genes essential for N_2O emission such as *norB* (nitric oxide reductase) and *nirK* (nitrite reductase) was performed and phylogenetic analysis has been used to compare diversity of these functional genes and of 16S rRNA gene sequences in betaproteobacterial AOB. In addition, functional *norB* and *nirK* genes in cultured AOB have been compared with homologous genes in other denitrifiers and provide information of the evolution of nitrifier denitrification in bacteria.

EM 20 Selective attachment of human gut bacteria to insoluble substrates

E. Carol McWilliam Leitch[1], Alan W. Walker[1], Sylvia H. Duncan[1], Grietje Holtrop[2] & Harry J. Flint[1,2]

[1]Microbial Ecology Group; [2]BioSS, Rowett Research Institute, Aberdeen

The main substrates available to bacteria in the human colon are insoluble plant polysaccharides and endogenous mucin. The object of this study was to determine whether specialized communities colonize such substrates. PCR-amplified 16S rRNA sequences from planktonic and particulate fractions of human faecal samples were analysed. The composition of these bacterial communities differed significantly, with particulate fractions containing comparatively more clostridial cluster IV sequences and less *Bacteroides*. Similarly, the colonization of bran, starch and mucin was examined in colon-simulating fermentors. Bran-associated sequences were dominated by clostridial cluster XIVa bacteria, mainly the *Eubacterium rectale/Roseburia* group and an uncultured group related to *Clostridium hathewayi*. The most prolific starch-attached sequences were *R. bromii*, *Bifidobacterium adolescentis*, *B. breve* and *E. rectale*. Uncultured bacteria related to *R. lactaris* and *B. bifidum* were the predominant sequences from mucin. This study suggests that only certain specialized groups of bacteria provide the primary colonizers of insoluble substrates found in the colon.

EM 21 Investigating microbial diversity in an acid tar lagoon

Leanne Sunter & D. James Gilmour

Dept of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN

Hoole Bank acid tar lagoon, situated in North West England, was formed when large amounts of waste from benzoate refining were deposited in an excavated clay pit. The lagoon is up to 9 m deep and is covered by approximately 0.9 m rainwater, which has an average pH between 2.6 and 2.8. The chemical composition of the original tar is 44 % sulfuric acid, 42 % oil residues, 8 % sulfated oil residues and 6 % water. Samples have been collected from a range of sites in and around the lagoon and analysed for microbial diversity using both classical and molecular techniques. Initially, fresh lagoon samples were spread plated onto a selection of solid media. Any colonies formed were re-streaked and pure cultures obtained for further study. Continuous culture methods have also been utilized as a method for isolating micro-organisms. Several organisms have been isolated thus far and putatively identified on the basis of 16S rRNA sequences. Molecular methods employed to assess microbial diversity have focused on temperature gradient gel electrophoresis (TGGE). Initial results suggest that the biodiversity of the acid tar lagoon is fairly restricted.

EM 22 Metagenomics of nitrate reduction in the hypernutrified Colne estuary

A. Houlden[1], C. Smith[2], J.A. Wilson[1], X. Liang[1,3], D.B. Nedwell[2] & A.M. Osborn[1]

[1]Dept Animal & Plant Sciences, University of Sheffield; [2]Dept Biological Sciences, University of Essex; [3]Inst. Geochemistry, Chinese Academy of Science, P.R. China

The hypernutrified Colne estuary (Essex) receives significant inputs of NO_3^- and NH_4^+ from sewage treatment works and agricultural fertilizers. In marine sediments bacterial denitrification removes nitrogen from water reducing eutrophication, via conversion of NO_3^- to N_2O or N_2 during anaerobic respiration, with denitrification rates correlating to NO_3^- levels along the Colne. PCR analysis of nitrate reduction genes from Colne sediments has revealed such genes are distinct from those in cultured model denitrifying bacteria. In this current study, metagenomics is being used to access and characterize the nitrate reduction gene pool in Colne sediments. High molecular weight DNA from sediments was isolated, by direct and indirect lysis methods, confirming quality by PFGE. rRNA-PCR fingerprinting indicated a reduction in eukaryotic DNA content using indirect lysis, whilst prokaryotic diversity was unchanged. Following subsequent construction of a library in pCC1-Fos, the screening and initial characterization of nitrate reduction sequences in the metagenomic library is in progress.

EM 23 Metagenomics of marine polysaccharide-degrading micro-organisms

Michael J. Cox[1], Jennifer L. Edwards[1], Ian R. Joint[2], Jon R. Saunders[1], Clive Edwards[1] & Alan J. McCarthy[1]

[1]School of Biological Sciences, Biosciences Building, University of Liverpool, Crown Street, Liverpool L69 7ZB; [2]Plymouth Marine Laboratory, West Hoe, Plymouth PL1 3DH

Polysaccharides are an important source of organic carbon in the marine environment, but require cleavage into more labile substrates before they can be utilized by marine micro-organisms. Cellulose and chitin are globally the two most abundant organic polymers in the marine environment, but there is little information on the microbiology of their degradation. The project is part of the Aquatic Microbial Metagenomics and Biogeochemical Cycles consortium and

aims to investigate the diversity, abundance and activity of marine chitin and cellulose degrading micro-organisms using a principally molecular ecological approach.

Oligonucleotide probes are being produced based on domains of the modular and highly diverse polysaccharide hydrolase genes for which there is an established classification. These will be used to rapidly screen metagenomic libraries of DNA extracted from colonized polysaccharide baits recovered from coastal marine and estuarine locations. Complemented by 13C cellulose DNA stable isotope probing, metaproteomic analyses, molecular microbial ecology and traditional microbiological approaches we hope to provide a primary analysis of the aquatic biogeochemistry of these two polysaccharides.

EM 24 Novel method to investigate spatiotemporal trends in rhizosphere bacterial communities

Paul G. Dennis, Antony J. Miller & **Penny R. Hirsch**

Rothamsted Research, Harpenden

Rhizobacteria are assumed to use root exudates as their primary substrate; up-regulating their metabolism thus allowing them to function and proliferate. Carbon translocated from plant shoots to roots is predominantly allocated to meristematic tissue; therefore, as exudation is assumed to be passive, the apical region is a predicted exudation hotspot. Bacterial numbers and diversity have been speculated to reflect the exudation pattern of the associated root location.

A non-destructive, microscale sampling method for bacteria has been developed; the scale is relevant to the field of influence of zones on the root that have contrasting exudation patterns. The method revealed greater bacterial numbers in the basal region than in the apical region. Accumulation curves of bacterial types originating from apical and basal root zones revealed that the small samples inventories poorly represented the true diversity of the communities.

^{14}C labelling confirmed that carbon allocation was low in the basal region and high in the apical region; therefore predicted sites of more diverse and intense exudation were not found to correlate with bacterial community structure.

EM 25 Monitoring active bacterial communities in soil

Ian M. Clark[1], W. Richard Cookson[2], Daniel Hampshire[1] & Penny R. Hirsch[1]

[1]Rothamsted Research, Harpenden; [2]Centre for Land Rehabilitation, UWA, Australia

Soil is a reservoir for microbial DNA: whether extracellular, within viable, non-viable or dead cells; survival depends on biotic and abiotic factors. DNA-based soil microbial community studies are complicated by resilient DNA from non-viable populations. Archived (dried/stored) soils contain viable cells and information about past populations, raising questions of survival time. In situ incorporation of the thymidine analogue bromo-deoxyuridine (BrdU) enables extraction of labelled DNA arising from the actively growing bacterial population. Comparison of 16S rRNA gene profiles from total soil community and BrdU-labelled DNA, using PCR and DGGE, identifies the active population and indicates which groups were active in the past, and which are currently active.

Growth on selective agar and qPCR with specific primers compared presence and survival of *Pseudomonas* spp. in archived soils from the Broadbalk field experiment dating back to 1846. In a separate study using BrdU, the total and active microbial populations in grassland and arable soils and those under beech and pine forests, were compared.

Results confirm soil community DNA profiles do not reflect active populations.

EM 26 Manipulation of microbial biomass activity to control nutrient mineralization

G.L. Sailo, P.J. Harris & P.C. Brookes

School of Science and Environment, Coventry University, CV1 5FB

The growing medium of a plant is the most important factor in a plant's life and, soil microbes play vital roles in nutrient mineralization. However, due to the chemical and physical stabilization of organic matter, microbes can only metabolize it very slowly. Plant nutrient supply through mineralization of organic matter was investigated under greenhouse and laboratory conditions. The hypothesis of this study is that the microbial biomass in a growing media is directly proportional to the nutritional status of the media and that manipulating the biomass in order to optimize the nutrient release for plant growth was possible. The nutrient status of the media was manipulated by addition of 'trigger molecules' in the form of cellulose at different levels of carbon. A direct correlation between the amounts of C added per pot to the dry weight of plants and also with the total nitrogen uptake was found. The laboratory soil incubation showed that with increased C level of addition, the biomass carbon decreased, whilst the organic nitrogen level increased after 10 days of aerobic incubation suggesting that the addition of the cellulose accelerated the rate of mineralization. The present results show that microbial biomass can be activated by amendment of the growing medium and its activity may be managed to facilitate continuous mineralization of nutrients for the plants.

EM 27 Effect of polycyclic aromatic hydrocarbons and bioremediation protocols on bacterial community composition in soil

Lorraine Muckian, Russell Grant, Evelyn Doyle & Nicholas Clipson

Dept of Industrial Microbiology, University College Dublin, Dublin 4, Ireland

Polycyclic aromatic hydrocarbons (PAHs) are listed as priority pollutants by the Environmental Protection Agency (EPA) and constitute ~85 % of the chemical mixture creosote. Creosote has been used for wood preservation for over a century and consequently soil and groundwater underneath wood treatment plants and adjacent areas surrounding wood preservation sites are often highly contaminated with PAHs. Bioremediation is a potentially cost effective and safe alternative for the rehabilitation of contaminated sites, involving exploitation of the degradative versatility of the indigenous microflora. However, little information is available concerning the type and diversity of bacterial communities involved during the stimulation of PAH degradation, which could be beneficial in the rational design of effective bioremediation technologies. This study is concerned with the elucidation of microbial community dynamics in a microcosm study investigating phenanthrene and fluoranthene degradation, with respect to aeration and fertilization of soil. Both culture-dependent and -independent techniques were employed; enumeration of culturable bacterial on modified tryptone soya agar was performed, with molecular fingerprinting of bacterial community compositions carried out using terminal restriction fragment length polymorphism (TRFLP). Results indicated that PAH type and treatment type had the most significant effect on compositional shifts observed in the microbial communities.

EM 28 Bacterial community changes in a silage fermentation

J. McEniry[1,2], P. O'Kiely[1], N.J.W. Clipson[2], P.D. Forristal[3] & E.M. Doyle[2]

[1]Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland; [2] School of Biology and Environmental Science, UCD Dublin, Belfield, Dublin 4, Ireland; [3]Teagasc, Crops Research Centre, Oak Park, Co. Carlow, Ireland

The two methods most commonly employed for ensiling forage are conventional precision-chop silage stored in horizontal silos and

individual bales of silage wrapped in polythene stretch film. Grass ensiled in bales is more conducive to the activities of undesirable micro-organisms and detrimental changes associated with these micro-organisms cause a quantitative and qualitative loss of feedstuff, as well as creating a niche for the potential proliferation of pathogens. The objective of this study was to identify chemical and/or microbiological processes that could contribute to the fermentation differences. Grass with a mean DM of 302.2 (s.d. 27.89) g/kg and pH of 6.3 (s.d. 0.15) was subject to two ensilage treatments: ensilage in bales, and in experimental silos with precision chopping, respectively. On days 2, 6, 14, 35 and 98 of ensilage, triplicate units from each treatment were sampled. Terminal restriction fragment length polymorphism (TRFLP) was successfully applied to analyse changes in bacterial community structure over the course of the fermentation. Abundance analysis studies revealed a difference in bacterial community structure between baled and conventional silage.

EM 29 Effect of 2,4-DCP on microbial communities in soil

L. Lillis, N.J.W. Clipson & E.M. Doyle

School of Biology and Environmental Science, UCD Dublin, Dublin 4, Ireland

Chlorophenols, such as 2,4-dichlorophenol (2,4-DCP) are of major environmental concern due to their persistence in nature, and their possible carcinogenic and mutagenic properties. 2,4-DCP is produced in soil following the oxidation of the herbicide 2,4-D. It has also been found to have a toxic effect on aquatic organisms and may cause long-term effects in the aquatic environment. The objective of this work was to study the effect of 2,4-DCP on bacterial community structure in soil. A microcosm was set up containing soil contaminated with 100mg/kg of 2,4-DCP. 2,4-DCP degradation was monitored over a period of 42 days. DNA and RNA were extracted from the soil at selected time points and TRFLP was used to compare the effect of 2,4 DCP on the total bacterial community (16s rDNA) with the active community (16s rRNA) in the soil.

EM 30 Bacterial community dynamics during the biodegradation of Pristane

John A. Reynolds, Nicholas Clipson & Evelyn Doyle

School of Biology and Environmental Science, UCD Dublin, Belfield, Dublin 4, Ireland

Crude oil is vital to modern industry. Over 3.5 x 10^9 tonnes p.a. are consumed globally. Petrochemicals are transported worldwide and spillages occur frequently during transport and storage. A number of cleanup options are available when these spills do occur and one of these is bioremediation. Bioremediation has considerable potential but is an unpredictable technology as our ability to investigate microbial populations implicated in biodegradation processes has until recently been limited to culturable soil micro-organisms. However, the application of molecular biological techniques to studies of soil microbiology allow us to study micro-organisms involved in biodegradation without the constraints of organism culturability. This study is concerned with microbial community changes occurring during the biodegradation of Pristane, a branched alkane and a component of crude oil. Soil microbial community activity was assayed using a dehydrogenase assay and community structure was assessed using tRFLP. Multivariate analysis was employed to examine the effects of Pristane on bacterial community members.

EM 31 Biochemistry and genetics of Gram positive phosphonoacetate metabolizing bacteria: Involvement of carbon catabolite repression in the regulation of biodegradative pathways

S.N. O'Loughlin, G. McMullan & N.G. Ternan

School of Biomedical Sciences, University of Ulster, Coleraine, Co. Londonderry BT52 1SA

Organophosphonate compounds contain a direct covalent carbon to phosphorus (C-P) bond and most are utilized only as P sources, under condition of P limitation. Studies have shown that microbes utilizing organophosphonates as sole P sources for growth do not generally release any substrate P into culture supernatants as Pi, as the biodegradation is tightly regulated by the phosphate starvation-inducible pho regulon. We have characterized a strain of *Agromyces fucosus* that can mineralize phosphonoacetate as sole C and P source in a phosphate starvation 'deregulated' manner: in which case phosphate release to the medium is observed. Intriguingly we have, for the first time, evidence that mechanisms associated with carbon catabolite repression plays a major role in the metabolism of this organophosphonate compound. We now report the effect that carbon source has upon the regulation of transport and metabolism of phosphonoacetate and propose the mechanism by which such a phenomenon, unique in organophosphonate metabolism, occurs.

EM 32 Survival of *Campylobacter* in waterborne protozoa

W.J. Snelling[1], J.P. McKenna[2], D.M. Lecky[1], J.E. Moore[3] & J.S.G. Dooley[1]

[1]School of Biomedical Sciences, University of Ulster, Co. Londonderry BT521SA; [2]Veterinary Sciences Division, Department of Agriculture and Rural Development, Stoney Road, Belfast BT43SD; [3]Northern Ireland Public Health Laboratory, Dept of Bacteriology, Belfast City Hospital, Belfast BT9 7AD

The failure to reduce the *Campylobacter* contamination of intensively reared poultry may be partially due to *Campylobacter* resisting disinfection in water following their internalization by water-borne protozoa. *Campylobacter jejuni* and a variety of water-borne protozoa, including ciliates, flagellates and alveolates, were detected in the drinking water of intensively reared poultry by a combination of culture and molecular techniques. An *in vitro* assay showed that *C. jejuni* remained viable when internalized by *Tetrahymena pyriformis* and *Acanthamoeba castellanii* for significantly longer (up to 36 h) than when they were in purely a planktonic state. The internalized *Campylobacter* were also significantly more resistant to disinfection than planktonic organisms. Collectively, our results strongly suggest that protozoa in broiler drinking water systems can delay the decline of *Campylobacter* viability and increase *Campylobacter* disinfection resistance, thus increasing the potential of *Campylobacter* to colonize broilers.

EM 33 Uncovering the diversity of bacterial degradation of the plant hormone indole 3-acetic acid (IAA)

Johan Leveau

Netherlands Institute of Ecology (NIOO-KNAW), Boterhoeksestraat 48, 6666 GA Heteren, The Netherlands

Pseudomonas putida 1290 has previously been put forward (Leveau and Lindow, 2005, Applied and Environmental Microbiology 71: 2365-2371) as a model organism for the study of bacterial degradation of the plant hormone indole 3-acetic acid (IAA). Genes necessary for IAA metabolism have now been identified in this organism. Transposon mutagenesis revealed the involvement of a two-component regulatory system and confirmed the role of catechol as an intermediate in the IAA degradation pathway. Complementation of *P. putida* KT2440 (not able to degrade IAA) with genomic DNA of *P. putida* 1290 identified a 16-kb DNA fragment

that conferred the ability to grow on IAA as a sole source of carbon. Sequence analysis of this DNA fragment exposed several candidate genes coding for the conversion of IAA to catechol. The complementation strategy was also used to recover from metagenomic libraries DNA fragments with IAA-degrading capacity. This approach will uncover much of the diversity of genes involved in IAA breakdown and add to our understanding of the role of bacterial IAA degraders in plant-microbe interactions.

EM 34 Aerobic anoxygenic photosynthetic communities in East Mediterranean Sea screened by PCR-DGGE with *pufM*-specific degenerate primers

Natalya Yutin[1], Rotem Guez[1], Marcelino T. Suzuki[2] & Oded Béjà[1]

[1]Dept of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel; [2]Chesapeake Biological Laboratory, University of Maryland, Solomons, MD 20688, USA

Aerobic Anoxygenic Phototrophic bacteria (AAnP) are diverse and widely distributed members of the marine microbial community. We designed general new primers to target a fragment of the *pufM* gene and applied these primers in a denaturing gradient gel electrophoresis (DGGE) study of spatial and seasonal distribution of AAnPs in East Mediterranean. We used PCR-DGGE technique to compare AAnPs communities coming from different environmental samples as well as to analyze clone libraries made from the same samples. Clones from representative bands were sequenced and analyzed phylogenetically. We found significant differences in AAnP assemblages coming from deep and surface water, while horizontal and seasonal differences were less pronounced. Surprisingly, different DGGE patterns between DNA and RNA-based analyses of the same samples were observed. Unequal *puf*-operon expression of different AAnP groups may suggest that certain environmental factors turn-on and off the photosynthetic machinery and that not all AAnPs observed in a given environment are actively engaged in photosynthesis.

EM 35 Anaerobic microbial communities associated with PCB dechlorination in the Chesapeake Bay

L.K. Kelly[1], S.K. Fagervold[2], H.D. May[3], K.R. Sowers[2] & **J.E.M. Watts**[1]

[1]Towson University, Towson, MD, USA. [2]Center of Marine Biotechnology, Baltimore, MD, USA; [3]Medical University of South Carolina, Charleston, SC, USA

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants. The Chesapeake Bay has a long history of industrial activity, resulting in regions of PCB contaminated sediments. Previous studies have shown anaerobic dechlorination in the presence of bacterial species from the *o*-17/DF-1 group within the Chloroflexi. Recently, two sets of 16S rRNA gene probes were developed to detect these micro-organisms in sediments. Microbial community 16S rRNA gene analysis was performed on sediments with varying PCB concentrations. The same sites were sampled two additional times over one year to further identify the effects of varying seasonal changes on community composition. This study tests the efficiency of these primers and determines what species are involved with *in situ* dehalogenation of PCBs in a contaminated tributary of the Chesapeake Bay. The results obtained can be used to aid the assessment of the dechlorinating potential of other contaminated sites.

EM 36 Detection of novel genes in environmental bloom strains of *Escherichia coli* aiding their persistence in the external environment

Jane Littlefield & David Gordon

School of Botany & Zoology, Australian National University Canberra, Australia

Escherichia coli is a common inhabitant of the mammalian gastro-intestinal tract and commonly used as an indicator of faecal contamination within environmental systems. The success of *E.coli* as an indicator bacterium has been questioned in recent years with numerous studies reporting their persistence in the external environment in the absence of faecal input.

E. coli strains have been responsible for multiple bloom events at different sampling localities throughout Australia. Three bloom strains have been isolated with unique genotypic and phenotypic characteristics when compared to strains originating from a vertebrate host. A common characteristic of all bloom strains is the presence of a group1K capsule. It is thought that the capsule aids the strain's survival external to a host. The persistence of these strains in the external environment indicates that they exhibit a free-living lifestyle and in doing so potentially encode a suite of genes in addition to the capsule genes that enhance their survival in the secondary environment.

We employed genomic subtraction to detect novel genes in all three bloom strains. All strains were subtracted against an environmental isolate positive for the group 1K capsule. Tester-specific fragments were screened across 500 *E. coli* strains representing isolates from vertebrates and the natural environment including soil, sediment and water to determine the frequency of the unique genes. The continued isolation of genes unique to the bloom strains supports the conclusion that these strains occupy a free-living lifestyle and can persist in the absence of a vertebrate host population.

Posters

Fermentation & Bioprocessing Group

FB 01 Investigation of the role of the alternative NADH dehydrogenase in submerged cultures of *Aspergillus niger* B1-D

Andrew O'Donnell, Linda M. Harvey & Brian McNeil

Strathclyde Fermentation Centre, Dept of Bioscience, University of Strathclyde, 204 George Street, Glasgow G1 1XW

The electron transfer chain (ETC) of many filamentous fungi is highly branched, containing numerous 'core' and alternative respiratory enzymes, such as the alternative oxidase (AOX) and alternative NADH dehydrogenases. Although the precise physiological role of the alternative respiratory enzymes in fungi is somewhat obscure at present, they have been implicated in various cellular mechanisms, for example, reduced reactive oxygen species (ROS) generation in *Aspergillus niger* by enhanced activity of the alternative NADH dehydrogenases. In order to better understand the physiological relevance of the alternative NADH dehydrogenases in fungi, the effects of inhibition of these enzymes in submerged cultures of *A. niger* was investigated. Cultures were grown in the presence of a known inhibitor of the alternative NADH dehydrogenases of plants, 7-iodoacridone 4-carboxylic acid (IACA), and culture metabolism and performance was assessed. In IACA-pretreated cultures, both growth and glucose consumption rates decreased, the yield of biomass on glucose and intracellular protein concentrations increased, although intracellular ATP concentrations were lower. These distinct differences are consistent with an increased electron flux via Complex I of the 'core' ETC paired with AOX, and accordingly further work will focus on investigation of this relationship.

FB 02 Fermentation strategies for improvement of submerged cultivation of a basidiomycete fungus

Mariana Fazenda, Linda M. Harvey & Brian McNeil

Strathclyde Fermentation Centre, Dept of Bioscience, University of Strathclyde, 204 George Street, Glasgow G1 1XW

Basidiomycete fungi are an interesting source of bioactive compounds, especially polysaccharides, because of their various biological and pharmacological activities including anti-tumour, imuno-stimulating and hypoglycaemic activities. In order to obtain polysaccharides from higher fungi submerged culture has recently received much attention among researchers as an alternative to soil cultivation. Submerged culture gives rise to potential advantages of higher mycelial production in a compact space and shorter time. Problems associated with submerged cultivation in basidiomycete fungi include: high viscosity resulting from the production of exopolysaccharide (EPS) or filamentous morphology, slow growth rate, and growth variability due to a high sensitivity to environmental factors. In this work, a fed-batch strategy in a 10-L bioreactor for a *Ganoderma* species was developed. The aim was to enhance biomass and metabolite productivity using a suitable fermentation strategy based on the information given by previous batch cultures. The fed-batch strategy led to an increase in the cell productivity as well as in the EPS production. A comparison between batch and fed-batch strategies and the complete characterization of substrate profiles and kinetics gave more insight into *Ganoderma* metabolism and will allow for greater control over its cultivation.

FB 03 Monitoring of an antibiotic bioprocess using Attenuated Total Reflectance Mid Infrared Spectroscopy (ATR-MIRS)

Payal Roychoudhury, Linda M. Harvey & Brian McNeil

Fermentation Centre, University of Strathclyde, Royal College Building, Glasgow G1 1XW

At-line ATR-MIR has been employed to attain efficient monitoring and control of Streptomyces clavuligerus ATCC 27064 grown in a complex medium for the production of the economically important antibiotic, clavulanic acid. Quantitative models have been developed for the key analytes glycerol, clavulanic acid and biomass using multivariate statistical techniques, over the entire time course of the bioprocess. The models were validated externally using samples not used in the original modelling exercise. The analytes chosen here are important variables in optimization of the progress of the bioprocess. The analytical challenges posed in this study were twofold; first, to assess the feasibility of modelling multiple analytes in a complex, multi-phase fluid, secondly, to assess the complexity of the models required to predict the concentration of the selected analytes and evaluate their performance relative to the degree of complexity. Despite the challenging heterogeneous nature of the sample matrix, and the complexity of the spectral information, robust models were developed giving high correlation coefficient and low prediction error values for these key analytes.

FB 04 Adaptation to peroxide stress in submerged cultures of *Aspergillus niger* B1-D

Qiang Li, Linda M. Harvey & Brian McNeil

Strathclyde Fermentation Centre, Dept of Bioscience, University of Strathclyde, 204 George Street, Glasgow G1 1XW

H_2O_2, a potent oxidant, is an unavoidable by-product of the aerobic lifestyle, but has deleterious effects on cells. Micro-organisms grown under vigorously aerated conditions, e.g. fungi such as *A. niger* used to express recombinant proteins, may thus experience oxidative stress from reactive oxygen species (ROS). In the present study, we investigated the effects of H_2O_2 upon *A.niger* B1-D (an expression system for lysozyme). Our findings indicate that *A. niger* B1-D, can become adapted to H_2O_2. Pre-treatment of H_2O_2 at a non-lethal concentration (100 μM) in early exponential phase of *A. niger* B1-D prevented subsequent H_2O_2 killing at a lethal concentration (10mM). A number of antioxidant enzymes activities, CAT, GPx, GR, were induced by the pre-treatment, of which CAT activity was the most significant. The concentration of antioxidant GSH was also increased in 'adapted' cultures, as was the oxidized form, GSSG, accompanied by a decrease of GSH/GSSG ratio. Also, both intracellular and extracelluar non-specific proteolytic activities increased in response to H_2O_2, resulting in a simultaneous decrease of intracellular protein concentration. In 'adapted' cultures, the enhancement of antioxidant activities, enzymatic or non-enzymatic, helped the cells to detoxify H_2O_2 rapidly and completely; while the induction of proteolytic activities seemed to correlate to their tolerance to lethal levels of H_2O_2.

FB 05 NIR-spectroscopy 'at-line' model development of indicator metabolites in Escherichia coli batch fermentation processes

Parveen Kousar[1], Alvin W. Nienow[1], Graham Poulter[2], Reg Wilson[3], Henri Tapp[3] & Christopher J. Hewitt[1]

[1]Biochemical Engineering, Centre for Formulation Engineering, School of Engineering (Chemical Engineering), University of Birmingham, Edgbaston B15 2TT; [2]Specac Ltd, River House, 97 Cray Avenue, Orpington, Kent BR5 4HE; [3]Institute of Food Research, Norwich Research Park, Norwich NR4 7UA

For the cultivation of any bacterium a suitable biochemical and biophysical environment must be provided. Defined media consist of the exact nutrients (including any growth factors) needed by the organism for growth. Complex media usually provide the full range of growth factors that may be required by an organism, the exact chemical constitution of the medium is not known. The optimization of a fermentation process requires the organism to be cultivated under desirable conditions, which also depends on how well the fermentation process is controlled. Rapid analysis of substrates and indicator metabolites in a fermentation process is critical for optimal control and growth. In this study, NIR-spectroscopy has been applied to monitor the concentrations of glucose, acetate, formate, succinate, lactate, ammonium hydroxide and biomass in the cultivation of *E. coli* W3110 in defined and complex media.

FB 06 The use of multi-parameter flow cytometry for the characterization and monitoring of insect cell-baculovirus fermentations in a mechanically-agitated bioreactor

Bojan Isailovic[1], Alvin W. Nienow[1], Ian W. Taylor[2], Ryan Hicks[2] & Christopher J. Hewitt[1]

[1]School of Engineering (Chemical Engineering), University of Birmingham, Edgbaston B15 2TT; [2]AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

Bacteria and mammalian cells have been traditionally used as hosts for commercial recombinant protein production. In recent years, the insect cell-baculovirus system has emerged as a potentially attractive recombinant protein expression vehicle. This route is attractive because baculovirus-infected insect cells are able to perform post-translational modification while accommodating very abundant expression of recombinant protein. Although flow cytometry has been used widely for analysis of mammalian and microbial cell physiology and morphology, there is very little information on applications of this powerful and highly efficient technique in insect cell culture. Here we have compared cell ratiometric counts and viability of Sf-21 cell cultures using a flow cytometer to those determined by more traditional methods using a haemocytometer and the trypan-blue exclusion dye. There was good agreement between the two counting methods but the former technique proved to be a more reliable and statistically robust viability indicator.

FB 07 The use of flow cytometry to study the impact of fluid mechanical stress on *Corynebacterium glutamicum* during continuous cultivation in an agitated bioreactor

Saethawat Chamsart[1], Alvin W. Nienow[2] & **Christopher J. Hewitt**[2]

[1]BBERG Bangsaen Biochemical Engineering Research Group, Dept of Biology, Faculty of Science, Burapha University, Chon Buri 20131, Thailand; [2]Biochemical Engineering, Centre for Formulation Engineering, School of Engineering (Chemical Engineering), University of Birmingham, Edgbaston B15 2T

Anecdotal reference to 'shear damage' is frequently made to explain detrimental changes in bioprocessing when mechanical agitation and aeration are introduced into a bioreactor as compared to the non-agitated and non-aerated conditions in a shake flask. However, such references have often been based on poor experimental protocols, which do not substantiate that explanation. The impact of fluid dynamic stress whether due to fluid turbulence associated with agitation or stresses from bursting bubbles is not clearly resolved. In this work the effect of mechanical stresses generated by an extreme agitation intensity (1–25 KW/m^3) or a high aeration rate (1-3vvm) on growth parameters and cell physiology were studied during continuous cultivation. It is concluded that variations in agitation, aeration rate, or dO_2 concentrations down to about 1 % of saturation do not damage cells of *C. glutamicum* or cause a significant change in physiological response, even though the cell size was slightly reduced.

FB 08 Individuals behave differently – multi-parameter flow cytometry for monitoring *Bacillus cereus* batch fermentation processes

Andrew Want[1], **N. Helen Foxall**[1], Suart Stocks[2], Owen R.T. Thomas[1], Colin R. Thomas[1] & Christopher J. Hewitt[1]

[1]Biochemical Engineering, Centre for Formulation Engineering, School of Engineering (Chemical Engineering), University of Birmingham, Edgbaston B15 2TT; [2]Novozymes A/S, Smørmosevej 25, 2880, Bagsværd, Denmark

Microbiology is important to both human health and industry, therefore many methods have been developed to count micro-organisms in the process environment. Accurate measurements relating to cell proliferation and viability are essential if informed decisions about a process are to be made, since process performance will depend largely upon cell number and individual cell physiological state. The development of multi-parameter flow cytometric techniques in our laboratories has led to a functional classification of the physiological state of single celled micro-organisms. This classification is based on the presence or absence of an intact fully polarized cytoplasmic membrane and the transport systems across it. Using these techniques it is possible to resolve a cells physiological state, beyond culturability to include metabolic activity enabling assessment of population heterogeneity. Importantly results are available in real-time, 1-2 minutes after a sample is taken enabling informed decisions to be taken about a process.

FB 09 Mini-monoclonals: antibody fragments as targeting molecules

Christopher J. Tape

Cambridge Antibody Technology (CAT)

The large majority of current monoclonal antibody (mAb) therapeutics relies heavily on whole immunoglobulin molecules as targeting agents. This has been proven effective in many mAb treatments (e.g. Humira®), but due to the large size of whole immunoglobulins (~150kDa) and their relatively large half-lives as a result of this size, the use of smaller antibody fragments (such as scFv and Fabs) have become a popular alternative to avoid many of the side effects caused by toxin conjugated antibodies spending long periods of time in the blood. A scFv contains a VH chain linked to a VL chain by a poly Glycine peptide and like normal whole antibodies, it is specific to its epitope. However, as scFv are much smaller (~30kDa) than whole IgG's, they can be degraded much faster by the body. This can result in a much more efficient use of toxin conjugated molecules, as they do not have the chance to spend long periods of time circling the blood steam. The relatively small size of scFv also allows them to be used more efficiently as internalizing agents. When a toxin which is only effective on internal cellular machinery is used (e.g. transcription at the ribosomes) as the conjugate, it is possible to produce mAb targeting systems which could revolutionize the oncology and R&I fields.

Again due to their small size, the production of antibody fragments can be performed using microbial expression systems. Various methods exist depending on the use of toxin and inactive inclusion bodies, several of which will be displayed.

FB 10 Production of chiral epoxides by using mutant mono-oxygenases expressed in *Streptomyces lividans*

Ashlee Perry & **Thomas J. Smith**

Biomedical Research Centre, Sheffield Hallam University, Howard Street, Sheffield S1 1WB

The multicomponent binuclear iron active-centre monooxygenases, which include stereoselective alkene mono-oxygenases (AMOs) and the soluble methane mono-oxygenases, have been suggested as biocatalysts to perform a wide range of oxygenation reactions. A number of these enzymes, including the AMO from *Rhodococcus rhodochrous* B-276, have not been obtained in an active form when cloned in *Escherichia coli*, and so alternative expression hosts must be employed if genetic methods are to be used to modify their properties for specific applications. By further developing a *Streptomyces lividans* expression system for AMO that was described previously, we have created a system for directed evolution and site directed mutagenesis of AMO and expression of the mutant enzymes in *S. lividans*. By using gas chromatography-based screening techniques, we have used this system for manipulation of the enantiomeric excess of epoxides produced by oxygenation of alkene substrates.

Posters

Food & Beverages Group

FdBev 01 Investigations into the biological succession of the infant faecal microbiota

L.C. Roger[1], M.J. Kullen[2] & A.L. McCartney[1]

[1]Microbial Ecology and Health Group, Food Microbial Sciences Unit, School of Food Biosciences, University of Reading, Reading RG6 6AP, Reading; [2]Wyeth Pharmaceuticals, 500 Arcola Road, Collegeville, Pennsylvania, 19426, USA

From birth onwards, infants are challenged by a vast quantity and variety of micro-organisms. The impact of diet on the gut microbiota is of particular interest, especially in relation to the health of the infant. The objective of this work was to monitor the bacterial succession of the infant GI tract during the developmental stages of gut physiology and microbiology. The diversity and dynamics of the infant gut microbiota were analysed, both at the community level and with regard to specific populations of interest (including *Bifidobacterium*). Regular faecal samples were collected from five breast-fed infants, from 4 weeks of age to 9 months of age. Predominant bacterial groups were monitored using modern molecular techniques such as fluorescence *in situ* hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE). The data demonstrated both inter- and intra-individual differences in microbiological profiles. Furthermore, the introduction of solid foods in the diet (i.e. weaning) was shown to elicit modulation of the faecal microbiota of breast-fed infants.

FdBev 02 *In vitro* investigations of the effects of orlistat on the human faecal microbiota

L. Hoyles[1], B.P. Daggy[2], M.H. Gordon[1], G.R. Gibson[1] & A.L. McCartney[1]

[1]School of Food Biosciences, University of Reading, PO Box 226, Reading RG6 6AP; [2]GlaxoSmithKline, 1500 Littleton Road, Parsippany, NJ, 07054 USA

Orlistat (tetrahydrolipstatin) is a non-systemically acting anti-obesity agent that inhibits gastric and pancreatic lipase, thereby reducing the absorption of dietary fats by approximately 30 %. Associated with the consumption of orlistat with fatty meals, in some individuals, are side-effects related to its mechanism of action, such as oily evacuations, increased urgency and more frequent defecation. In general, side-effects tend to occur early in the use of orlistat and diminish with time, suggesting that the microbiota may have a role in tolerability of the drug. Therefore, the aims of this study were to determine (i) whether increased availability of lipids affects the composition of the faecal microbiota, (ii) whether orlistat has an effect on these profiles and/or the ability of the commensal organisms to degrade lipids, and (iii) to identify components of the microbiota that improve tolerability of orlistat. Initial batch culture experiments were run to determine the rate of degradation of olive oil (1 and 5 %, v/v) by intestinal bacteria. Gas chromatography (GC) analysis was used to follow the degradation of triglycerides to di- and monoglycerides, while density gradient gel electrophoresis (DGGE) was used to monitor changes in microbiota profiles. Subsequent batch culture experiments were run with three different doses of orlistat (10, 20 and 50 mg) and olive oil (1 %, v/v). Again GC and DGGE were used for chemical and bacterial community analyses, along with fluorescence *in situ* hybridization analysis of the bacterial populations of selected samples. The results of this study indicate that increased availability of lipids affects the composition of the faecal microbiota, and that orlistat affects the ability of the intestinal microbiota to degrade triglycerides.

FdBev 03 *In vitro* antipathogenic activities of selected probiotic strains against *Clostridium difficile* and associated mechanisms

Christos Gougoulias, Kieran Tuohy, Robert A. Rastall & Glenn R. Gibson

Food Microbial Sciences Unit, School of Food Biosciences, University of Reading, Whiteknights, Earley Gate, Reading, Berkshire RG6 6AP

Clostridium difficile is a motile bacterium frequently found in the intestinal microflora. Diarrhoea often occurs following use of broad-spectrum antibiotics, which lower the relative amount of other normal gut microflora. This allows *C. difficile* to proliferate in the large intestine releasing enterotoxins that destroy the intestinal lining and cause diarrhoea. *C. difficile* is responsible for the most severe cases of antibiotic-associated diarrhoea.

The aim of this research was to examine *in vitro* the potential anti-pathogenic effects of 17 probiotics (10 *lactobacillus* strains and 7 *bifidobacterium* strains) against *C. difficile* and to investigate the associated mechanisms. Here, we demonstrate that two strains, *Lactobacillus casei* 608 and *Bifidobacterium bifidum* 508, when co-cultured with *C. difficile* in a pH-controlled environment (pH = 6.5) exhibited strong inhibitory activity against *C. difficile*. *L. casei* 608 and *B. bifidum* 508 reduced *C. difficile* bacterial counts from 10^9 CFU/ml to 10^7 CFU/ml and from 10^9 CFU/ml to 10^6 CFU/ml, respectively in 24 h broth co-cultures, when compared with the control treatment. Moreover, we present evidence that the neutralized spent culture supernatant of strains *L. casei* 608 and *B. bifidum* 508 (*L. casei* 608-SCS and *B. bifidum* 508-SCS) contained antibacterial components, other than just the drop of the pH, active against *C. difficile*. The SCS of the two strains were concentrated at different levels by freeze drying and then filter sterilized. The *L. casei* 608-SCS and *B. bifidum* 508-SCS antimicrobial activity acted in a dose dependent manner. *L. casei* 608-SCS 1.5-fold concentrate exhibited a bacteriostatic effect, whereas *L. casei* 608-SCS 3-fold concentrate exhibited a strong bacteriocidal effect. *B. bifidum* 508-SCS 1.5-fold concentrate showed almost no effect in *C. difficile* growth whereas, *B. bifidum* 508-SCS 3-fold concentrate exerted a bacteriostatic effect. The *L. casei* 608-SCS and *B. bifidum* 508-SCS antimicrobial activity was insensitive to heat treatment at 95 °C for 40 min and was not inactivated by proteinase K and trypsin treatments. Future work will aim to identify and characterize the component(s) responsible for this antimicrobial activity.

FdBev 04 Potential prebiotic effect of bergamot (*Citrus bergamia* Risso) oligosaccharides

G. Mandalari[1], C. Nueno Palop[2], K. Tuohy[3], K. Waldron[2], C.B. Faulds[2], G. Bisignano[1] & A. Narbad[2]

[1]Dip. Farmaco-Biologico, Universita' di Messina, Messina, Italy; [2]Institute of Food Research, Norwich NR4 7UA; [3]School of Food Biosciences, University of Reading, Reading RG6 6AP

Bergamot (*Citrus bergamia* Risso) peel is the primary by-product of the processed fruit after extraction of the essential oil. If not processed further, it becomes waste and can give rise to many economic and environmental problems because of its fermentability. In this study we describe the potential prebiotic effect of enzyme solubilized pectic oligosaccharides derived from bergamot peel (BOS)

and compared this with the effect of fructooligosaccharides (FOS). Sugar analysis of the BOS indicated that it was mainly composed of galacturonic acid. Studies with pure and mixed cultures using human *in vitro* gut model showed that addition of BOS resulted in a high increase in the numbers of bifidobacteria and lactobacilli whereas the clostridial population decreased. A prebiotic index (PI) value of 6.90 was obtained for BOS and this was higher than that of FOS (6.12). These results indicate that bergamot oligosaccharides have prebiotic properties and may be used as functional food ingredients.

FdBev 05 Effects of a dietary fructo-oligosaccharide additive on gut lactic acid bacteria in finishing pigs

Narut Thanantong, Lorraine Salmon, Sandra Edwards & Olivier Sparagano

School of Agriculture, Food, and Rural Development, University of Newcastle, Newcastle upon Tyne NE1 7RU

Fructo-oligosaccharides (FOS) are used as dietary additives to improve enteric health in pigs. The intestinal lactic acid bacteria changes were studied in relation to the presence or not of a FOS additive in finishing pigs using a 16S-based PCR and nine species-specific probes in a reverse line blot (RLB) hybridization assay. Two groups of five finishing pigs were fed a cereal/soya based diet with similar composition except for inclusion of the FOS product at 0.1 % of the diet. The digesta from the ileum, caecum, and colon were collected at the time the pigs were slaughtered (125 kg liveweight), rectal samples were also collected. The RLB hybridization results showed that in the control group *Lactobacillus acidophilus* was present more frequently (73.7 % of samples) compared to the treated group (20 %). On the other hand *Lactobacillus reuteri* was more often present in the treated group (25 %) compared to the control group (5.3 %). *Streptococcus alactolyticus* was detected more often in samples derived from pigs fed a FOS diet (35 %) than for the control diet (15.8 %). Moreover, *Streptococcus hyointestinalis* was ubiquitously present in the majority of the gut samples from both groups. The present study demonstrated that a FOS diet may provide a favourable environment for a *S. hyointestinalis* growth but not for *L. acidophilus*. There was no difference between gilts and boars and between pen numbers.

FdBev 06 Different mechanisms of metabolic cross-feeding between human gut bacteria

Alvaro Belenguer, Sylvia H. Duncan, Graham Calder, Grietje Holtrop, Petra Louis, Gerald E. Lobley & Harry J. Flint

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB

Resistant starch and fructo-oligosaccharides (FOS) have been reported to result in a butyrogenic fermentation in the large intestine. It has been suggested that metabolic cross-feeding between different components of the gut microbial community may contribute to butyrate production. Metabolic cross-feeding was investigated here in co-cultures between *Bifidobacterium adolescentis* and strains of butyrate-producing bacteria that are by themselves unable to utilize starch or FOS for growth. ^{13}C-acetate or ^{13}C-lactate labelling confirmed that *E. hallii* was able to convert acetate and lactate into butyrate in such co-cultures. A non lactate-utilizing *Roseburia* strain however produced also butyrate in co-cultures with *B. adolescentis*, implicating a second mechanism of cross-feeding. We conclude that two different mechanisms of cross-feeding may operate in gut ecosystems between *B. adolescentis* and other bacteria, one due to consumption of lactate and acetate and the other due to cross feeding of partial breakdown products from complex substrates.

FdBev 07 Involvement of CspA paralogues in post-transcriptional expression of σ^s

J.L. Reyner, I.W. Hutchinson & M.P. Gallagher

Institute of Structural & Molecular Biology, School of Biological Sciences, King's Buildings, Edinburgh EH9 3JT

In cold temperatures, the survival of *Salmonella enterica* sv. Typhimurium (*S. typhimurium*) requires the action of protective cold shock protein A paralogues. These are thought to melt misfolded ribonucleic acids, facilitating their translation at low temperatures.

The general stress σ subunit of RpoS plays an important role in adapting cells to low temperatures, oxidative stress and stationary phase resistance. Under such conditions, RpoS acts as an 'emergency co-ordinator', subsequently inducing transcription of the necessary stress response genes. RpoS is regulated post-transcriptionally by at least three small RNAs (sRNAs): DsrA, RprA and OxyS.

In the present study we examine whether CspA paralogues play a role in regulating RpoS expression in *S. typhimurium*. Using a combination of mutants, with deletions in genes encoding CspA paralogues, and immunoblotting with anti-σ^s antibodies, we examine the levels of σ^s before and after exposure to relevant stresses, or when cells are in different growth phases. The results of these experiments will be reported and possible models, involving sRNAs, CspA paralogues and σ^s, will be presented.

FdBev 08 Genetic diversity among antibiotic resistant bacterial isolates collected from pigs over 21 weeks

Melanie H. Thompson, Helen M. Miller & John Heritage

Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT

Antibiotic resistant bacteria have the potential to contaminate food intended for human consumption. They may contribute to human infections originating from food. Additionally, they may act as a pool for resistance genes, thus having the potential to compromise human antibiotic therapy. This study aimed to determine the genetic diversity of antibiotic resistant bacteria isolated from pigs over a growth cycle. Eight groups of pigs exposed to different farming practices were monitored from birth until 'finishing'. Samples were grown on selective media. Randomly selected isolates were subjected to ERIC PCR. BioNumerics software was used to create a 'UPGMA' tree from the genetic profiles. Ampicillin and tetracycline resistant isolates produced nine different clusters at the 50 % similarity point, whereas the chloramphenicol resistant isolates produced three. The major cluster with five antibiotics included isolates from all eight groups of pigs from each week. However, the major cluster with the chloramphenicol resistant isolates was dominated with isolates from the indoor pigs. The results illustrate that bacteria resistant to a specific antibiotic are not members of a dominant clone and illustrate that diverse farming practices may exert different selective pressures depending on the nature of the resistance determinant being selected.

FdBev 09 Effects of hospital cleaning agents on spore formation by US and UK outbreak *Clostridium difficile* strains

S. Underwood[1], K. Stephenson[1], W.N. Fawley[1], J. Freeman[1], S. Baines[1], R.C. Owens Jr[2] & M.H. Wilcox[1]

[1]Microbiology, LGI and University of Leeds; [2]Maine Medical Centre, Portland, MA, USA

Clostridium difficile (CD) causes major nosocomial infections of antibiotic-associated diarrhoea and pseudomembranous colitis, particularly in elderly patients. Highly virulent strains have resulted in outbreaks in the US, Canada and in the UK. Evidence is lacking on the sporulation capacity of epidemic strains, a possible virulence factor of this organism, and the optimal decontamination agents to

remove CD from the hospital environment. US and UK outbreak isolates (CD027), the UK epidemic strain (CD001a) and 3 non-outbreak isolates were cultured in filter-sterilized, human faecal emulsion in the presence of 5 different hospital cleaning agents. CD027 and CD001a produced significantly more spores than non-outbreak strains. Mean sporulation capacity of all strains was exacerbated by exposure to Hospec, Dispatch and G-force, but not by Chlor-clean or Sanichlor. In conclusion, sporulation levels of outbreak CD strains exceeded those of other strains. Choice of cleaning agent may have a substantial effect on the persistence of CD spores in the hospital environment.

FdBev 10 Shiga toxin encoding bacteriophages – ecology and role in disease

L.M. Riley, D.L. Smith, P.C.M. Fogg, J.R. Saunders, H.E. Allison & A.J. McCarthy

School of Biological Sciences, University of Liverpool

Shigatoxigenic *Escherichia coli* (STEC) *e.g.* O157:H7 are a global health concern and infection can lead to symptoms including severe bloody diarrhoea. In some cases downstream sequalae such as haemolytic ureamic syndrome (HUS), haemorrhagic colitis (HC) and thrombocytopenic purpura (TTP), which can be fatal, may also develop. The major virulence determinant of STEC is the Stx toxin, which is encoded by lambdoid bacteriophage (Stx-phage). To date, over 100 serotypes of *E. coli* have been found to produce this toxin as well as other Gram-negative bacteria including strains of *Vibrio cholerae, Citrobacter freundii, Enterobacter cloacae* and *Aeromonas spp*. Over 500 environmental STEC strains, isolated from a well established farm network study site in Cheshire, were subjected to norfloxacin-based phage induction under category 3 containment. The carriage rate of inducible and infective Stx-phage was found to be ca. 20 %, which is a similar order of magnitude to the rate described for other STEC collections. The induced Stx-phage were characterized by PCR using a bank of oligonucleotide primers designed to identify suites of phage genes including those involved in infection immunity, integration, lytic infection and phage assembly. The objective is to determine the degree of heterogeneity in extant Stx phage populations and correlate the occurrence and distribution of *E. coli* hosts and specific Stx-carrying bacteriophages in the wider farm environment.

FdBev 11 Some factors influencing the photoreactivation of *Listeria monocytogenes*

M.N. Lani, J.G. Anderson, S.J. MacGregor & G.A. Woolsey

The Robertson Trust Laboratory for Electronic Sterilization Technologies (ROLEST), University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW

Photorepair, or photoreactivation, processes have been examined for several micro-organisms following exposure to continuous UV light, but there is no information available in the literature concerning the photorepair of *L. monocytogenes* following UV damage. This examination of photoreactivation involved inactivation of samples of *L. monocytogenes* using pulsed UV light from a xenon flashlamp, followed by photorepair using continuous non-UV light from a bank of fluorescent lamps in a light cabinet. The following studies were carried out, with the results documented and discussed.

The effect of the initial dose of pulsed UV light was used to establish the susceptibility of *L. monocytogenes* to UV damage. Post-exposure conditions were investigated to compare the extent of light and dark repair. The influence of growth phase (exponential and stationary phase) prior to treatment was examined and shown to have no significant effect. Other aspects studied were the influence of growth temperature on the photorepair process and observations of the motility of *L. monocytogenes* during photoreactivation.

The results demonstrate that *L. monocytogenes* exhibits an effective light-repair mechanism, and this study also confirms that dark repair of *L. monocytogenes* is negligible.

FdBev 12 Comparison of continuous and pulsed light sources for photoreactivation of *Listeria monocytogenes*

M.N. Lani, J.G. Anderson, S.J. MacGregor & G.A. Woolsey

The Robertson Trust Laboratory for Electronic Sterilization Technologies (ROLEST), University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW

Photoreactivation is a process that generates repair of ultraviolet-radiation (UV) damage in micro-organisms. Repair involves the DNA photolyase enzyme, which uses energy provided by light of wavelengths between 300 and 450 nm to reverse many types of DNA damage. Most studies of photoreactivation have relied on irradiation with continuous-wave (CW) light. This study examines the repair of UV-induced damage using both CW light from a bank of fluorescent lamps in a light cabinet, and pulsed light provided by a xenon flashlamp.

The bacterium chosen for the study is *Listeria monocytogenes* for which no data on photoreactivation has previous been reported. The pulsed xenon flashlamp which has a spectrum with an intense UV component was first used to inactivate some bacterial samples with initial concentrations of around 10^8 CFU/ml. Some samples were then subjected to CW fluorescent light with wavelengths above 320 nm and some to pulsed light from the xenon flashlamp using different long-pass filters with 50 % cut-off in light transmission at 320 nm, 345 nm, 375 nm, acrylic lid (approx 380 nm), 400 nm, 450 nm, 500 nm and 550 nm. The levels of photoreactivation obtained using these different light sources will be discussed. The results suggest that photoreactivation of *L. monocytogenes* occurs at wavelengths between 340 nm and 450 nm.

With appropriate choice of long-pass filter, the xenon flashlamp is a convenient source for both inactivation and photoreactivation studies.

FdBev 13 Effect of the enzymic hydrolysis of skim milk on the growth of *Bifidobacterium lactis* (Bb12)

Nedal A. Swidan, Ara D. Kanekanian & Adrian C. Peters

School of Applied Sciences, University of Wales Institute, Cardiff, Western Avenue, Cardiff CF5 2YB

Bifidobacterium lactis (Bb12) is an anaerobic, probiotic bacterium used increasingly in fermented diary products. There is evidence that enzymic hydrolysis of milk protein enhances the growth of the organism and that increased growth and metabolic activity of the probiotic may enhance its health effect. This study examines the effect of enzymic pre-treatment of skim milk on *Bifidobacterium* growth.

Skim milk (UHT) was hydrolysed with trypsin (enzyme to substrate ratio of 1:100) for 1 hour at 37 °C before inactivation by heating to 90°C for 10 minutes. Milk, cooled to 37 °C, was inoculated with log 3 cfu/ml *Bifidobacterium lactis* (Bb12) and incubated anaerobically at 37 °C for up to 30 hours. Controls were conducted in milk treated identically without the adding of enzyme. Population densities were estimated by plate count during lag and early logarithmic growth and lag time and specific growth rate calculated. Results showed that enzymic hydrolysis decreased lag and increased specific growth rate. These results are discussed in relation to the activity of *Bifidobacterium* in fermented milks.

FdBev 14 Probiotics and iron bioavailability

Z. Khodaii, A.M. Snelling & **H.I. Dodson**

Dept of Biomedical Sciences, University of Bradford, Bradford BD7 1DP

Low iron bioavailability is the most common cause of iron deficiency. Phytate, which forms insoluble complexes with iron, is probably the most important inhibitory factor in the diet. Phytase enzymes are often used to upgrade the nutritional quality of phytate-rich foods and feeds such as grains. Phytase activity may thus be a useful additional attribute of probiotics to be used as food supplements.

To investigate the potential of strains of lactobacilli (n= 38) and bifidobacteria (n= 5) to improve iron bioavailability, phytase activity of 43 isolates from commercial probiotic preparations, dairy products and type strains were measured. In MRS broth, enzyme activity ranged between 1.1–5.4 mU and was strain not species specific. Three lactobacilli isolates with markedly different levels of phytase activity were selected for further study in a colonic cell culture model. Iron can be stored in Caco-2 cells as ferritin, and its uptake is decreased by phytate. Ferritin accumulation in Caco-2 cell monolayers was quantified using an ELISA method, after exposure to different concentrations of iron, phytate and bacteria. The strain with high phytase activity (*L. acidophilus* NCIMB 701748) had the greatest effect in overcoming the inhibitory effect of phytate. Overall, the results suggested probiotic strains have potential for improving iron bioavailability where the diet is rich in the anti-nutrition substance phytate.

Posters

Microbial Infection Group

MI 01 The use of *in silico* techniques to identify generic vaccine candidates

Melanie Duffield[1], Chris Stubben[2], Jian Song[2], Rob Leach[2], Jason Gans[2] & Murray Wolinsky[2]

[1]Biomedical Sciences, Dstl, Porton Down, Salisbury, Wilts; [2]Bioscience Division, Los Alamos National Laboratories, Los Alamos, New Mexico, USA

The 'holy grail' of vaccine development is to identify a single antigen that would provide protection across several species of pathogens – a generic vaccine. To this aim, Dstl and the Los Alamos National Laboratories have been collaborating to develop methods, including the Toxin and Virulence Factors database (TVFac) and other techniques, to compare proteins from pathogen and non-pathogenic genomes. In this way we hope to identify proteins present only in pathogens which may therefore be involved in virulence. This poster will describe the TVFac tool more fully and comment on progress made so far.

MI 02 Immunization with *Clostridium perfringens* α-toxoid protects against heterologous α-toxins

B.N. Neeson, H.S. Atkins, G.C. Clarke & R.W. Titball

Dstl, Porton Down, Salisbury, Wilts SP4 0JQ

The *Clostridium perfringens* α-toxin is a phospholipase C with sphingomyelinase and haemolytic activity. The α-toxin is a major toxin in a number of the diseases resulting from infection with *C. perfringens* and is vital for *Clostridial* myonecrosis to occur. Protection against homologous *C. perfringens* α-toxin challenge is afforded by immunization with the C-terminus ($cpa_{247\text{-}370}$) in mice. Similar phospholipase C toxins are found in a number of other toxigenic *Clostridia* and contribute to the diseases caused by these species. In this study we present data demonstrating the *in vivo* protective efficacy of a GST-$cpa_{247\text{-}370}$ fusion protein against heterologous α-toxins from *C. absonum*, *C. bifermentans* and a variant *C. perfringens* strain. Following intraperitoneal immunization of Balb/c mice with GST-$cpa_{247\text{-}370}$ in Freund's Incomplete Adjuvant 80 % protection was observed against variant *C. pefringens* SWAN α-toxin challenge. Similarly immunization provided complete protection against *C.bifermentans* α-toxin challenge and partial protection against *C. absonum* α-toxin challenge. This demonstrates the ability of the *C. perfringens* α-toxoid to cross-protect against heterologous *Clostridial* α-toxins.

MI 03 The identification of surface proteins in *Burkholderia pseudomallei*

S. Harding[1], M. Sarkar-Tyson[1], S. Smither[1], Y. Liu[2], R. Wait[3] & R.W. Titball[1]

[1]Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ; [2]DMERI@DSO, DSO (Kent Ridge) Building, 27 Medical Drive, #13-01, Singapore 117510; [3]Kennedy Institute of Rheumatology Division, Faculty of Medicine, Imperial College London, 1 Aspenlea Road, Hammersmith, London W6 8LH

Burkholderia pseudomallei, the causative agent of the human disease melioidosis, is a pathogen endemic in the Southern hemisphere, that can cause infection leading to fatal pneumonia and septicaemia. *B.pseudomallei* is resistant to many antibiotics, and there is currently no vaccine available for use. Protein antigens present on the cell surface of bacteria are potential candidates for a sub-unit vaccine. Surface proteins are exposed to the immune system upon infection, and have been used successfully as vaccine candidates against a variety of micro-organisms This study aims to identify immunogenic surface proteins of *B.pseudomallei*, for evaluation as vaccines against melioidosis. Cells were labelled with biotin, then the outer membrane proteins were extracted and separated by 2D gel electrophoresis. Proteins that were found to be both immunoreactive and biotin labelled, were excised and identified using peptide mass fingerprinting and mass spectrometry. A panel of proteins have been identified by this method and after purification will be taken forwards as potential candidates for a sub-unit vaccine against *B. pseudomallei*.

MI 04 Polysaccharides in *Burkholderia pseudomallei*

M. Sarkar-Tyson, T. Atkins, J. Prior & R.W. Titball

Microbiology, Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ

Burkholderia pseudomallei is the causative agent of melioidosis, an infectious disease of man and animals. Currently there are no vaccines to protect against *B. pseudomallei*. Polysaccharides are essential surface components in bacteria and are the immunodominant antigens of many Gram-negative bacteria. Several vaccines are based on surface polysaccharides and have been proven to be effective in protection against disease. In *B. pseudomallei* there are two polysaccharides which have been characterized in detail, the capsule polysaccharide and lipopolysaccharide (LPS) also known as O-PS I and O-PS II respectively. Previous studies have shown that both the LPS and capsular polysaccharide are virulence determinants and have been identified as potential vaccine candidates. In order to understand the roles of these polysaccharides and to validate them as potential vaccine candidates, the O-PSII operon was disrupted. Western analysis using a monoclonal antibody against LPS demonstrated lack of expression in the mutant strain. However, silver stained gels still showed the presence of a ladder indicative of LPS in these strains, although in reduced amounts. Availability of the *B. pseudomallei* genome sequence has facilitated the identification of two further putative polysaccharide clusters, designated polysaccharide cluster III (PSIII) and polysaccharide cluster IV (PSIV). To elucidate the roles of these putative polysaccharide clusters in virulence and ability to illicit protective immunity, strains harbouring disruptions in each polysaccharide operon were constructed and characterized.

MI 05 Development of a combined vaccine for salmonellosis and necrotic enteritis in poultry

A.M. Johnson[1], B.N. Neeson[1], H.S. Atkins[1], M.J. Woodward[2] & R.W. Titball[1]

[1]Dstl, Porton Down, Salisbury SP4 0JQ; [2]VLA, New Haw, Surrey KT15 3NB

The *Clostridium perfringens* α-toxin is known to be a major virulence factor in gas gangrene in man, and more recently in necrotic enteritis (NE) in poultry. NE is currently controlled by the use of antimicrobial growth promoters in feed. However, as these are phased-out from next year, a rise in the incidence of the disease is expected. We have previously developed a vaccine against gas gangrene and it is proposed that this α-toxoid protein (C-terminal domain of α-toxin)

may also protect poultry against NE. One way to deliver α-toxoid would be orally using *Salmonella* as a vaccine vector. *Salmonella* have frequently been used in this way as they stimulate long-lived cellular and humoral immune responses. A *Salmonella*-based vaccine would have a further advantage in that it could provide protection against both NE and Salmonellosis infections. Laying chickens are routinely vaccinated against *Salmonella*, and some of the current licensed vaccine strains are used in this investigation. This study demonstrates expression of α-toxoid in a range of *Salmonella* strains.

MI 06 ABC transporters as targets for the development of anti-bacterial vaccines and therapies

H.S. Atkins[1], D.N. Harland[1], M. Tanabe[2], N.J. Walker[1], B. Byrne[2], K.A. Brown[2] & Richard W. Titball[1]

[1]Dstl, Porton Down, Salisbury SP4 0JQ; [2]Imperial College London, London SW7 2AZ

ATP-binding cassette (ABC) systems are widespread among living organisms and comprise one of the largest protein families. In bacteria, ABC systems carry out a diverse range of functions that may be required in response to the environments in which different bacteria find themselves. For example, they play roles in nutrient uptake and drug export. However, there is increasing evidence that these systems play either direct or indirect roles in the virulence of bacteria. Thus, we hypothesized that they may constitute targets for the development of anti-bacterial vaccines or therapies. In this study, protein components of ABC systems from various bacteria have been evaluated as vaccine candidates for their protective efficacy in mouse models of infection. Novel protective antigens against *Yersinia pestis* and *Burkholderia pseudomallei* have been identified.

MI 07 ORT-VAC: antibiotic-free plasmid stabilization for live vaccine delivery with attenuated *Salmonella*

Helen S. Atkins[1], Matthew W. Leckenby[2], Kate F. Griffin[1], Stephen J. Elvin[1], Rosa R. Taylor[1], M. Gill Hartley[1], Julian A.J. Hanak[2], E. Diane Williamson[1] & **Rocky M. Cranenburgh**[2]

[1]Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ; [2]Cobra Biomanufacturing Plc, The Science Park, Keele, Staffordshire ST5 5SP

Oral vaccines based on live bacteria have the potential to be effective, cheaper and easier to administer than conventional methods. However, the plasmid expressing the recombinant protein antigen is often highly unstable *in vivo*, and regulatory concerns make antibiotic resistance genes unacceptable. Therefore we have engineered a strain of *Salmonella*, SLDAPD, which utilizes Operator-Repressor Titration (ORT) technology that enables selection and maintenance of plasmids that do not require antibiotic or any other expressed selectable marker genes. We demonstrate stable maintenance of a plasmid in SLDAPD that was unstable in a conventional strain in mice. We have also successfully protected mice against bubonic plague with a single immunization using SLDAPD containing an antibiotic resistance gene-free plasmid expressing the F1 antigen from *Yersinia pestis*. ORT-VAC has thus solved key problems in heterologous antigen delivery using live vaccine vectors.

MI 08 Effect of anthrax vaccine pre-immunization on immune responses to an anthrax toxin-based intracellular vaccine delivery system

N. Walker[1], H. Flick-Smith[1], S.A. White[1], A.J. Gates[1], S.C. Rowe[2] & K.F. Griffin[1]

[1]Biomedical Sciences, Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ; [2]Institute of Animal Health, Compton, Newbury, Berkshire RG20 7NN

We have constructed a delivery system for a viral sub-unit vaccine candidate which exploits the ability of anthrax toxin to gain entry to the cytosol of the host cell. This allows the antigen to be processed and presented in the correct way to stimulate the CD8 T cell response required to provide protection against intracellular pathogens. However, there is a possibility that the delivery system would be inactive in individuals with pre-existing antibodies to anthrax toxin components. To determine if this was the case we have carried out a pre-vaccination study to determine if pre-vaccination will affect the efficacy of our delivery system.

MI 09 Recombinant expression of anthrax toxin components in non-toxigenic *Bacillus anthracis* hosts

R. Mackie, M. Herbert, G. Anlezark, J. McGlashan, M.J. Hudson, S. Charlton & B. Hallis

Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury, Wiltshire SP4 0JG

Anthrax continues to be a problem both in the case of naturally and occupationally acquired infections by *Bacillus anthracis* and recently through actual and deliberate release. Oedema Factor [EF], Lethal Factor [LF] & Protective Antigen [PA] are the three components of anthrax toxin and are also the main constituents of the anthrax vaccine.

Novel expression vectors for production of the three individual anthrax toxin components were constructed. The non-toxigenic host (*Bacillus anthracis* UM23C1-1) was employed for expression, resulting in high levels of expression of each individual native toxin component in the absence of contamination from other toxin components. Proteolytic cleavage, commonly observed with alternative expression hosts was avoided. This novel system has allowed the production of highly purified toxin components, which retain functional and antigenic properties. These reagents have been used within our group to develop a range of sensitive, specific immunoassays which are used at CEPR to characterize anthrax vaccine, its production and the immune response generated on vaccination.

MI 10 The physiology of *Bacillus anthracis* Sterne during manufacture of the UK anthrax vaccine and the effect of growth parameters on vaccine antigen production

S. Noonan, M. Herbert, M.J. Hudson, J. McGlashan, J. David, K. West, P. Jones, A. King, P. Ford, B. Hallis & S. Charlton

Health Protection Agency, Centre for Emergency Preparedness and Response, Salisbury, Wiltshire SP4 0JG

The manufacturing process of the UK anthrax vaccine has remained unchanged and uncharacterized for the past 40 years. The vaccine is a largely undefined mixture of cellular components from culture supernatants of *Bacillus anthracis* Sterne $34F_2$, which is grown in multiple 500ml static cultures in a semi-defined medium containing charcoal. Sterile culture supernatants are precipitated using alum. The majority of work carried out to date at CEPR has been directed towards characterization of the final product.

Recent work has undertaken a comprehensive analysis of the vaccine manufacturing process, concentrating on the production of toxin components by *B. anthracis* Sterne $34F_2$. Toxin components (PA, LF and EF) and S-layer proteins (Sap and EA-1) have been analysed by SDS-PAGE and Western blotting. In addition, immunoreactive LF and PA have been quantitated using qualified ELISAs. The functional activity of the three independent toxin components and the two binary toxins is described.

Additionally, we are assessing the effects of growth parameters on the production of vaccine components. Parameters under investigation include glucose levels, the role of charcoal and the

possibility of supplementing the media with cyclodextrins. These data represent the first in-depth study of UK anthrax vaccine production.

MI 11 Assays for the evaluation of immune responses to anthrax vaccines

H. Cuthbertson, T. Patel, H. Shuttleworth, R. Baker, P. Jones, S. Noonan, G. Anlezark, R. Munns, B. Hallis & M.J. Hudson

Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury, Wiltshire SP4 0JG

A range of antibody enzyme linked immunosorbent assays (ELISAs) has been developed to monitor immune responses to anthrax toxin components protective antigen (PA) and lethal factor (LF). Assays were developed using highly purified toxin components as antigens and hyper-immune antisera as reference and QC sera. These ELISAs have been qualified by assessing the following parameters: Specificity, Standard Curve and Range, Linearity/Accuracy and Precision and Intermediate Precision. Acceptance criteria for these assays including %CV for test samples, mean background and reference curve fit have also been established.

The qualified assays have been applied to measure the immune response generated following immunization with the UK licensed acellular anthrax vaccine. Further applications of these assays will also be described.

MI 12 Novel antibodies designed to evaluate cytokine expression in the guinea pig following vaccination

J. Oldfield & J. Vipond

Health Protection Agency, Porton Down, Salisbury SP4 0JG

The guinea pig aerosol infection model is often used to test new vaccines against tuberculosis, due to both the close resemblance of the resultant pathology to human disease and its discriminative properties based upon the guinea pig's high susceptibility to *M. tuberculosis* aerosols. However, a major disadvantage of this model is the lack of commercially available reagents for further immunological analysis. Therefore, we sought to generate a selection of guinea pig cytokine-specific antibodies.

By using standard molecular techniques, we have cloned, expressed and purified four key cytokines (IL-1β, TNFα, IFNγ and TGFβ) and out-sourced them for antibody production; additional cytokines are currently being expressed. The levels of antibody made for three of the cytokines were very high and their specificity is being tested at present. The generation of these antibodies will enable us to measure guinea pig cytokine production following vaccination, and ultimately provide a valuable insight into the immunological profile and mechanisms of vaccine-induced protection against disease where the guinea pig is the model of choice.

MI 13 Development of *in vitro* and *in vivo* models to study latent tuberculosis infection

E. Shaw, J. Allnutt, J. Bacon, S. Clark, J. Vipond, A. Williams & P.D. Marsh

Health Protection Agency, Porton Down, Salisbury SP4 0JG

Mycobacterium tuberculosis primary infection can lead to a condition termed latency, in which the organism evades the host immune response and remains viable, but does not cause active disease. After years of dormancy the bacteria can reactivate and establish symptomatic disease. Therefore, we sought to investigate potential gene targets involved in latency, which could be used as a new vaccine or as a diagnostic target of latent TB.

A nutrient-starved, batch culture of *M. tuberculosis* has been developed and monitored over an extended stationary growth phase (>100 days). Microarray analysis on extracted RNA indicated large shifts in the gene expression profile as nutrients were depleted. The development of an *in vivo* model of chronic persistent TB infection in guinea pigs is underway, using BCG to restrict the growth of *M. tuberculosis* in the lungs. This will enable us to examine correlation between the two models and identify genes that are recognized by the immune system, thus indicating potential vaccine or therapeutic targets.

MI 14 Comparison of mycobacterium-specific IFN-γ responses to BCG vaccination and *Mycobacterium tuberculosis* infection in rhesus and cynomolgus macaques

S. Dowall[1], S. Sharpe[1], H. McShane[2], A. Williams[1], M. Dennis[1] & P.D. Marsh[1]

[1]Health Protection Agency, Porton Down, Wiltshire SP4 0JG; [2]Centre for Vaccinology and Tropical Medicine, University of Oxford

Establishment of a primate model will assist the global effort to develop novel vaccination strategies against TB disease.

This study investigated the mycobacterium-specific IFN-γ profiles induced in two primate species (rhesus macaque, cynomolgus macaque) following immunization with BCG, or aerosol infection with *M. tuberculosis*. The frequency of IFN-γ secreting cells was determined by ELISpot and secreted IFN-γ by ELISA. The ELISpot results in primate species were compared with those measured in humans similarly immunized with BCG in a clinical trial.

The responses induced by BCG or *M. tuberculosis* infection in rhesus macaques were greater than those induced in cynomolgus macaques. Following aerosol challenge, both species made responses to antigens ESAT6 and CFP10 which are diagnostic of *M tuberculosis* infection in humans. The responses in rhesus macaques induced by BCG vaccination more closely resembled the response seen in humans than those induced in cynomolgus macaques.

MI 15 Analysis of a putative adhesion sequence in *Mycoplasma amphoriforme*

M.J. Pond[1], C.L. Ling[1], D. Webster[1], S.H.Gillespie[2], & T.D. McHugh[2]

[1]Royal Free Hospital NHS Trust, Pond Street, London NW3 2QG; [2]Royal Free and University College, Medical School, Rowland Hill, London NW3 2PF

Mycoplasma amphoriforme is a novel *Mycoplasma* that is associated with lower respiratory tract infections. We have previously reported the presence of a 300 base pair genomic fragment region with homology to the P1 family of *Mycoplasma* adhesins. Members of the *Mycoplasma* family are known to adhere to cells via adhesin proteins located within tip organelles and the presence of a P1 family homologue within *M.amphoriforme* suggests that it may adhere to human cells via a similar mechanism. Using a 'gene-walking' method we have sequenced regions surrounding the 300 bp fragment, extending the sequence to greater than 1 kbp. This region has equal nucleotide homology to the P1, P1-like MgPa adhesin genes of *M. pneumonaie*, *M. pirum* and *M. genitalium* respectively. Putative translations suggest that region also has homology to the *gatC* (subunit C of glutamyl-tRNA amidotransferase) gene family at the amino acid level. Therefore we are currently conducting further work to confirm the identity of this region and its possible function in *M. amphoriforme*.

MI 16 Failure to respond to ESAT6 stimulation in tuberculosis patients

R.J. Shorten, R.A. Breen, C. Jenkins, S.H. Gillespie, & T.D. McHugh

Centre for Medical Microbiology, University College London, Hampstead Campus, London NW3 2PF

The secretory protein ESAT-6 is the target of the ELISpot assay for the diagnosis of *M. tuberculosis* infection and thought to be essential for virulence. Our group has demonstrated that not all tuberculosis patients give a positive response to this test, despite having active disease. The response to ESAT-6 stimulation may be a combination of mycobacterial and host factors. For example, polymorphisms in the ESAT6 gene may result in variation in transcription levels.

Seventeen clinical *M. tuberculosis* isolates from patients with a range of defined responses to ESAT-6 stimulation were analysed. PCR was used to identify the presence or absence of the ESAT-6 gene, which was then sequenced to identify polymorphisms and mapped using homology-modelling methods.

All 17 strains possessed the ESAT6 gene. Initial sequence analysis showed no polymorphisms, indicating that anomalies observed in ESAT-6 stimulation response may be due to differences in DNA transcription or protein expression. We are currently assessing the level of transcription in these isolates using Quantitative Reverse Transcriptase PCR.

MI 17 Inhibition of adhesion of *Streptococcus mutans* to hydroxyapatite by enzyme-modified whey products

R.M. Halpin[1], M.M. O'Connor[2], C. Boughton[2], E.D. O'Riordan[2], M. O'Sullivan[2] & D.B. Brady[1]

[1]School of Biomolecular & Biomedical Sciences, University College Dublin, Ireland; [2]School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Ireland

Adhesion and colonization of the tooth surface by *S. mutans* is the first step in the pathogenesis of dental caries. Recent studies have shown that enzyme-modified whey products can inhibit adhesion of this organism. In the present study the effect of a range of enzyme-treated whey products on the adhesion of *S. mutans* to hydroxyapatite was examined, using a fluorescence-based microtitre plate assay.

Neither whey protein isolate nor demineralized whey reduced adhesion at concentrations up to 1mg/ml. Whey protein concentrate (WPC) 35 showed little inhibition at levels up to 0.5mg/ml but inhibited adhesion by 50 % at a concentration of 1mg/ml. Acid WPC80 significantly inhibited adhesion at levels > 0.25 mg/ml and gave complete inhibition at a concentration of 1mg/ml. Sweet WPC80 was the most effective inhibitor examined and exhibited substantial inhibition of adhesion at concentrations >0.125mg/ml. Thus, enzyme-modified whey protein products may be useful ingredients in treatments for the prevention of dental caries.

MI 18 Identification of browning related bacterial components in mushroom viral patch disease

Juluri R. Rao[1,2] & David Nelson[1]

[1]Applied Plant Science Division, Department of Agriculture and Rural Development and the School of Agriculture, [2]Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX (Email jr.rao@dardni.gov.uk)

Pseudomonas tolaasii is a principal causal agent for mushroom browning (blotch) disease. KB agar incubated with diseased mushroom virus x (MVX) tissue scrapings yielded predominantly *P. tolaasi*, *P. putida* and *P. savastoni* and plaques. Bacteria were distinguished by PCR assays for specific amplification of tolaasin genes and were confirmed for their tolaasin production (lipodepsipeptide) via culture plate precipitation assay and mass spectral analysis. Molecular analyses of RNA isolated from plaques indicated they correspond to a dsRNA carrying bacteriophage phi-6, whose host, a phytopathogen, *Pseudomonas syringae* pv phaseolicola is found in compost straw. Hybridization experiments indicated that the plaque RNA sequences shared homology with the four diagnostic MVX (browning) dsRNA bands (2.0-0.6 Kbp). A rapid mushroom bioassay for browning was performed using one-day old *Agaricus bisporus* cubes of cap tissue inoculated with single cultures of *P. tolasii* and appropriate controls. Co-infection tests indicated that all pseudomonads isolated from MVX tissues were susceptible to phi-6 phage. Our data suggests that the *P. tolasii* contributed to browning symptoms and via transfection acted as potential carriers of the dsRNAs of phage originating from *P. phaseolicola* in compost straw. A mechanism involving homologous mushroom viruses as helper viruses has been proposed leading to the culmination of alien dsRNAs as 'defective' viral particles in the diseased mushrooms.

MI 19 Anaerobic bacteria in cystic fibrosis pulmonary infection

T.R. Field[1], A. McDowell[2], S. Patrick[2], J.S. Elborn[3] & M.M. Tunney[1]

[1]Clinical and Practice Research Group, School of Pharmacy; [2]School of Medicine; [3]Respiratory Medicine Research Group, Queen's University of Belfast, Belfast BT9 7BL

Pseudomonas aeruginosa pulmonary infection is the leading cause of morbidity and mortality in cystic fibrosis (CF) patients. The reduced oxygen concentration observed in sputa couple with respiration of *P. aeruginosa* is believed to create anoxic zones within the CF lung. If the airway mucus of CF patients in anaerobic, there is the potential that these anoxic zones contain obligate anaerobes which may also contribute to the infection. In this study, we used strict anaerobic bacteriological culture techniques to anaerobic bacteria in sputa samples from CF patients colonized with *P. aeruginosa*. Potential anaerobes were checked for oxygen sensitivity and identified by sequencing of the 16S rRNA gene. To date, 57 samples representing 40 patients have been examined and anaerobes detected in 45 (79 %) of these samples. The total viable count (TVC) of the majority of anaerobes isolated from each individual patient was equal to or exceeded the TVC of *P. aeruginosa* isolated from that same patient. Anaerobes isolated have belonged to the genus' *Bifidobacterium, Prevotella* and *Veillonella*. These results indicate that anaerobes are present in the lungs of CF patients in significant numbers and may, therefore, contribute to a polymicrobial infection in the lungs of CF patients.

MI 20 Preclinical characterization of a MenA conjugate vaccine for Africa

A. Martino[1], C. Mattick[1], D. Granoff[3], K. Burkin[1], D. Crane[1], X. Lemercinier[2], C. Jones[2], I.M. Feavers[1] & B. Bolgiano[1]

[1]Division of Bacteriology; [2]Laboratory for Molecular Structure, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Herts EN6 3QG; [3]Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland, CA 94609, USA

An increased interest in preventive vaccines for Africa, effective in infants, and with long-term immunological memory, has lead to the development of a monovalent meningococcal A-tetanus toxoid conjugate vaccine by the Meningitis Vaccine Project. A preclinical characterization of the MenA conjugate vaccine during the developmental and clinical phase is presented. The vaccine was assayed by spectroscopic and chromatographic techniques; also immunogenicity was tested in mice. The starting PS and tetanus toxoid was of satisfactory quality. The molecular size profile of MenA conjugate was a broad peak with a small aggregate contribution. Low levels of unconjugated PS were detected. Also,

the carrier protein was resistant to conjugation-induced conformational changes. A MenA developmental lot was immunogenic in mice and increased antibody responses between dose2 and dose3 suggested affinity maturation. Lyophilized, adjuvanted MenA clinical batches had good lot-to-lot consistency. Sizeable anti-PS IgG and SBA titres were seen after dose2, increasing only slightly after dose3. This study confirmed the overall quality of this vaccine. Grateful thanks to Marc LaForce and the vaccine manufacturer for providing material and support.

MI 21 Inflammation as a result of heat-resistant *Campylobacter* fraction

Michael Loughlin, Ken Mellits & Ian Connerton

Food Microbiology, Dept of Biosciences, University of Nottingham, LE12 5RD

Correct cooking reduces food poisoning by elimination of viable bacteria, however *Campylobacter* and other enteric organisms have an inflammatory component highly resistant to heat.

Washed bacteria are boiled to release components examined for ability to induce NF-κB in HeLa cells by means of a luciferase reporter assay. Low molecular weight (<3kDa) protease-treated components from Campylobacter and other species were prepared and pre-treated with antibodies raised against *C. jejuni* 11168 boiled components. Live bacteria were added to HeLa and colonic HCA7 cells to determine levels of induction by live organisms and gentamicin assay performed to determine if there is a link between NF-κB activation and invasion.

All enteric pathogens examined had NF-κB activation in live bacteria and heat released components, retained 90 % activity in <3kDa fraction, and were resistant to proteinase. No correlation was determined between invasion and activation of NF-κB with some invasive species having the lowest activity, whilst pre-treatment with antibody reduced activity of components from a number of bacteria.

NF-κB activation by heat resistant component is not limited to campylobacter, nor linked to invasion of eukaryotic cells but can be blocked by antibody treatment.

MI 22 Infection control in radiology – an audit

B. Thomas, N. Vishwanath & H.C. Burnett

Manchester Royal Infirmary

Purpose It is an NHS and government priority to prevent dissemination of infection whenever possible. Healthcare associated infections (HCI) are on the rise in UK in the last decade especially MRSA and *C. difficile*. Arrangements for surveillance, prevention and control of infections are provided within the trust.

Interventional radiology departments manage numerous patients with infection risk but there is no specific infection control policy for the department of radiology. The caseload and workflow of intervention radiology department is unique as radiologist do not have direct access to patients until the patient is in the department and relies entirely on the relevant clinical details communicated by the team caring for the patient. Infection control risks may not be mentioned. This pilot prospective audit looks at existing practice and identifies pitfalls and suggests changes including an infection control policy pertaining to Interventional radiology in particular and radiology in general.

Materials and methods Audit period June 2005 to August 2005. This prospective audit was performed using a questionnaire and information on the request cards for radiological procedures. The data collected was analysed and any pitfalls will be identified

Results Analysis of:

1. Percentage of patients attending the department with infection risk
2. Adequacy of clinical communication of infection risk
3. Waiting time in the department
4. Quality of infection control practice by radiologists, nurses and other staff
5. Effect on the list of failures in current practise
6. Action plan for prevention
7. Infection control policy for radiology
8. Training of all medical and non medical staff
9. Risk classification of patients and healthcare workers
10. Promote hand hygiene and aseptic no-touch techniques

Conclusion Existing practice is scrutinized and pitfalls identified. We developed a robust and practical strategy for effective infection control in Radiology. Created a departmental policy to this effect and will reaudit following implementation.

MI 23 Cellular localization of HSP90 in *Candida albicans*

Ahmed Albarrag, Issam Alshami & James P. Burnie

University of Manchester, School of Medicine, Manchester M13 9PT

Candida albicans is both commensal and opportunistic pathogen of humans. In immunocompromised individuals it causes a wide range of infections. Heat shock protein 90 (HSP90) is now recognized as an immunodominant antigen in *C. albicans* and plays a key role in systemic candidiasis. Antibody to HSP90 is found to be associated with recovery in patients with invasive candidiasis. Mycograb, a human genetically recombinant antibody targeting fungal HSP90, has been developed by *Neu*Tec pharma therapeutic company. Mycograbe has been proved to have a synergistic activity with amphotericin B against invasive candidiasis.

In this study, this human recombinant antibody against the HSP90 was used in immunoelectron microscopy and immunofluorescence to demonstrate the cell wall location of the hsp90 in yeast and hyphal cells of *C. albicans*. Two different methods of immunoelectron microscopy were used, pre-embedding immunolabeling and post-embedding immunolabeling. The hsp90 was found on the surface, and within the cell wall of both yeast and hyphal cells of *C. albicans*. The extracellular location indicates that the HSP90 of *C. albicans* is naturally exposed and readily available to antibodies, without the necessity for cellular breakage.

MI 24 Development of a mucosal vaccine against group B *Streptococcus* using live recombinant *Lactococcus lactis*

Kate Carlisle, Jerry Wells, Ravin Seepersaud & Sean Hanniffy

Institute of Food Research, Norwich NR4 7UA

Group B *Streptococcus* (GBS; *Streptococcus agalactiae*) is the leading cause of neonatal bacterial infection in the developed world. High levels of GBS colonization and carriage in the urogenital tract of the mother is a significant risk factor in the development of the disease, which usually manifests itself in the form of septicaemia, pneumonia and meningitis. While no vaccine is currently available, maternal mucosal immunization that elicits both systemic and mucosal immunity has been proposed as the ideal vaccination strategy.

The highly conserved LrrG antigen, previously shown to provide protection against GBS infection in mice (Infect Immun. 2005; 73(3):1671-83), was cloned and expressed in the food grade bacterium *L. lactis* using an inducible expression system. When intranasally administered to mice, this live recombinant *L. lactis* vaccine elicited high levels of LrrG-specific antibody in serum and conferred marginal but significant protection against lethal infection with GBS. Given these findings and the safety profile of *L. lactis*, there is considerable potential to further develop this vaccine for use in humans.

MI 25 Comparison of virulence factors in *Campylobacter* isolates from human, poultry and ruminant sources

Karen Stanley, Thomas J. Smith, Prisca Thornton & Lucy Hoareau

Biomedical Research Centre, Sheffield Hallam University, Howard Street, Sheffield S1 1WB

Campylobacter jejuni is the most common cause of food borne disease in the UK and other industrialized countries yet the epidemiology of *C. jejuni* infection has been poorly understood. It is widely held that the eating and handling of contaminated poultry is a major risk factor for acquiring infection. However, the ruminant reservoir of *C. jejuni* is overlooked. The distribution of *Campylobacter* is described as ubiquitous but it is not clear whether all strains of *C. jejuni* are capable of causing disease. Previously molecular typing studies using PFGE and *fla* Gene RFLP revealed that *C. jejuni* isolates from ruminants were genotypically indistinguishable from those causing disease in the community.

To investigate whether there are differences in the ability of isolates from different sources to cause disease, we are using PCR to investigate the presence of known virulence genes including *cadF*, *ceu*, *iam* and *cdt* in previously typed isolates from human, chicken and ruminant sources. We are also investigating the incidence of polymorphism in the *cdt* gene. Bioinformatics tools are being used to indicate possible functional significance of the observed variations in gene sequences.

MI 26 Case notes of MRSA positive patients: a vehicle of MRSA spread

Numan Hamza[1], George Bazoua[1], Yosra Al-Shajerie[1], Elizabeth Kubiak[1], Peter James[1] & Christopher Wong[2]

[1]Royal Gwent Hospital, Cardiff Road, Newport NP20 2UB; [2]Frenchay Hospital, Frenchay Park, Bristol BS16 1LE

Aim Methicillin-resistant *Staphylococcus aureus* (MRSA) has received much publicity and remains a major problem for the health service. This study aims to determine whether case notes of MRSA positive patients can act as a vehicle for MRSA transmission.

Method A prospective study of patients with active MRSA infection identified from Department of microbiology database. Two swabs were taken from the cover of the case notes and the page of current note entry for identification of MRSA using MRSA ID (Chromogenic agar medium), Pastorex and DNase tests. Positive case notes were re-swabbed 72hrs later.

Results Fifty MRSA positive patients were identified from surgical, medical, gynaecological, High dependency and intensive care wards. Three sets of notes were MRSA positive (3/50, 6 %). MRSA was isolated from the spine of the case notes in 2 cases and from the inside of the case notes in the third case. None of the positive case notes were positive on re-swabbing at 72hrs.

Discussion This study has demonstrated that case notes of MRSA positive patients can potentially spread MRSA to both healthcare and non-healthcare workers without direct contact with infected patients. We recommend extending the universal precautions to the handling of case notes belonging to MRSA infected patients.

MI 27 Characterizing the genomic island contents of blood stream infection-associated *Escherichia coli* isolates

Ali Bin Thani, Hong-Yu Ou, Michael R. Barer & Kumar Rajakumar

Dept of Infection, Immunity and Inflammation, University of Leicester

Escherichia coli are a leading cause of blood stream infections (BSIs) in both developed and developing countries. The genomic structure of BSI-associated isolates, excluding uropathogenic strains, has been poorly studied. In this study we focussed on blood culture isolates derived from patients with no laboratory evidence of concurrent urinary tract infections. We hypothesized that the chromosomes of these isolates would comprise core genome interspersed with multiple genomic islands and prophages that lay immediately downstream of selected tRNA genes, known hotspots for the integration of horizontally acquired DNA. We utilized PCR-based interrogation coupled with chromosome walking and sequencing to investigate sixteen tRNA loci in ten *E. coli* BSI isolates. Eighty four GIs that were grouped into 35 distinct GI 'families' based on GI-extremity signatures were identified. Eleven were related to UPEC islands, with an equal number resembling elements in EAEC, EHEC, EPEC and *Shigella* pathotypes of *E. coli*. Six GIs were novel to *E. coli* and five of these did not possess any counterparts in the entire DNA database.

MI 28 Genomic island discovery in *Shigella* by tRNA site interrogation

James Lonnen, Hong-Yu Ou, Michael R. Barer & Kumar Rajakumar

Dept of Infection, Immunity and Inflammation, University of Leicester

To date only four *Shigella* genomic islands (GIs) have been well characterized. Given the possible emergence of new pathogenic variants, an efficient screen for islands dispersed amongst large numbers of strains that detects dissemination of known GIs and discovers novel elements that may play a role in virulence and/or resistance acquisition could be outstandingly valuable. We used a PCR-based screen called tRNA site Interrogation for Pathogenicity islands (tRIP) to investigate 16 tRNA genes in ten *Shigella* strains representative of the four species. These loci had been shown to be hotspots for the integration of GIs following bioinformatic analysis of sequenced genomes. Ninety eight sites were identified as potentially occupied, with at least 89 loci confirmed as harbouring islands following chromosome walking and sequencing experiments. Approximately half the islands discovered were novel to *Shigella*, highlighting the extent of the *Shigella* dispensable genome. GIs identified in this study may represent pathotype-specific markers, signatures of evolution-in-progress or merely transient passengers, and an investigation of their roles and origins would be highly informative.

Sponsored by BBSRC Studentship

MI 29 Use of a primary oviduct epithelial cell culture model to study host–pathogen interactions in avian salmonellosis

F.T.W. Jordan & **Paul Wigley**

Dept of Veterinary Pathology, University of Liverpool, Leahurst, Neston CH64 7TE

Infection of the chicken reproductive tract by *Salmonella enterica* serovars *Enteritidis* and *Pullorum* and subsequent transmission to eggs is a public and animal health issue. The factors involved in reproductive tract infection are poorly understood. Whole animal models of reproductive tract infection are difficult to reproduce consistently and require long-term maintenance of experimental animals. In order to study host-pathogen interactions in detail we have developed a primary chicken oviduct epithelial cell model. Using cells derived from mature laying hens, we demonstrate that the *S. enteritidis* phage type 4 strain 125109 attaches well to these cells *in vitro*. However, both this strain and *S. pullorum* 449/87 are poorly invasive into these cells though both strains are capable of producing reproductive tract infection *in vivo* and readily invade mammalian cells. In contrast *S. typhimurium* SL1344, which does not cause reproductive tract infections *in vivo*, invades chicken reproductive tract epithelial cells in high numbers as it does mammalian cells. These initial findings indicate that this cell model is a useful tool

in studying *Salmonella* infection of the avian reproductive tract and that the ability to invade the oviduct epithelium in high numbers is not a requisite for reproductive tract infection and vertical transmission.

MI 30 New antimicrobial agents from *Lactobacillus acidophilus*

H. Mkrtchyan

Institute of Biotechnology, Armenia, Yerevan

Antagonistic properties of the *Lactobacillus acidophilus* strains against the pathogenic micro-organisms causing diseases in digestive tract (DT) are well known. Most of these strains have the capability to produce substances with direct antimicrobial action only; organic acids, hydrogen peroxide and bacteriocins with inhibiting effect on pathogens. We elaborated the technology of cultivation of lactic acid bacteria on the nutrient media containing the cheap sources of raw materials.

The sensitivity of cell-free supernatant obtained from lactic acid bacteria were tested to proteolytic and other enzymes (proteinase K, pepsin, trypsin, catalase). The influences of pH on the active substances were estimated by adjusting the pH supernatant samples in the range 3-8. The sensitivity to temperature was determined by heating supernatant at 60-130 ^{0}C 120 min.

The supernatant was heat stable even after 130^{0}C 120 min. No significant differences were observed at pH 3-6, however a significant reduction in activity was observed at pH 7-8 values. Complete inactivation was observed after treatment of supernatant with proteinase K, trypsin, pepsin, which indicated the proteinaceous nature of the active agent.

The catalase did not affect on the activity of supernatant, indicating that hydrogen peroxide did not account for the observed antimicrobial activity.

The supernatant was precipitated by ammonium sulfate. The pellets were resuspended in Na-phosphate buffer (pH=5) and dialyzed. The active fraction was applied to a Toyopearl HW-55 which had been equilibrated with 0.01N K-Na phosphate buffer. Tree fractions had the same inhibiting effect. Active fractions were treated for heat stability. Only one was heat stable and observed antimicrobial activity after heat treatment.

The active fraction was used for the final purification by reversed-phase HPLC on a C_{18} Nucleosil column. After chromatographed on an HPLC the eluted peaks were collected and checked for activity. The results indicated that the activity corresponded to the peak eluted at 38 min. The active fraction was used for gel-electrophoresis (SDS-PAGE) in 15 % gel. The molecular weight of the biologically active substance was estimated to be 2.2 KDa.

The active substance has shown high inhibiting effect against number of EDI pathogens *Yersinia pestis, Vibrio cholerae eltor, Y. enterocolitica, Brucella abortus, Bacillus anthrocoides, etc., as well as Pseudomonas, Shigella sonnei, Salmonella, Staphylococcus aureus, Staphylococcus typhimurium, E. coli* and some other pathogenic micro-organisms.

At present we are engaged in elaboration of the technology for producing a biological preparation and small-scale production at the Institute.

MI 31 Reduction of ganglioside binding activity of tetanus toxin HC fragment destroys immunogenicity: implications for development of novel tetanus vaccines

Omar Qazi[1], Dorothea Sesardic[2], Robert Tierney[2], Zahra Söderbäck[2], Dennis Crane[2], Barbara Bolgiano[2] & Neil Fairweather[1]

[1]Centre for Molecular Microbiology and Infection, Imperial College London, London SW7 2AZ; [2]Division of Bacteriology, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire EN6 3QG

The immunogenicity of the non-toxic H_C fragment of tetanus toxin, and derivatives lacking ganglioside binding activity, were compared with tetanus toxoid after sub-cutaneous immunization of mice. H_CWT and toxoid both elicited strong antibody responses against toxoid and H_C antigens, and provided complete protection against toxin challenge. Mutants of H_C containing deletions essential for ganglioside binding elicited lower responses than H_CWT. H_CM115, containing two amino acid substitutions within the ganglioside binding site, provided reduced protection against tetanus toxin challenge compared with H_CWT, consistent with lower anti-H_C and anti-toxoid antibody titres. CD spectroscopy and intrinsic fluorescence spectroscopy showed minimal structural perturbation in H_CM115. We conclude that the presence of the ganglioside binding site within H_C may be essential for induction of a fully protective anti-tetanus response comparable to that induced by tetanus toxoid by sub-cutaneous injection.

MI 32 Permeability changes in human umbilical vein endothelial cells caused by pertussis toxin

Kirstie Manderson, Dorothy Xing & Michael Corbel

National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG

Pertussis toxin (PT) is one of the toxins that contribute to the toxicity of *Bordetella pertussis*, the causative agent of whooping cough. PT in its detoxified form is an important component of both acellular and whole cell pertussis vaccines that are currently used to prevent the disease. Vaccines with a high residual PT content have been claimed to cause neurological reactions in some children. To assure the absence of significant residual toxicity or reversion of the toxoid to toxin in pertussis vaccines, safety tests are required by regulatory authorities. Currently, the *in vivo* histamine-sensitization test is used for this purpose. The overall objective of this research is to explore the possibility of using an *in vitro* testing method for determination of PT content in vaccines, as an alternative to the *in vivo* system.

Human umbilical vein endothelial cells (HUVEC) have been used to determine the effects of PT on vascular endothelial cells. Tetrazolium salt (MTT) and lactate dehydrogenase release tests were used to determine levels of PT that were cytotoxic to cells. Further studies with HUVEC have been performed to assess the effects of PT on the permeability of endothelial cells and the consequences of histamine incorporation on permeability. PT at concentrations of 2.5 and 5µg/ml were found to increase the permeability of HUVEC. The method could potentially be used to develop a novel *in-vitro* assay as an alternative to histamine sensitization tests.

Posters

Physiology, Biochemistry & Molecular Genetics Group

PBMG 01 Exoprotein expression and its diversity in *Enterococcus faecalis*

Rachel G. Walker & Malcolm J. Horsburgh

School of Biological Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZB

Enterococci are important human pathogens, yet there is currently little understanding of the role and regulation of excreted protein synthesis in *Enterococcus faecalis*. GelE and SprE are two important secreted proteases that have been associated with infection derived strains. These proteases are positively regulated by the *fsrABC* locus, which shows homology to the density-sensing *agrABC* locus of *Staphylococcus aureus*.

We have examined the variability in *E. faecalis* exoprotein expression using laboratory strains OG1RF and JH2-2 and a set of strains from two distinct PFGE clusters with high-level ciprofloxacin and gentamicin resistance, collected in the UK and Ireland. Characterization of isolates from these two clusters revealed that each had a distinct excreted protein profile corresponding to the laboratory strains OG1RF and JH2-2. We have also seen a distinct difference in the temporal synthesis of these proteins, suggesting they are Fsr-dependent. In addition to Fsr-dependent regulation, the excreted proteome is modulated temporally by GelE and SprE as determined by inactivation of the two major secreted proteases, which has led to the identification of a GelE-regulated protein.

PBMG 02 Multi-replicon genome architecture of *Lactobacillus salivarius*

Marcus Claesson[1], Yin Li[1], Sinead Leahy[1], Carlos Canchaya[1], Jan Peter van Pijkeren[1], Ana M. Cerdeño-Tárraga[2], Julian Parkhill[2], Sarah Flynn[1], J. Kevin Collins[1], Des Higgins[3], Fergus Shanahan[1], Gerald F. Fitzgerald[1], Douwe van Sinderen[1] & **Paul W. O'Toole**[1,2]

[1]Dept of Microbiology & Alimentary Pharmabiotic Centre, University College Cork, Ireland; [2]Pathogen Sequencing Unit, Wellcome Trust Sanger Centre, Cambridge; [3]Conway Institute, University College Dublin, Ireland

Lactobacillus salivarius subsp. *salivarius* strain UCC118 is a probiotic of human origin. It has desirable properties including acid and bile resistance, adhesion to human cell lines, and potent antimicrobial activity. UCC118 has demonstrated colonization ability and therapeutic effect in human subjects. It has been shown to reduce arthritis, inflammation and tumor development in mouse models. *L. salivarius* is part of a distinct clade in 16S-based phylogenetic trees of the genus, well separated from other sequenced *Lactobacillus* species. To examine the phylogenomics of UCC118, and to provide a platform for exploring probiotic traits in this strain, we employed a shotgun approach to sequence the genome.

The *L. salivarius* UCC118 genome comprizes a 1.83 Mb chromosome, a 242 kb megaplasmid (pMP118), and two previously known smaller plasmids. pMP118 is the first reported megaplasmid in lactic acid bacteria or probiotic species. Annotation of the genome sequence indicated an intermediate level of auxotrophy compared to other sequenced lactobacilli. No single-copy essential genes were located on the megaplasmid. However, contingency amino acid metabolism genes and carbohydrate utilization genes, including three genes for completion of the pentose phosphate pathway, were megaplasmid encoded. The megaplasmid also harbored genes for the Abp118 bacteriocin, a bile salt hydrolase, a presumptive conjugation locus, and other genes potentially relevant for probiotic properties. The fact that pMP118 contains a repertoire of contingency genes that likely confer metabolic flexibility or competitive advantage, seen in the context of significant megaplasmid size variation in other strains, strongly suggests that the multi-replicon genome architecture of *L. salivarius* bestows upon the species a dynamic and flexible genetic complement that relates to environmental adaptation.

PBMG 03 Isolation and expression of oxytetracycline gene cluster in heterologous hosts

Awatif Almuteirie, Paul R. Herron & Iain S. Hunter

Dept of Bioscience, University of Strathclyde, Glasgow G1 1XW

Streptomcyes rimosus is a Gram-positive filamentous actinomycete which produces of the broad-spectrum aromatic polyketide antibiotic oxytetracycline (OTC). Antibiotic production is encoded by 34kb gene cluster, which is located 600kb from the end of the unstable linear chromosome. The *otc* gene cluster consists of a core of otc biosynthetic genes flanked by resistance genes. For the isolation of the *otc* gene cluster, a cosmid library was constructed using the vector pSupercos1 and around 4000 cosmid colonies were obtained. The library was screened by Southern blot hybridization using the two resistance genes as probes to identify colonies containing the entire *otc* gene cluster. Such a cosmid containing the entire *otc* cluster was modified by *in vitro* transposition using an engineered transposon which contains an origin of transfer (*oriT*), the attachment site and integrase of actinophage-ϕC31 and a selectable marker. This modified cosmid was then conjugated from *E. coli* to a *Streptomyces rimosus* strain in which the *otc* cluster has been deleted and to different *Streptomyces* species to study the expression of the *otc* gene cluster in heterologous hosts.

PBMG 04 Structural and functional studies of the novel TRAP family of transport proteins

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

[1]Dept of Biology (Area 10), University of York, PO Box 373, York YO10 5YW; [2]Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT

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 in 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* are being overexpressed and purified for biochemical and biophysical studies, with aim of elucidating the protein-protein interactions between the membrane components and also their interactions with the ESR.

PBMG 05 Completing the *Escherichia coli* transport phenotype: characterization of extracytoplasmic solute receptor dependent ABC transporters

Richard Horler & Gavin Thomas

Dept of Biology, University of York (Area 10), PO Box 373, York YO10 5YW

There are over 70 ABC transporters annotated in *E. coli* yet roughly 50 % are uncharacterized. Using a medium throughput method to clone, express and purify the extracytoplasmic solute receptor (ESR) proteins from uncharacterized *E. coli* ABC transporters we have been able to identify ligands for two of the remaining systems by tryptophan fluorescence spectroscopy and electrospray mass spectroscopy. We will report he ligand binding properties of these ESRs and attempts to characterize their physiological roles in *E. coli*.

PBMG 06 The *Haemophilus influenzae* sialic acid TRAP transporter SiaPQM

Emmanuele Severi[1], Gaynor Randle[2], Derek Hood[2], Richard Moxon[2], Dave Kelly[3] & Gavin Thomas[1]

[1]Dept of Biology, University of York, York YO10 5YW; [2]Molecular Infection Diseases Group, University of Oxford, Dept of Paediatrics, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS; [3]Dept of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN

The bacterial human pathogen *Haemophilus influenzae* possesses the genes for three tripartite ATP-independent periplasmic (TRAP) transporters. TRAP transporters are a novel group of prokaryotic solute transporters that typically contain three components: an extracytoplasmic solute receptor protein, a 12-transmembrane helix (TMH) integral membrane protein of the ion transporter (IT) superfamily, and 4-TMH protein of unknown function. We have previously demonstrated that the *H. influenzae* SiaPQM TRAP transport system is a sialic acid transporter necessary for lipopolysaccharide (LPS) sialylation in *H. influenzae* and for increased resistance to the bactericidal action of normal human serum. Together with the occurrence of the *siaPQM* genes in pathogenic strains of *H. influenzae* these results underline the importance of elucidating the physiological role of the SiaPQM system in *H. influenzae* as well as the mechanism of sialic acid uptake.

We now report our characterization of the function of the SiaPQM sialic acid transporter in whole cells of *H. influenzae* strain Rd, both in the WT strain and in mutant derivatives defective in sialic acid metabolism. Furthermore we report structure-function studies of SiaPQM heterologously expressed in *E. coli*.

PBMG 07 Mutational analysis of ϕC31 integrase

Paul Rowley & Maggie Smith

University of Aberdeen

The *Streptomyces* temperate bacteriophage ϕC31 encodes an integrase required for site-specific recombination with the host chromosome. Control of integration versus excision is essential for efficient progression through the lytic and lysogenic life cycles. The observation that integrase can only recombine *attB* and *attP* (i.e. integration) *in vitro* to form *attL* and *attR* (the substrates for excision) indicates that the recombinase itself is highly regulated. Excision does not occur *in vitro* because integrase fails to form a synapse with *attL* and *attR*.

ϕC31 integrase is a member of the serine recombinase family and has, at its N-terminus, a resolvase/invertase-like catalytic domain. Currently the structure of the large C-terminal domain is unknown and the N-terminal domain is entirely informed by the structures of γδ resolvase. In order to understand the basis for directional control of ϕC31 integrase we have directed our attention to elucidating residues that are important in forming the synaptic interface. We propose that amino acids required for synapsis in ϕC31 integrase may be in equivalent positions to those that form the synaptic interface in γδ and Tn3 resolvases. To test this we isolated a range of ϕC31 integrase mutants that are either defective in *attP* and *attB* recombination or hyperactive by demonstrating recombination with *attL* and *attR* (excision). The defective mutants have substitutions in both N- and C-terminal domains. The hyperactive mutants isolated to date all map to a position in the uncharacterized and non-conserved C-terminal region of ϕC31 integrase. These observations suggest that the C-terminal domain is directly or indirectly involved in forming the synaptic interface and we are currently testing this with further biochemical characterization.

PBMG 09 Molecular variation of downy mildew pathogenicity factors

Julia Meitz[1], Laura Rose[2], Rebecca Allen[1], Peter Bittner-Eddy[1] & Jim Beynon[1]

[1]Warwick HRI, University of Warwick; [2]University of Munich LMU, Germany (Email Julia.Meitz@warwick.ac.uk)

Downy mildew – infected *Brassicaceae* show disease resistance visible as a hypersensitive response (HR); a localized necrosis killing the infected host cell follows. Some potential novel *H. parasitica* (At) pathogenicity genes show high levels of amino acid variability between *H. parasitica* isolates, implying positive selection for change. Five of these novel *Hyaloperonospora parasitica* (host specific to *Arabidopsis thaliana*) genes were assessed for allelic variation by sequencing alleles from up to 16 pathogen isolates. Analyses of levels of variation allowed grouping of genes into those showing diversifying, neutral or purifying selection. One of these genes was shown to be the avirulence gene *ATR13*. I am now attempting to isolate orthologues of these genes from isolates of *H. parasitica* from *Brassica oleracea* (Bo) to determine if the same evolutionary pressures are being exerted and if they are recognized by the host.

PBMG 09 Cloning and characterization of an (HSL) acetylesterase from *Streptomyces coelicolor* A3(2)

Sameh Soror & John Cullum

LB Genetik, University of Kaiserslautern, Postfach 3049, 67653 Kaiserslautern, Germany

Because of their rich secondary metabolism, *Streptomyces* species offer a relatively untapped source of interesting esterase enzymes. The gene *estA* was annotated as a potential lipase/esterase in the genome sequence of *S. coelicolor* A3(2). Sequence comparison showed that it belongs to the HSL family whose founder member is the human hormone sensitive lipase. EstA was cloned and expressed in *E. coli* as a His-tagged protein. The protein was purified and could be recovered in its non-tagged form after digestion with factor Xa. The enzyme was only active on acetate esters and not on larger substrates. The pH optimum was 7.5 and the enzyme was stable over a pH range 6.0-10.5. The activity increased with temperature showing maximum activity at 55 °C. It was thermostable, with a half-life at 50 °C of 4.5 hours. Comparison with other members of the HSL family suggested targets for site-directed mutagenesis. Mutants showed changes in pH and temperature sensitivity and substrate specificity.

PBMG 10 Purification and biochemical characterization of a putative D-2-hydroxyacid dehydrogenase from *Rhizobium etli* CNPAF512

Maxime Ndayizeye, Karen Vos, Maarten Fauvart, Kristien Braeken, Jos Vanderleyden & Jan Michiels

Centre of Microbial & Plant Genetics, Katholieke Universiteit Leuven, 3001 Heverlee, Belgium

Rhizobia are gram-negative aerobic bacteria belonging to several genera including *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Bradyrhizobium* that form N_2-fixing symbioses with legume plants. Through a study of symbiotic genes of *Rhizobium etli* CNPAF512, we identified a gene encoding a putative hydroxyacid dehydrogenase protein. This protein was highly homologous to bacterial D-2-hydroxyacid dehydrogenases, D-glycerate dehydrogenase and D-3-phosphoglycerate dehydrogenase. The 2-hydroxyacid dehydrogenase from *R. etli* was expressed in *Escherichia coli* as a His-tag fusion protein and purified with Ni-metal affinity chromatography. From SDS-PAGE, the molecular mass of this protein was estimated to be 37 kDa, which is in agreement with the calculated molecular mass of the protein deduced from the nucleotide sequence. In vitro, biochemical studies using recombinant protein showed that this enzyme exhibited activity towards phenylpyruvate, 2-oxovalerate, 2-ketobutyrate, pyruvate, oxaloacetate and hydroxypyruvate. The determination of the kinetic parameters showed that phenylpyruvate was the preferred substrate among all 2-ketocarboxylic acids tested. When phenylpyruvate was the substrate, the optimal pH for the enzyme was neutral (pH 7.0). The conversion of phenylpyruvate into phenyllactate was confirmed by HPLC. This new enzyme can be used for the production of D-hydroxycarboxylic acids (e.g. D-phenyllactic acid).

PBMG 11 Task distribution between the EAL-domain proteins in the regulatory network controlling multicellular behaviour of *Salmonella* Typhimurium

Roger Simm, Astrid Lusch, Kader Abdul, Mats Andersson & Ute Römling

Microbiology and Tumorbiology Center (MTC), Karolinska Institutet, Box 280, SE-171 77 Stockholm, Sweden

See page 28.

PBMG 12 Characterization of the C-terminal thioesterase domains in arthrofactin synthetase: a prototype of two internal thioesterases

Niran Roongsawang, Kenji Washio & Masaaki Morikawa

Division of Biosphere Science, Graduate School of Environmental Science, Hokkaido University, Sapporo 060-0810, Japan

Nonribosomal peptide synthetases (NRPS) are modular multifunctional enzymes that synthesize an incredibly diverse set of biologically active peptides and lipopeptides. Macrocyclization is occurred at the last step of synthesis and is usually catalyzed by a single C-terminal thioesterase (Te)-domain. Arthrofactin synthetase (Arf) from *Pseudomonas* sp. MIS38 represents a new type of NRPS, which consists of three large NRPS subunits, ArfA/B/C, containing unique two C-terminal Te-domains with no epimerization domain. In order to analyze the function of ArfCTe domains, site directed mutagenesis at the serine residue in GXSXG motif, S91 and S383, was performed. Productivity of arthrofactin in the mutants was analyzed by HPLC/MS. Peaks corresponding to arthrofactin and its derivatives were found in the sample from MIS38, while they were completely missing in that from mutant S91A. Interestingly, the mutation at S383 affected on the structure of arthrofactin. These results suggested that S91 residue is essential for cyclization of the peptide and S383 may be involved in the control of fatty acid chain lengths of arthrofactin.

PBMG 13 Analysis of a histidine-containing phosphotransfer protein-mediated phosphorelay pathway in *Pseudomonas aeruginosa*

Jye Lin Hsu & **Hwan You Chang**

Institute of Molecular Medicine, National Tsing Hua University, 101 Kuang Fu Road, 2nd Sec. HsinChu, Taiwan 30016

The histidine-containing phosphotransmitter (Hpt) domain, normally present in sensor kinases, has been implicated in multistep phosphorelay. In *Pseudomonas aeruginosa* PAO1, 3 Hpt proteins that do not contain any kinase domain were noted. The aim of this study is to identify the phosphorelay pathway regulated by the Hpt proteins. Initially 12 hybrid sensors, 3 Hpt and 9 orphan regulators were expressed, purified and subjected to in vitro phosphorelay assays. Five of the sensors displayed an autophosphorylation activity and 3 (PA1611, PA1976 and PA2824) of them could further transfer the phosphate group to HptB (PA3345). Neither HptA nor HptC could be phosphrylated by any of the tested sensors. Furthermore, HptB could transfer the phosphate to PA3346 but not to other tested regulators. PA3346 is a ser/thr phosphatase-like protein, which is likely to regulate PA3347, a protein resembles anti-sigma factor antagonists. Analysis of HptB, PA3346 and PA3347 mutants indicate that the HtpB phosphorelay pathway is involved in regulation of biofilm synthesis, twitching motility, swimming and chemotaxis responses.

PBMG 14 Expression of the ligninolytic enzyme-coding genes during solid-state-fermentation of edible mushroom *Pleurotus pulmonarius*

Kam-Che Chan & Siu-Wai Chiu

Dept of Biology, Chinese University of Hong Kong, Shatin, Hong Kong SAR, China

Pleurotus pulmonarius secretes laccase (lac) and manganese peroxidase (MnP). The expression profiles of its 5 *mnp* and 8 *lac* genes during solid-state-fermentation were characterized by multiplex RT-PCR. All genes were unexpectedly and constitutively expressed in all developmental stages examined. Enzyme activities were maximal during vegetative growth and declined sharply at fruiting stage and raised in the intercrop period between two flushes. This indicates the important role of ligninolytic enzymes in degrading substrate for providing resource for growth and fruiting. Gene *lac*6 had the highest transcription level while *lac*2, *lac*3 and *lac*7 dropped their expression levels in mycelial compost along development. Caps were characterized with higher transcript levels of *lac*1, *lac*4 and *lac*8. Genes *mnp*1 and *mnp*5 maintained low constitutive expression levels throughout development while remaining *mnp* genes showed high transcription levels in both mycelial stages and mushrooms. Good correlation existed between lac activity and *lac*5 and *lac*6 transcriptional levels. In contrast, no correlation was observed between *mnp* transcript levels and MnP activities. This may reflect post-transcriptional regulation.

PBMG 15 The role of the sigma factor σS in the tripartite lifecycle of *Photorhabdus luminescens* TT01

R.J. Watson, Y. Wei, I. Glazer & D.J. Clarke

University of Bath

Photorhabdus luminescens TT01 is a bacterium of the family *Enterobacteriacae* that has both the ability to kill insect larvae and to form a symbiosis with the nematode, *Heterorhabditis*. From the bacterial point of view exponential growth has been shown to be

important for pathogenicity and stationary phase to be important for symbiosis. As part of our ongoing molecular analysis of symbiosis we constructed a knock-out of *rpoS,* a gene encoding σS, a sigma factor identified in other enteric bacteria to be important in regulating gene expression in stationary phase. This Δ*rpoS* mutant showed increased sensitivity to hydrogen peroxide induced oxidative stress and viability was also severely reduced over 72h growth. Pathogenicity and nematode growth and development were all unaffected in the Δ*rpoS* mutant. Subsequent analysis revealed a major deficiency in the fraction of total nematodes that were colonized with Δ*rpoS* bacteria. These results suggest that σS is not required for the production of symbiosis factors but is likely to be required to maintain bacterial viability in the insect cadaver.

PBMG 16 Characterization of VVA0326 and VVA0328 respectively encoding a GGDEF and an EAL domain protein in *Vibrio vulnificus*

Gary Chang & **Hwei-Ling Peng**

Dept of Biological Science and Technology, National Chiao Tung University, 75 Po-Ai Street, Hsin Chu, Taiwan 30050

In *Vibrio vulnificus* YJ016 genome, a gene cluster highly homologous to the three-component system VieSAB of *V. cholerae* was identified. This consists of VVA0329, encoding a VieS homolog, VVA0328 and VVA0327, exhibiting homology with the N- and C-terminal parts of VieA, respectively, and VVA0326, encoding a protein with a GGDEF domain. Unlike VieA, that has been reported to negatively regulate expression of vps and biofilm in *V. cholerae*, deleting the EAL domain in VVA0328 conferred no change in *V. vulnificus* phenotype. On the other hand, the VVA0326 mutant carrying a deletion of the GGDEF domain synthesized less capsular polysaccharide and biofilm, and produced a larger swarming zone in comparison with the wild type. The recombinant *E. coli* JM109 expressing either VVA0326-GFP or VVA0328-GFP exhibited green fluorescence at the cell poles implying a role in establishment of the signaling gradient for compartmentalization. Intracellular levels of c-diGMP in these bacteria are being determined for the roles of the proteins in modulating the second messenger.

Posters

Plenary

PS 01 Characterization of the *Ureaplasma parvum* AmtB1 protein

Tim Fulford & Mike Merrick

John Innes Centre, Colney Lane, Norwich, Norfolk NR4 7UH

The pathogenic Mycoplasma *Ureaplasma parvum* is believed to possess a unique metabolism whereby the hydrolysis of urea, and subsequent export of ammonium, is used to generate the proton motive force and thereby ATP. Export of ammonium has been proposed to occur via two ammonium transport (Amt) proteins identified from the *U. parvum* genome sequence. However recently solved structures of the *Escherichia coli* AmtB protein suggest that Amt proteins function as gas channels for the import of NH_3. Multi site-directed mutagenesis of *U. parvum* $amtB_1$ was used to overcome the unusual codon usage characteristic of mycoplasmas and to enable expression of $amtB_1$ in *E. coli* and *Saccharomyces cerevisiae*. In *S. cerevisiae,* growth complementation and ^{14}C-methylammonium transport assays demonstrated that $UpAmtB_1$ is a functional Amt protein that can facilitate ammonia uptake when heterologously expressed. These experiments also suggest, albeit indirectly, that $UpAmtB_1$ may be able to function bi-directionally. The property of bidirectional ammonia movement has also been proposed for the Rhesus proteins, the Amt family representatives in humans and other animals.

PS 02 Hypervariability of a phase variable type III restriction-modification system

Chris Bayliss, Martin Callaghan & Richard Moxon

Molecular Infectious Diseases Group, Weatherall Institute for Molecular Medicine, University of Oxford, Oxford OX3 9DU

Phase variation refers to the rapid and reversible generation of phenotypic variants. The *mod* gene of *Haemophilus influenzae* strain Rd is phase variable due to the presence of a 5'AGTC repeat tract present in the reading frame (De Bolle *et al.* 2000 Mol. Micro. 35 p211). This *mod* gene forms an operon with a *res* gene indicating that this locus encodes a type III restriction-modification system. These genes were sequenced from a set of 16 diverse non-typeable *Haemophilus influenzae* strains. The central portions of the *mod* gene could not be aligned demonstrating that this portion of the gene is hypervariable whilst other regions of *mod* and all of *res* exhibited limited variation. Thus variation in the *mod* gene is generated by both intragenomic (phase variation) and intergenomic (natural transformation) mechanisms. The significance of this variation is discussed relative to the potential functions of these genes, i.e. effects on gene expression (Srikhanta *et al*., 2005 PNAS 102 p.5547), transformation frequency and bacteriophage resistance.

PS 03 Horizontal gene transfer and evolution of the *Streptomyces* chromosome

Atiya Ul-Hassan, S. Tolba, D. Kallifidas & E.M.H. Wellington

Dept of Biological sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL

Streptomyces species are well known for their ability to produce an array of secondary metabolites which have antibacterial, antifungal and antitumour properties. The ability of one strain to produce different compounds which have both clinical and agricultural importance led to over speciation of the genus. A number of classification methods were developed to overcome this problem. Williams *et al.,* (1983), carried out a comprehensive study of the genera using numerical taxonomy. This enabled *Streptomyces* species to be clustered based on the phenotype, however this method was unable to accurately resolve the relationships between closely related strains. It is widely accepted that molecular methods are required to define the intrageneric relationships between closely related strains and to improve the understanding of species relatedness within the *Streptomyces* genera.

The linear chromosome of *Streptomyces* species is genetically unstable and is highly prone to genomic rearrangements including insertions, deletions, amplifications and horizontal gene transfer events which occur mainly in the terminal regions. The aim of this research is to define the phylogenetic structure of the Cluster 21 *S. violaceoruber* clade by carrying out an extensive phylogenetic analysis using house-keeping genes (16S rDNA, *recA*, *trpB* and *gyrB*) and adaptive genes (*strA*). This study has identified the transfer of the streptomycin gene cluster from a putative *S. griseus* (Cluster 1) source to ASB37, a Cluster 21 soil isolate. The possible location of the cluster was determined in ASB37 by PCR and sequencing. This analysis identified the presence of a number of genes showing high sequence similarity to membrane proteins of *S. coelicolor* M145 being located in the middle of the Sm cluster in ASB37. The structure of this cluster in ASB37 will be discussed. RT-PCR and Western blot analysis has shown this cluster to be silent and DNA/DNA microarrays have been carried out to determine the structural integrity of ASB37 chromosome. This has revealed a number of significant differences between this strain compared to *S. coelicolor* M145.

PS 04 Biogeography of alkalitolerant streptomycetes

S. Antony-Babu, J.E.M. Stach & M. Goodfellow

Division of Biology, University of Newcastle, Newcastle-upon-Tyne NE1 7RU

Biogeography deals with questions relating to the distribution of species, usually at regional levels, but sometimes at the continental scale. The cosmopolitan hypothesis postulates that 'Everything is everywhere-but nature selects'. To this end we are testing this hypothesis on a regional spatial scale. A transect of samples were collected from sand-dune and littoral systems at Ross Links, County Northumberland. Many alkalitolerant streptomycetes, isolated on starch-casein-nitrate agar at pH 10.5, were dereplicated by assigning them to groups based on their ability to produce pigments on oatmeal agar and melanin production on peptone-iron-yeast-extract agar. Pearson and UPGMA cluster analyses were done on the whole-genome fingerprint of the *rep*-PCR profile of the isolates using BIONUMERICS. Excellent congruence was found between colour and the fingerprint groups. The fingerprint analysis showed that alkalitolerant streptomycetes show strict site endemism. Partial sequences of the 16S rRNA genes were obtained from representatives of the defined groups, and the resultant phylogenetic tree showed that six isolates from four different sites showed 100 % similarity. These strains were the subjects designed to investigate species endemism over small geographical scales.

PS 05 Do taxonomically related strains produce similar antibiotics?

Y. Kumar[1], S. Robinson[2], E. Lacey[2], A.C. Ward[1] & M. Goodfellow[1]

[1]Division of Biology, University of Newcastle, Newcastle upon Tyne NE17RU; [2]Microbial Screening Technologies, Smithfield, Australia

Actinomycetes are an excellent source for novel antibiotics. The discovery of novel structures compared to the number of actinomycetes screened declined over the past two decades, making it increasingly necessary to understand the distribution of secondary metabolites across bacterial diversity. One hypothesis is that taxonomic diversity is a surrogate for chemical diversity. The alternative hypothesis is that taxonomically closely related strains produce structurally different kind of compounds, as exemplified by the proposition that *Streptomyces hygroscopicus* strains produce 46 structurally different compounds. The aim of the present investigation was to determine S. *hygroscopicus* strains produce different metabolites. It was evident that four of the ten tested strains were misclassified so much so that they fell outwith the *S.violaceusniger* clade; a phyletic line that encompasses bona fide *S.hygroscopicus* strains. The four authenticated strains produced the same metabolites. The remaining strains did not produce metabolites characteristic of the *S.violaceusniger* clade. These preliminary results suggest that in the case of *S.hygroscopicus* strains taxonomic diversity is a surrogate of chemical diversity.

PS 06 MLST analysis of enterotoxigenic *Escherichia coli*

Sue Turner, Roy Chaudhuri, Charles Penn, Mark Pallen & Ian Henderson

University of Birmingham, Edgbaston, Birmingham B15 2TT

Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of *E. coli*-mediated human diarrhoea worldwide, particularly affecting children living in the developing world and visiting travellers and military personnel. ETEC is also major pathogen of cattle and neonatal and post-weaning piglets. Plasmid encoded colonization factors and enterotoxins are implicated in the major virulence mechanisms of ETEC although more recently chromosomally encoded genes have been associated with increased adherence to and invasion of epithelial cells. MLST analysis based on seven housekeeping genes, *adk, fumC, gyrB, icd, mdh, purA* and *recA* was used to look at the population structure of ETEC. Isolates were identified in the A, B1, B2, D and E groups of the *E. coli* phylogenetic structure, suggesting that no specific genetic background is required for the acquisition of plasmids encoding the colonization factors and toxins. This data was combined with, data on the distribution of the toxins and chromosomally encoded putative virulence factors to reveal the population structure of ETEC.

PS 07 A novel strategy for identification of genomic islands by comparative analysis of the contents and contexts of tRNA sites in closely related bacteria

Hong-Yu Ou, Michael R Barer & Kumar Rajakumar

Dept of Infection, Immunity and Inflammation, University of Leicester

We devised software tools to systematically investigate the contents and contexts of bacterial tRNA and tmRNA genes, which are known insertion hotspots for genomic islands (GIs). The strategy, based on MAUVE-facilitated multigenome comparisons, was used to examine 87 *E. coli* MG1655 tRNA and tmRNA genes and their orthologues in *E. coli* EDL933, *E. coli* CFT073 and *S. flexneri* Sf301. Our approach identified 49 GIs that mapped to 18 tRNA genes. All these GIs had many strain-specific CDS, anomalous GC contents and/or significant dinucleotide biases, consistent with foreign origins. Our analysis demonstrated marked conservation of sequences flanking both empty tRNA sites and tRNA-associated GIs across all four genomes. *In silico* PCR analysis based on conserved flanking regions was also used to interrogate hotspots in another eight completely or partially sequenced *E. coli* and *Shigella* genomes. The tools developed are ideal for the analysis of other bacterial species and will lead to *in silico* and experimental discovery of new genomic islands.

PS 08 Diversity of anaerobic cellulose-degrading microbial communities in landfill

James E. McDonald, R.J. Lockhart, H.E. Allison & A.J. McCarthy

School of Biological Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZB

Cellulose is the most abundant carbon polymer in the environment and is a major component of landfill waste. However, little information is available on the micro-organisms responsible for primary cellulose degradation in anaerobic environments such as landfill sites. Difficulties in the isolation and cultivation of strictly anaerobic micro-organisms can be circumvented by the use of molecular biological techniques. We are using a molecular approach to characterize the activity and diversity of cellulose-degrading micro-organisms in a number of municipal solid waste (MSW) landfill sites. PCR primers targeting the 16S ribosomal DNA of known groups of cellulose degraders, including members of the genus *Clostridium*, are applied to DNA and reverse-transcribed RNA extracted from landfill leachate. TTGE (Temporal Thermal Gradient Gel electrophoresis) profiling of PCR amplification products has provided information on the community structure and identity of cellulose-degrading populations across sites, and on the temporal and spatial distribution of these organisms. In addition, we are using 16S ribosomal-RNA targeted oligonucleotide probes to provide quantitative data on the relative abundance of cellulose-degrading microbes. Further work includes targeting the fibrobacters, an important group of cellulose-degraders in the rumen. Molecular ecological data suggests a wider distribution of *Fibrobacter* spp. and their relatives in the environment, but their role in cellulose degradation outside the rumen has been little-studied.

PS 09 A proteomic investigation of the obligate thermophile *Geobacillus thermoleovorans* T80

C.E. Pollock, R.L. Graham, N.G. Ternan & G. McMullan

School of Biomedical Sciences, University of Ulster, Coleraine, Co. Londonderry BT52 1SA

Thermophilic bacteria belonging to Bacillus genetic group 5 have been reclassified as members of the *Geobacillus* gen. Nov., with *G. stearothermophilus* as the type strain. There is an abundance of genomic information available for this genera with sequencing projects at various stages of completion for isolates of *G. stearothermophilus* and *G. kaustophilus*. Within our own laboratory we have completed sequencing the genome of *G. thermoleovorans* T80, an isolate obtained from temperate Irish soil. In this paper we present the pertinent features of the T80 proteome and hope to widen our knowledge and understanding of how this micro-organism adapts and survives in extreme environments. We report the development of gel-based and shotgun protein and peptide separation strategies for the analysis of the T80 proteome, prior to identification via mass spectrometry. Such strategies have led to the characterization of more than10 % of the theoretical T80 proteome. Of particular interest has been our ability to use proteomics as a tool to recurate the genomic information available for *G. kaustophilus* HTA426.

PS 10 Description of the genome of *Geobacillus thermoleovorans* T80 and comparison with related organisms

S.J. Matzen[1], N.G. Ternan[1], I.M. Banat[1], I. Zhulin[2], R. Marchant[1] & G. McMullan[1]

[1]School of Biomedical Sciences, University of Ulster, Coleraine; [2]Oakridge National Laboratory, USA

Thermophilic bacteria belonging to *Bacillus* genetic group 5 have been reclassified as being members of *Geobacillus* gen. nov., with *G. stearothermophilus* as the type strain. There is an abundance of genomic information available for this genera with sequencing projects of the isolates *G. stearothermophilus*, and *G. kaustophilus* HTA426. Within our own laboratory we have been sequencing the genome of *G. thermoleovorans* T80, an isolate obtained from temperate Irish soil. The genome of *G. thermoleovorans* T80 is the smallest at 3.38 Mbp, of all Bacillus related species so far sequenced including *G. kaustophilus* (3.54 Mbp). G+C content was 52.3 %, equivalent to that of *G. kaustophilus* (52.1 %) and higher than that found in other non-thermophilic Bacilli. The genome of *G. thermoleovorans* had 3830 predicted protein coding sequences (CDSs). Given that *G. kaustophilus* had only 3498 CDSs despite having a larger total genome size, it is predicted that the average gene length in our isolate is 771 bp compared with 862 bp in *G. kaustophilus*.

PS 11 *Pedobacter aquatilis* sp. nov., a novel bacterium isolated from drinking water

V. Gallego, M.T. García & **A. Ventosa**

Dept of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain

The culturable and non-culturable bacterial population from the tap water of four different points of the distribution system in Sevilla (Spain) have been studied in order to monitor the microbiological water quality. Four sampling campaigns during the period of one year where completed, one each season. Drinking water samples (25 l) were concentrated by using a tangential flow filtration system and plated on Plate Count Agar (PCA-Difco) and R2A (Difco). Plates were incubated at 28 °C for 7 days and different morfological colonies were subsequently plated in order to obtain pure cultures. Amongst organisms isolated in this study (about 600 pure cultures), strain AR107^T was obtained from the sampling campaign of January 2004 and it was maintained on R2A medium. This strain was studied taxonomically by using a polyphasic approach, by a combination of phenotypic, genotypic and phylogenetic features. The phylogenetic analysis of the 16S rRNA showed the highest similarity to members of the genus Pedobacter. The genus *Pedobacter* belongs to the family Sphingobacteriaceae and currently it includes seven species: *Pedobacter africanus*, *P. caeni*, *P. cryoconitis*, *P. heparinus*, *P. himalayensis*, *P. piscium* and *P. saltans* with *P. heparinus* as the type species. Members of this genus have been isolated from soil, fish, a nitrifying inoculum and glacier samples from the Tyrolean Alps (Austria) and an Himalayan mountain. The level of 16S rRNA gene sequence similarities with respect to the type strains of the genus Pedobacter were between 95.2 % with respect to the type strain of *P. heparinus* and *P. piscium*, and 94.1 % with respect to the type strain of *P. himalayensis*. A low (90.0 %) sequence similarity with *P. saltans* was observed. The DNA G+C content of this strain is 38 mol%, a value that is within the range described for the genus *Pedobacter*. Strain AR107T is a strictly aerobic Gram-negative rod producing pink-pigmented colonies. Temperature growth range is between 4 and 30 °C. Contains cytochrome-c oxidase, catalase, acid and alkaline phosphatases, esterase lipase, leucine arylamidase and β-galactosidase. Urease, lipase, arginine dihydrolase, β-glucuronidase and α-fucosidase are not present. Nitrate is not reduced. Does not produce H2S from thiosulfate nor indol from tryptophan. Hydrolyses aesculin but not gelatin. The predominant fatty acids were iso-C15:0, iso C17:0 3-OH and summed feature 3 (iso-C15:0 2-OH/C16:1ω7C). On the basis of this phenotypic, phylogenetic and genotypic study we propose to place this isolate as a novel species of the genus *Pedobacter*, for which the name *Pedobacter aquatilis* sp. nov. is proposed.

PS 12 Effect of cultivar and soil type on the methanogenic archaeal community inhabiting rice roots

R. Conrad, M. Klose, D. Kemnitz, M. Noll & P. Bodelier

Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany

Rice fields are an important source for the global budget of the greenhouse gas methane. Plant-derived carbon entering soil as root exudates and debris drives much of the methane production in rice field soil. Rice cluster I methanogens inhabiting the roots of rice plants were found to play a key role in methane production. We studied the effects of eight different rice cultivar and two different soil types on the composition of the methanogenic community on the rice roots. We targeted the genes of archaeal 16S rRNA and methyl coenzyme M reductase (*mcrA*) by terminal restriction fragment polymorphism (T-RFLP), cloning and sequencing. As soils we used a genuine rice field soil from Italy and a riparian wetland soil from the Netherlands. These soils differed in the structure of the methanogenic community, as the rice field soil mainly contained Rice Cluster I methanogens and *Methanosarcinaceae*, while the riparian soil was dominated by *Methanomicrobiales*. We found that the methanogenic community structure on the rice roots was mainly determined by the respective community structure of the soil, but was in addition affected by the rice cultivar. This result appears plausible, as the soil acts as seed bank from which the root environment selects the microbes.

PS 13 Diversity of lignan-converting human intestinal bacteria

Thomas Clavel[1,2], Gemma Henderson[1], Joël Doré[2] & Michael Blaut[1]

[1]German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; [2]French National Institute for Research in Agriculture, Jouy-en-Josas, France

Little is known about the human intestinal bacteria that metabolize the dietary lignan secoisolariciresinol diglucoside (SDG) to the active oestrogen-like compounds enterodiol and enterolactone. The aim of the present work was to identify, characterize and enumerate bacteria catalysing the four reactions required for SDG conversion. Eleven phylogenetically and functionally distantly related anaerobic species isolated from human faeces or obtained from bacterial culture collections catalysed the O-deglycosylation, O-demethylation, dehydroxylation or dehydrogenation step of SDG conversion. Most of these species were members of dominant intestinal microbiota, as determined by most probable number and fluorescent *in situ* hybridization enumerations. They included strains of the genera *Bacteroides*, *Clostridium*, *Eggerthella*, *Eubacterium* and *Peptostreptococcus*, but also one strain of a possible novel species and one strain of a possible novel genus, as determined by 16S rRNA gene sequence analysis. The ability to catalyse the O-deglycosylation, O-demethylation and dehydroxylation step of SDG conversion was conserved within several strains of the species *Bacteroides fragilis*, *Peptostreptococcus productus* and *Eggerthella lenta*. Conversely, close phylogenetic or functional relatives of SDG-converting strains did not metabolize SDG. The present work provides the basic knowledge on the diversity of SDG-converting bacteria, the activity of which is relevant to other dietary lignans with possible beneficial health effects.

PS 14 Enterosome-mediated utilization of 1, 2-propanediol by *Escherichia coli*

S.D. Dinesh[1], H. Luensdorf[2], A. Bleloch[3], J.M. Warren[4], E. Raux-Deery[4], A. Rambach[4] & M.B. Prentice[1]

[1]Dept of Microbiology/Pathology, UCC, Cork, Ireland; [2]Dept of Microbiology, GBF, Braunschweig, Germany; [3]SuperSTEM facility, Daresbury Laboratories, Daresbury; [4]Dept of Biosciences, University of Kent, Kent

Bacteria differ from eukaryotic cells by the absence of organelles. However, carboxysomes in chemoautotrophic cyanobacteria and the related enterosomes in heterotrophic bacteria have organelle-like properties. *E. coli* K-12 chromosome possesses enterosome-associated ethanolamine utilization genes but it lacks genes for the utilization of propanediol. The *pdu* operon from *Citrobacter freundii* was cloned into *E. coli* and its phenotype was investigated. In growth conditions that induce the *pdu* operon, polyhedral inclusions resembling carboxysomes (enterosomes) were seen on electron microscopy of the recombinant *E. coli*. Biochemical assays confirmed the vitamin B_{12}–dependent utilization of propanediol by the recombinant *E. coli*. This is the first demonstration that a recombinant *pdu* operon can produce functional enterosomes in *E .coli*. Preliminary SuperSTEM investigations suggested regular surface protein arrangement consistent with an assembled organelle.

PS 15 Regulation of transfer of the *Mesorhizobium loti* strain R7A symbiosis island

Joshua P. Ramsay, John T. Sullivan, Kate Williman, Iain Lamont & **Clive W. Ronson**

Dept of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

The *Mesorhizobium loti* symbiosis island is a 500-kb genomic island containing genes required to form a nitrogen-fixing symbiosis with *Lotus* plants. It is able to excise and transfer by conjugation to non-symbiotic mesorhizobia, We have identified several genes required for transfer, many of which are conserved amongst a family of genomic islands found in alpha- and beta-proteobacteria. Using a quantitative PCR assay, it was found that excision was increased in stationary-phase cultures and plant nodules. A bioinformatics approach identified *msi109* as likely encoding a recombination directionality factor. Mutation of *msi109* greatly reduced excision, while overexpression of *msi109* resulted in curing of the symbiosis island. *msi109* is located in an operon with transfer genes, indicating that excision and transfer are co-ordinately regulated. Further genes conserved amongst related islands include homologues of the quorum-sensing regulators *traR* and *traI* (*msi174* and *msi173*) and a relative of the *parB* family, *msi150*. Msi174 was found to be a positive regulator of excision, while disruption of *msi150* resulted in the presence of excised islands in as high as 50 % of cells. *msi150* mutants also displayed an 100-fold increase in transfer of a plasmid-borne symbiosis island *oriT* relative to that of the wild-type. These results offer an initial insight into a complex regulatory system for genomic island excision and transfer.

PS 16 The distribution ecology of a model micro-organism along an estuarine gradient

Robert J. Hawkins[1] & Kevin J. Purdy[2]

[1]School Biological Sciences, University of Reading, PO Box 228, Whiteknights, Reading RG6 6AJ; [2]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

Microbial Ecology has, to date, been primarily descriptive in nature with a limited grounding in ecological theory, the result being that the structure and function of microbial communities and their importance in the global ecosystem are poorly understood. One aim of this project is to start to address this issue by using an indigenous model organism as a tool to investigate the relationship between microbial community structure and function.

The sulfate reducing bacteria (SRB) play a vitally important role in the global carbon and sulfur cycles (Postgate, 1984; Purdy *et al*., 2002). One genus of the SRB – *Desulfobulbus* – is thought to be ubiquitous in its range and is a major propionate utilizing micro-organism in the environment. It is also unknown what else, if anything, these organisms are doing in the environment but evidence suggests that they utilize sulfate as an electron acceptor in the marine end of the estuary but not at the freshwater end (Purdy *et al*., 2003).

The major aim of the project is to investigate the effects on the bacteria that changes in the dynamic environmental factors found in the estuarine environment cause as they fluctuate down an estuarine gradient and determine what the bacteria are capable of doing in the environment.

The River Colne estuary, Essex, UK was the chosen project site, of which a body of background information is already known (Purdy *et al*., 2003). DGGE profiling at strategic points along the estuary has produced community fingerprints which reveal that different bacterial communities exist at opposing ends of the watercourse. Phylogenetic analysis of cloned PCR products indicate that distinct groups of *Desulfobulbus* are found in different stretches of the estuary.

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PS 17 Polyphasic approach to the taxonomy of 13 *Clostridium* spp. strains by means of multivariate data analysis: strategy for describing a new species

Z.R. Suárez, B. Cháves, F. Aristizábal & D. Montoya

Instituto de Biotecnología Facultad de Agronomí,. Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia

This work presents a polyphasic approach to the taxonomy of thirteen solventogenic *Clostridium* spp. strains isolated from Colombian soils by means of multivariate data analysis. Analyzed data were obtained from phenotypic and genotypic techniques employed during characterization process (Biochemical tests, solvent production, cellulolytic activity, 16S rRNA sequencing, PFGE macro restriction profiles, AFLPs, and DNA-DNA hybridization). Multiple correspondence analysis (MCA) was used for categorical variables, whereas principal component analysis (PCA) was used for continues variables. Multiple factor analysis (MFA) with a hierarchical cluster analysis was applied for the jointed set of data, considering that when a greater number of variables were used, it gave a comprehensive strains description. Obtained results showed a 10 native strains cluster clearly separated from reference strains in all analysis. This fact suggests that native strains may constitute a new species within the *Clostridium* genus. The strategy developed in this study may be the departure point to describe a new bacterial species with biotechnological potential.

Keywords Multivariate data analysis, molecular characterization, species description

Posters

Systematics & Evolution Group

SE 01 Molecular biological detection of anaerobic gut fungi (*Neocallimastigales*) involved in cellulose degradation in freshwater lake systems

R.J. Lockhart[1], J.P. Loughnane[1], H.E. Allison[1], R.W. Pickup[2] & A.J. McCarthy[1]

[1]School of Biological Sciences, The University of Liverpool, Crown Street, Liverpool L69 7ZB; [2]Centre for Ecology and Hydrology, Lancaster Environment Centre, Lancaster LA1 4AP

The anaerobic fungi (*Neocallimastigales*) comprise part of the normal rumen microbial community, where they make an important contribution to cellulose digestion. They have not been detected elsewhere and are therefore termed the anaerobic gut fungi. We have used nylon mesh bags containing cellulose, moored at the lake sediment interface and in the water column to act as bait for colonization by indigenous cellulose degraders. DNA was recovered and amplified with PCR primers designed to be specific for the 18S rRNA gene of *Neocallimastigales*, to demonstrate their presence in both water and sediment samples. Phylogenetic analysis of the sequences recovered confirms their assignment to the *Neocallimastigales*. Further analysis in which the ribosomal ITS1 sequence is used as the target will reveal the relationships between these sequences and those from rumen isolates, and provide information on the taxonomic and evolutionary significance of these findings.

Authors

Authors

Authors

Cells & Cell Surfaces Group

S-Layers: self-assembly systems for application in nanobiotechnology

Nicola Ilk, Eva Maria Egelseer, Carina Huber, Judith Ferner-Ortner, Seta Küpcü, Uwe B. Sleytr & Margit Sára

Center for NanoBiotechnology, University of Natural Resources & Applied Life Sciences, Vienna, Austria (Email nicola.ilk@boku.ac.at)

Many bacteria and archaea possess regularly structured protein lattices, so called S-layers as their outermost cell envelope component which completely cover the cell surface during all stages of bacterial growth and division. S-layer proteins contain all information necessary for their crystallization into lattices with either oblique, square or hexagonal symmetry. The structure-function relationship of distinct segments of selected S-layer proteins was investigated by the production of N- and C-terminally truncated forms. Amino acid positions that were found to be located on the outer S-layer surface were exploited as fusion sites for functional peptide sequences, such as the Fc-binding ZZ-domain, core streptavidin, EGFP, the variable domain of heavy chain camel antibodies or the major birch pollen allergen.

By exploiting the specific interaction between the N-terminal part of the S-layer protein and the secondary cell wall polymer (SCWP) which is the proper anchoring molecule in the bacterial cell wall, oriented binding of the S-layer fusion protein was achieved on solid supports precoated with chemically modified SCWP. Due to the crystalline structure of S-layer lattices, fused sequences show a well-defined distance down to the sub-nanometer length scale. The application of S-layer fusion proteins for the development of biosensors and DNA chips will be discussed.

Microbial Infection Group/ Clinical Microbiology Group

Predictive features of the genome-wide host response to infectious diseases

David A. Relman

Stanford University Medical Center, California, USA

Genomic tools and approaches have enabled a more detailed description of host–microbe encounters, and shed light on fundamentally important processes, including the cellular responses associated with infection. Genome-wide transcript-abundance profiles, like other comprehensive molecular readouts of host physiological state, provide a detailed blueprint of the host–pathogen dialogue during microbial disease, and can reveal functional gene-based modules associated with mechanisms of virulence and host defense. Studies of cancer based on genome-wide transcript-abundance profiles have led to novel signatures that predict disease outcome and serve as useful clinical classifiers. The highly dynamic and compartmentalized aspects of the host response to pathogens complicates efforts to identify predictive signatures for infectious diseases. Yet, studies of systemic infectious diseases so far suggest the possibility of successfully discriminating between different types (classes) of infection and predicting clinical outcome. In addition, host gene expression analysis could lead to the identification of early signatures associated with a protective immune response, both to natural infection and to vaccination. Early explorations in some of these areas indicate the potential feasibility of this approach but also point to important unmet challenges.

Non-parenteral vaccines: from concept to clinical validation

Alain Li-Wan-Po

Centre for Evidence-Based Pharmacotherapy, Aston University, Birmingham B4 7ET (Email a.liwanpo@talk21.com)

Although most medications are given orally, vaccines are usually administered by injection. This is due to the fact that the vaccine molecules inducing the protective responses are large molecular weight, usually unstable, compounds, which diffuse poorly across biological absorption barriers. Major exceptions are vaccines consisting of live attenuated microorganisms, which can transgress barrier membranes. An example is the Sabin polio vaccine, introduced over four decades ago. Despite the controversy about the continued use of the oral polio vaccine, the search for other vaccines effective by the oral route continues with great intensity because of the major advantages, which they theoretically confer.

In this presentation the development of non-parenteral vaccines is considered and their theoretical advantages and disadvantages discussed. The historical performance of marketed non-parenteral vaccines is compared against those theoretical characteristics. The scientific and regulatory challenges faced by developers of non-parenteral vaccines are reviewed and promising avenues for the development of such vaccines are surveyed.

Food & Beverages Group

Of microbes, mice and men: understanding the mammalian-microbial-metabolic axis

J.K. Nicholson

Imperial College London

Post-genomic technologies are being widely applied to improve the understanding of adverse drug reactions and the molecular basis of human disease. Metabonomics is an approach that enables multivariate profiling of the integrated metabolic responses of complex systems to patho-physiological stress, and so involves understanding the way the whole metabolic regulatory system varies with interventions thus providing complementary information to genomics and proteomics (1). Mammalian biochemistry is strongly influenced by gut microbial symbionts and indeed modulation of the gut microbiota may be important in a diverse range of diseases and also alter drug metabolism and toxicity (2–4). We have termed the study of these transgenomic interactions as ?global systems biology?. Because these interactions are mediated by a large number of co-metabolic processes the system state integrity can be evaluated indirectly via metabolic profiling of mammalian biofluids using advanced spectroscopic methods. The influence of the gut microbial metabolic activities on drug toxicity, the development of insulin resistance in experimental models will be described together with the use of metabonomic technologies for extracting information on the influences of gut microbial metabolism on the metabolic phenotypes of human populations. Understanding the microbial-mammalian-metabolic axis may be of great value in understanding problems related both to personalised healthcare and in molecular epidemiology.

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Virus Group

Coronaviruses and the nucleolus

Julian A. Hiscox

Institute of Molecular and Cellular Biology, University of Leeds

The nucleolus is a dynamic sub-nuclear structure which is crucial to the successful functioning of a eukaryotic cell. The nucleolus has a diverse range of functions including ribosome subunit biogenesis, RNA processing, control of cell growth and mediating stress responses. Interaction of viruses with the nucleolus is not restricted to any one particular group and examples can be found from DNA, retro and negative and positive strand RNA viruses. For the latter group, this is somewhat surprising, as the conventional paradigm of replication in the cytoplasm would suggest no involvement of the nucleus in virus infection. We have been studying the interaction of coronaviruses with the nucleolus both in the context of virus infection and investigating the trafficking of the viral nucleocapsid (N) protein. Using a combination of live cell and confocal microscopy coupled to deletion and substitution mutagenesis we have delineated a nucleolar retention motif in the N protein as well as a nuclear export signal. Our data also suggests that the nucleolar proteome and architecture is disrupted during virus infection which has downstream consequences for the host cell cycle.

Cells & Cell Surfaces group

CCS 13 Identification of lipoteichoic acid in the thermophilic actinomycete *Thermobifida fusca*

Obaidur Rahman, Oxana Pester & **Iain Sutcliffe**

Northumbria University, Newcastle upon Tyne

The cell envelopes of Gram-positive bacteria are characterised by the presence of membrane-anchored polymers (macroamphiphiles). Those of the high GC Gram-positive bacteria (*Actinobacteria*) typically contain lipoglycans (e.g. lipoarabinomannans) whereas low-GC *Firmicutes* typically contain lipoteichoic acids (LTA). We have investigated the macroamphiphile present in the filamentous thermophilic actinomycete *Thermobifida fusca* strain YX. Cells were extracted using a standard phenol-water procedure and the crude extracts purified by hydrophobic interaction chromatography. Analysis revealed the presence of a surprisingly phosphate-rich macroamphiphile which also contained significant amounts of glycerol. Cumulatively, these data are consistent with the presence of a polyglycerophosphate LTA rather than a lipoglycan. Western blot analysis using a monoclonal anti-LTA antibody gave a positive reaction that confirmed the likely presence of LTA in *T. fusca*. Reports of the presence of LTA in actinomycetes are rare and, to our knowledge, this is the first report of LTA in a thermophilic Gram-positive bacterium.

Plenary

PS 18 Diversity of rainbow trout (*Oncorhynchus mykiss*) intestinal microbial flora

Dohyung Kim, Jason Brunt & Brian Austin

School of Life Sciences, John Muir Building, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS

The diversity of the microflora present within rainbow trout intestine was investigated through the analysis of the 16S rRNA gene sequences of both cultivated and non-cultivated flora. Microbial counts were $4.8 \times 10^6 \pm 6.4 \times 10^6$ and $3.0 \times 10^6 \pm 4.6 \times 10^6$ recovered from intestinal contents and mucus, respectively. Forty one culturable microbial phylotypes (or operational taxonomic unit) (99 % minimum similarity), 39 sequences from 16S and 2 from 18S rRNA genes, were retrieved from the intestine. *Aeromonadaceae* (30 %) and *Enterobacteriaceae* (20 %) representatives were the dominant cultured bacteria (51 %) followed by *Pseudomonadaceae* (10 %) representatives. The genomic DNA isolated from intestinal contents and samples of mucus were used to generate 104 random clones that were subject to comparative phylogenetic analysis. The 104 sequences were classified into 32 phylotypes, and most of them were affiliated with a major phylum Proteobacteria (> 70 %). However, unlike library G, the phyla Bacteroidetes and Fusobacteria were not found in intestinal mucus (library M), indicating microbiota in the gut mucus may be quite different from that of intestinal contents. Also, many novel sequences that have not been previously recognised as part of the intestinal flora of rainbow trout were retrieved, suggesting the fish intestine may harbour a larger diversity than what was first thought.