society for general Microbiology

Spring Meeting

31 March–3 April 2008

Edinburgh International Conference Centre

Abstracts

Contents

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

Plenary Session

Bacterial secretion systems: commonality and diversity

Mechanism of ATP-driven translocation by the bacterial preprotein translocase

Arnold J.M. Driessen

Dept of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute and the Zernike Institute for Advanced Materials, University of Groningen, 9751 NN Haren, The Netherlands

About 25–30% of the bacterial proteins function in the cell envelope or outside of the cell. These proteins are synthesized in the cytosol and the vast majority is recognized as a ribosome-bound nascent chain by the signal recognition particle or by the secretion-dedicated chaperone SecB. Subsequently, they are targeted to the Sec-translocase in the cytoplasmic membrane, a multimeric membrane protein complex with a highly conserved protein conducting channel, SecYEG, and peripheral bound ligands, the ribosome or the ATP-dependent motor protein SecA. The Sec-translocase mediates the translocation of proteins across the membrane and the insertion of membrane proteins into the cytoplasmic membrane. Translocation requires the energy sources ATP and the proton motive force, while membrane protein insertion is coupled to polypeptide chain elongation at the ribosome. This presentation will discuss recent insights in the different structural requirements for translocase-mediated membrane protein insertion and protein translocation.

The bacterial Sec translocase nanomotor: structure and function

T. Economou

University of Crete

Abstract not received

The signal recognition particle targeting pathway in *Escherichia coli*

Harris D. Bernstein, Janine H. Peterson, Pu Tian & Cheryl A. Woolhead

Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda MD 20892, USA

The signal recognition particle (SRP) is a universally conserved ribonucleoprotein that recognizes signal sequences as they emerge during translation and then targets ribosome-nascent chain complexes to the SecYEG/Sec61p translocation channel. Mammalian SRP recognizes both cleaved signal peptides and the transmembrane segments of integral membrane proteins, which function as internal signal sequences. In contrast, we found that *E. coli* SRP recognizes only transmembrane segments and a few cleaved signal peptides that are exceptionally hydrophobic. Consequently, most proteins that contain cleaved signal peptides (periplasmic and outer membrane proteins) are targeted to the SecYEG channel post-translationally. In recent studies we have examined the export of cytoplasmic proteins fused to signal peptides. Previous work showed that cytoplasmic proteins must be targeted to the SecYEG channel co-translationally to prevent them from folding into a translocation-incompetent conformation. We found, however, that efficient export of several cytoplasmic proteins depends not only on the sequence of the signal peptide, but also on the sequence of residues adjacent to the signal peptide. Our results indicate that the initiation of protein export involves a second event (presumably the opening of the SecYEG channel) that is separable from the targeting reaction and that is sensitive to sequences both within and just beyond the signal peptide.

Inserting and assembling proteins into bacterial membranes by the YidC insertase

R. Dalbey

Ohio State University, USA

The YidC/Oxa1p/Alb3 family of proteins is a new class of proteins that play a role in the membrane assembly of proteins in bacteria, mitochondria, and chloroplasts. In bacteria, null mutations in YidC are lethal, indicating that YidC is essential. YidC was discovered to be critical for the membrane insertion of viral coat proteins that were previously thought to insert into the membrane spontaneously. In addition to acting as an independent insertase, YidC also assists in membrane protein insertion in the Sec pathway. In this talk, we will highlight what is known about the role of the YidC insertase in the membrane insertion of subunit II of cytochrome bo3 oxidase. We will also describe recent membrane insertion studies with *Streptococcal mutans* YidC family members and results of cold-sensitive mutants that indicate the importance of transmembrane segment 3 in YidC function.

Transport of folded proteins by the bacterial Tat (twin arginine translocation) pathway

Tracy Palmer

Division of Molecular & Environmental Microbiology, College of Life Sciences, University of Dundee, Dundee DD1 5EH

The Tat protein translocase transports fully folded and often oligomeric proteins across the bacterial cytoplasmic membrane and the thylakoid membrane of plant chloroplasts. Protein substrates are targeted to the Tat machinery by N-terminal signal peptides that contain a twin arginine motif. The Tat system of *Escherichia coli* comprises three membrane-bound components, TatA, TatB and TatC. Under resting conditions the machinery comprises two separate, high molecular weight complexes. One complex is made up of TatB and TatC which acts as the receptor for substrates. Disulfide cross-linking expermients indicate that TatB is arranged as a multimeric unit, at least a tetramer, within the TatBC complex. The second type of complex contains only the TatA protein. This complex is highly heterogeneous and forms a ladder of bands on blue native PAGE, ranging from less than 100 to greater than 600 kD. Analysis of the TatA complex by negative stain electron microscopy and random conical tilt reconstruction reveals that it forms ring-shaped structures of variable diameter. These structures enclose internal cavities which are large enough to accommodate folded Tat substrate proteins, strongly suggesting that TatA forms the protein conducting channel. The channel is closed by a lid, probably located at the cytoplasmic side of the membrane, which may gate substrate access.

The ESX-1 secretion pathway in virulent mycobacteria

J. Cox

University of California at San Francisco, USA

Abstract not received

Targeting proteins to the cell wall envelope of Gram-positive bacteria

Olaf Schneewind

Dept of Microbiology, University of Chicago, USA

The cell wall envelopes of Gram-positive bacteria represent a surface organelle that not only functions as a cytoskeletal element but also promotes interactions between bacteria and their environment. Cell wall peptidoglycan is covalently and noncovalently decorated with teichoic acids, polysaccharides, and proteins. The sum of these molecular decorations provides bacterial envelopes with species- and

strain-specific properties that are ultimately responsible for bacterial virulence, interactions with host immune systems, and the development of disease symptoms or successful outcomes of infections. Surface proteins typically carry two topogenic sequences, i.e. N-terminal signal peptides and C-terminal sorting signals. Sortases catalyse a trans-peptidation reaction by first cleaving a surface protein substrate at the cell wall sorting signal. The resulting acyl enzyme intermediates between sortases and their substrates are then resolved by the nucleophilic attack of amino groups, typically provided by the cell wall cross bridges of peptidoglycan precursors. Surface protein linked to peptidoglycan is then incorporated into the envelope and displayed on the microbial surface. Gram-positive bacteria elaborate several different sortase targeting systems that lead also to assembly of pili, to decorations of mother cell and spore envelope in bacilli, and to dedicated transport systems involved in scavenging of heme during infection.

Bacterial lipoproteins: to the membrane, through the periplasm and beyond!

Wolfram R. Zückert

The University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160 USA

Since the original description of a prokaryotic lipoprotein in the cell envelope of *Escherichia coli* almost four decades ago, this class of membrane-anchored proteins has been increasingly appreciated. Lipoproteins are found in all extracytoplasmic compartments of both mono- and diderm bacteria and can also be released into the extracellular environment. They have been shown to be important factors in envelope biogenesis and virulence, often acting as membrane-bound linkers to matrix molecules, but also as potent immunostimulatory mitogens. While Sec-dependent export through the cytoplasmic membrane and lipidation modification pathways appear conserved, a variety of secretory pathway branches subsequently ferry lipoproteins through the periplasmic space to – and through – the outer membrane of diderm bacteria. At the same time, recent work has challenged earlier conclusions that lipoprotein sorting signals are universal, at least at the current resolution. Exploring this diversity will yield important clues on the evolution of bacterial protein secretion mechanisms and may lead to novel intervention strategies for infectious diseases.

The evolution and diversity of protein secretion systems in bacteria

Milton Saier

Dept of Molecular Biology, University of California at San Diego, La Jolla, CA, 92093-0116, USA (Email msaier@ucsd.edu)

Gram-negative bacteria have evolved at least 16 types of protein secretion systems which appear to have evolved independently of each other in spite of the fact that certain protein components can be shared by more that one of them. Some of these systems, in divergent form, are retained in eukaryotic organelles that arose from bacteria in a degenerative endosymbiotic process. At least eight such independently functioning systems secrete proteins across or into the outer membranes of Gram-negative bacteria, and at least eight secrete proteins across or into the inner membrane; some of the latter export proteins across both membranes of the Gram-negative bacterial envelope in a single energy coupled step. A few of these systems are also found (in modified form) in Gram-positive bacteria.

It will be argued that the multicomponent systems arose as modular structures where the protein constituents were often recruited from other preexisting sources. Complexity was enhanced by intra- and extragenic duplication events as well as domain recruitment. Even for a single type of secretion system, the degrees of complexity vary tremendously, and homologues are sometimes difficult to detect because of extensive sequence divergence. Potential evolutionary pathways for the appearance of select systems will be presented.

Outer membrane biogenesis in Gram-negative bacteria

Thomas J. Silhavy

Princeton University, USA

The outer membrane (OM) of Gram-negative bacteria functions as a protective barrier. It is unusual because the OM bilayer is asymmetric; the inner leaflet is composed of phospholipids, but the outer leaflet is made of lipopolysaccharide (LPS). Two kinds of proteins are found in the OM. Lipoproteins are inserted into the inner leaflet of the OM by posttranslationally attached lipid moieties. Integral OM proteins are β-barrel proteins (OMPs). All of the components of the OM are synthesized inside the cell. They must be transported to, and assembled in the OM in the correct orientation to maintain barrier function, and all of this takes place outside the cytoplasm where there is no obvious energy source, such as ATP.

I will describe the genetic and biochemical strategies we have used to identify and characterize the cellular components required for LPS and OMP biogenesis. Together these methods have identified two OM protein complexes. One of these complexes contains the OMP Imp and an OM lipoprotein termed RlpB. The other complex contains the OMP YaeT and four lipoproteins, YfiO, YfgL, NlpB, and SmpA. Evidence will be presented showing that the Imp complex assembles LPS at the cell surface and that the YaeT complex assembles β-barrel proteins. Structural studies with YaeT suggest a mechanism for β-barrel assembly.

Structure and function of tripartite pumps for bacterial toxin export and multidrug efflux

Vassilis Koronakis

Dept of Pathology, University of Cambridge

Bacterial pathogens use TolC-dependent machineries to export toxins and enzymes by bypassing the periplasm via a tripartite apparatus in which the outer membrane TolC protein is recruited by a substrate-engaged inner membrane translocase of a traffic ATPase and an accessory or 'adaptor' protein. This establishes a contiguous export channel from the cytosol to the outside. Our work has focused on export of the large hemolysin toxin by uropathogenic and enterohemorrhagic *E. coli*, and I will summarize our view of this process. Closely related TolC-dependent tripartite machines are ubiquitous in the the cell envelopes of bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* and these expel antibacterial drugs and other small noxious chemicals, so helping the bacteria survive. These multidrug efflux pumps are important to the growing threat of bacterial resistance to chemotherapy. They function similarly by recruitment of a TolC family protein by energized drug-laden translocases in the inner membrane, though in this case the adaptor is typically coupled to a proton antiporter.

We have crystallized and solved the 3D structures of the TolC protein, revealing a trimeric exit duct anchored in the outer membrane and projecting across the periplasm, and also the periplasmic adaptor protein that recruits TolC. These structures reveal how the tripartite pumps assemble to a contiguous trans-envelope pores for virulence protein export and multidrug resistance, and we have now established how the recruitment and pump assembly is effected by interaction of TolC-adaptor coiled-coil interfaces in the periplasm. We have described how the periplasmic exit duct entrance can be opened in the assembled pumps to allow exit of toxins and drugs, and also shown how the entrance constriction can be blocked by potential inhibitors. This suggests it may be possible to develop new drugs to target the pumps and counteract multidrug resistance.

References Lobedanz *et al.* (2007) A periplasmic coiled-coil interface underlying TolC recruitment and the assembly of bacterial drug efflux pumps. *Proc Natl Acad Sci U S A* **104**, 4612–4617 / Higgins *et al.* (2004) Structure of the ligand-blocked entrance of the bacterial multidrug efflux protein TolC. *J Mol Biol* **342**, 697–702 / Eswaran *et al.* (2004) Three's company: component structures bring a closer view

of tripartite drug efflux pumps. *Curr Opin Struct Biol* **14**, 741–747 / Koronakis *et al.* (2004) Structure and function of TolC – the bacterial exit duct for proteins and drugs. *Annu Rev Biochem* **73**, 467–489 / Higgins *et al.* (2004) Structure of the periplasmic component of a bacterial antibiotic efflux pump. *Proc Natl Acad Sci U S A* **104**, 4612–4617 / Andersen *et al.* (2002) Transition to the open state of the TolC periplasmic tunnel entrance. *Proc Natl Acad Sci U S A* **99**, 11103–11108 / Koronakis *et al.* (2000) Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. *Nature* **405**, 914–919 / Thanabalu *et al.* (1998) Substrate-induced assembly of a contiguous channel for protein export from *E. coli*: reversible bridging of an inner membrane translocase to an outer membrane exit pore. *EMBO J* **17**, 6487–6496 / Koronakis *et al.* (1991) Energetically distinct early and late stages of HlyB/HlyD-dependent protein secretion across both *E. coli* membranes. *EMBO J* **10**, 3263–3272 / Koronakis *et al.* (1989) Isolation and analysis of the C-terminal signal directing export of *E. coli* hemolysin protein across both bacterial membranes. *EMBO J* **8**, 595–605.

Assembly of the *Yersinia* Ysc injectisome

Guy R. Cornelis

Focal Area: Infection Biology, Biozentrum, Universität Basel, Basel, Switzerland

The type III secretion injectisome is a nanosyringe that injects bacterial effector proteins into the cytosol of eukaryotic cells. It is related to the flagellum and consists of three rings spanning the bacterial membranes, connected by a central tube. On top of the rings, comes a short stiff needle ending with a tip structure made of LcrV, a protective antigen against plague. This structure is believed to act as a scaffold for the insertion of a pore, made of two hydrophobic proteins, into the target cell membrane. An export apparatus occupies the lower rings and serves for the export of the needle subunits and LcrV, first and the pore-formers and effectors, later, upon contact with a target cell. The length of the needle is controlled by a mechanism involving a protein thought to act both as a molecular ruler and a substrate specificity switch (YscP in *Yersinia*). When assembly of the needle is complete, the molecular ruler changes the substrate specificity of the export apparatus, which becomes ready to export pore formers and the effectors. Protein YscU from the export apparatus plays a role in the substrate specificity switch.

Agrobacterium type IV secretion

Peter J. Christie, Simon J. Jakubowski, Jennifer Kerr & Christina Martinez

Dept of Microbiology & Molecular Genetics, UT-Houston Medical School, 6431 Fannin, Houston, TX 77030, USA

The *Agrobacterium tumefaciens* VirB/D4 type IV secretion (T4S) system elaborates a transenvelope secretion channel and extracellular T pilus for translocation of oncogenic T-DNA and protein substrates to susceptible eukaryotic cells. A general model for substrate translocation posits that secretion substrates are recruited to the VirD4 receptor, which in turn co-ordinates its ATP binding/hydrolysis activities with those of the VirB4 and VirB11 ATPases to drive substrate transfer through an inner membrane channel composed of these ATPases, polytopic VirB6 and bitopic VirB8. Secretion substrates then pass through a channel composed of VirB2 pilin and an outer-membrane-associated complex of VirB7 lipoprotein and secretin-like VirB9. Recent findings suggest that a combination of substrate binding and ATP binding/hydrolysis by the VirD4 receptor triggers a structural transition in the bitopic energy sensor VirB10 required for productive engagement of inner and outer membrane subassemblies of this T4S machine. This talk will discuss domain requirements for VirB10 ATP energy sensing and VirB10-VirB9 complex formation, and the disposition of VirB9 at the outer membrane.

Membrane insertion and assembly of a bacterial secretin

Ingrid Guilvout[1], Martin Krehenbrink[1], Mohamed Chami[3], Andreas Engel[3], Séverine Colin[1], Nicolas Bayan[1,2], Alexandre Ghazi[2], Catherine Berrier[2], Frédéric Pecorari[1], Pedro Alzari[1] & **Tony Pugsley**[1]

[1]Institut Pasteur, Paris, France; [2]University of Paris-Orsay, Orsay, France; [3]Biozentrum, University of Basel, Basel, Switzerland

The secretin family of bacterial outer membrane proteins exhibits numerous characteristics that differentiate them from classical β-barrel outer membrane proteins. In an attempt to determine how these high-ordered oligomeric structures are targeted to and assemble in the *Escherichia coli* outer membrane, we have undertaken a genetic, biochemical, biophysical and structural approach to the study of the secretin PulD from the type II secretion pathway of *Klebsiella oxytoca*. The data show that PulD can multimerize and insert into lipid bilayers in the absence of endogenous membrane proteins. Thereafter, the protein can only be extracted from these membranes by ionic detergents that partially unfold the N-terminal periplasmic domain of the protein. Targeting to the outer membrane requires a specific chaperone, the liopoprotein PulS, which is targeted to the outer membrane by the Lol pathway used by all other outer membrane lipoproteins. We propose that the interaction between PulD and its chaperone PulS and between PulS and its chaperone LolA, PulS release from PulD, PulD multimerization and PulD insertion are temporally and spatially ordered events that lead to the assembly of PulD in the outer membrane. Surprisingly, other secretins seem to utilize different mechanisms to reach the outer membrane, despite their overall sequence and structural similarity with PulD

Type V secretion: barrel mediated protein translocation across the outer membrane

Ian R. Henderson

Division of Immunity and Infection, The Medical School, University of Birmingham, Edgbaston, Birmingham

The Type V secretion system is comprised of three main subfamilies represented by the classical autotransporters, the Two-partner secretion system and the trimeric autotransporters. These diverse proteins are produced by pathogenic and non-pathogenic Gram-negative bacteria and perform a wide variety of functions. Despite the apparent simplicity of these systems many aspects of the secretion process remain poorly understood. The mechanism of biogenesis of members of the three subfamilies will be compared and contrasted, with a particular focus on discussing recent structural and biochemical studies which have led to a greater comprehension of the steps in autotransporter biogenesis. In addition, recent evidence supporting a role for accessory factors in the biogenesis of these proteins will be discussed.

Type VI secretion systems: new players in the bacterial pathogenesis arena

Edward G. Dudley

The Pennsylvania State University, University Park, Pennsylvania, USA

Bacterial pathogens have evolved a number of diverse mechanisms for the translocation of proteins from the cytoplasm to the extracellular environment. During the past five years several groups reported on a new mechanism of protein secretion that was termed a type VI secretion system, or T6SS. These systems are best characterized in aquatic and mammalian pathogens, and in plant symbionts. However, bioinformatics searches indicate that T6SSs are found within a wide range of proteobacteria species, both disease-causing and commonly benign. The genomic regions encoding T6SSs are generally clusters of 16–23 genes that are co-regulated with other virulence determinants, and are responsible for the biosynthesis and translocation of at least two proteins into the supernatant and/or eukaryotic cells. Although T6SSs are still poorly understood, recent exciting reports reveal commonalities between systems, in terms of functions during pathogenesis and the mechanisms involved in protein secretion. This talk will cover these similarities and also discuss prominent differences that exist, suggesting that multiple categories of T6SSs exist.

Hot Topic Symposium

Influence of climate change on disease and microbial environmental processes: microbes and climate change

Microbes as climate engineers

Dave S. Reay

School of GeoSciences, Crew Building, West Mains Road, University of Edinburgh, EH9 3JN [Tel +44(0)131 6507723; Fax +44(0)131 6620478; Email David.Reay@ed.ac.uk]

The Nobel Laureate Paul Crutzen has described the epoch we now live in as 'The Anthropocene', an age where global climate is increasingly determined by humankind. Arrogant organisms that we are, it is easy to view this as something entirely novel in Earth's history – evolution's newest top consumers breaking the environmental shackles and dictating global climate. In truth of course, micro-organisms have been at it for billions of years. From the first molecule of oxygen released by a cyanobacterium in the turbulent oceans of a young Earth, to the methanogen-made methane belched from the warm bogs of the Carboniferous, microbes have long helped determine the composition of Earth's atmosphere and its climate. Here I examine the key role microbial life plays in the sinks and sources of carbon dioxide, methane and nitrous oxide, and how these microscopic climate engineers may respond to global change in the 21st century.

Soils and climate change – the role of microbiology

Pete Smith

Professor of Soils & Global Change, Institute of Biological & Environmental Sciences, School of Biological Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen AB24 3UU

Climate change has thrust soil science to the forefront of policy relevant international science. Climate change has impacts on soils. Increasing temperatures will tend to increase decomposition but this will be limited where the soil water balance becomes very low. Where increasing temperatures increase net primary production (NPP), carbon inputs to the soil may increase which will work to decrease the direct impact of climate change on soils and may increase soil carbon. Results from modelling studies will be presented to show how climate change is projected to change soil carbon stocks in Europe over the next 75 years. There is still disagreement over the temperature sensitivity of soil carbon decomposition. Implications of different temperature sensitivities will be discussed.

As well as soils being affected by climate change, improvements in soil management can be used to reduce greenhouse gas emissions or increase soil carbon stocks. Soil management can therefore be used as a climate mitigation option. Results from a recent global analysis of greenhouse gas mitigation options in agriculture, conducted for the IPCC Fourth Assessment Report, will be presented.

Potential roles for microbiology in examining soils and global change will be discussed.

Climate change and the recent emergence of vector-borne diseases

B. Purse

Centre for Ecology & Hydrology, Edinburgh

Climate change is widely predicted to increase the incidence and intensity of vector-borne disease transmission, largely through direct effects of climate on vector biology, abundance and distribution. However, there is little direct evidence that recently observed changes in vector-borne diseases have been precipitated by climate change and a growing recognition of the influence of other non-climatic abiotic and biotic factors on disease distributions. I will discuss how altered patterns in vector-borne diseases should best be attributed to climate change versus other drivers and how the framework of the basic reproductive number may help us identify those vector-borne diseases most likely to change and the direction of their responses. I will draw-on a range of case-studies across vector-borne diseases with particular focus on the dramatic emergence of a midge-borne virus – bluetongue virus – across Southern Europe since the late 1990s. Initial invasion of this disease was climate mediated – multiple strains of BTV entered Europe simultaneously from several different directions and increases in incidence overlapped with those areas that had warmed the most since the 1980s. However, altered biotic interactions with vector and host communities in newly invaded areas were found to be instrumental in allowing bluetongue virus to spread rapidly across Europe.

Predictive epidemiology, a case for cholera

R.R. Colwell

University of Maryland, USA

Abstract not received

A government and international perspective on climate change

J.M. Lynch

Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH

The first meetings of the UN Intergovernmental Panel on Climate Change (IPCC) took place in 1989. These discussions resulted in the UN Framework Convention on Climate Change (UNFCC), with the Kyoto Conference in 1997. In terms of the state of scientific knowledge for formulating policy, it seems to me that there are still some areas where more information is needed or alternative interpretations of existing knowledge could be made. For example the sequestration of carbon dioxide by plants in photosynthesis results in up to 40% of the carbon being released as rhizodeposition, with about half of this available for catabolism by micro-organisms, including the generation of methane. A further example where micro-organisms are involved in climate change mitigation is in the generation of first and second generation biofuels. The true value of this renewable source of energy can only be assessed when a full life cycle assessment is carried out. In reviewing this recently for the OECD, I have emphasized that these analyses are essential if a truly sustainable environment and lifestyle are to be achieved.

Climate change and impacts on above and below ground biodiversity

M.J. Bailey

Centre for Ecology & Hydrology, Oxford

Abstract not received

Coral reef bleaching events, microbial communities and climate change

John Bythell

School of Biology, Newcastle University, Newcastle upon Tyne NE1 7RU

Concurrent with mass coral bleaching events, there has been widespread concern over the emergence of coral diseases over the past 2–3 decades. Reef core data indicates that the mass mortality of two of

the dominant coral species in the Caribbean due to disease is unprecedented in at least the last 3000 years, which suggests a link to anthropogenic activities. Unfortunately, despite the clear relationships in the underlying factors associated with bleaching and disease, and the important role that the microbial flora plays in both processes, the two areas are largely being investigated independently by different groups of researchers using different approaches. This presentation examines the two phenomena and the potential for molecular microbial techniques to help address the underlying causes. Several potential pathogens have been identified using culture-independent approach and are currently being isolated and investigated via challenge experiments. A major emerging theme is the role played by the surface mucus layer (SML) as a barrier to microbial penetration and recent work examining the microbial populations of the SML will be presented.

Applying microbial metagenomics to investigate the effects of ocean acidification on marine microbes

Ian Joint & Jack Gilbert

Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH

Anthropogenic CO_2 in the atmosphere is causing large changes in ocean pH because, when it dissolves in seawater, CO_2 forms a weak acid. If anthropogenic CO_2 continues to be released at current rates, by the end of the century the pH of the surface ocean will be 7.8. It is more than 25 million years since the pH of seawater was so low. We have investigated the effects of ocean acidification in a mesocosm experiment by adjusting pCO_2 to that expected in 2100 (under the IPCC 'business-as-usual scenario'). The response of the microbial community was followed over 3 weeks. Primary production was reduced under high CO_2 conditions and there were fewer coccolithophores present. In an attempt to analyse the response of bacterioplankton communities to elevated pCO_2, we have applied both metagenomic and metatranscriptomic approaches using pyrosequencing. Four DNA and four mRNA samples resulted in a total of 323 million bp (equivalent to 19% of the sequences in the recent Global Ocean Survey). 120 Mbp of mRNA and 200 Mbp of DNA were used to compare how the metatranscriptome changed under high CO_2 conditions.

Cells & Cell Surfaces Group Session

Type V secretion

Secretion of hemoglobin protease

W. Jong & **J. Luirink**

Dept of Molecular Microbiology, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1085, 1081HV Amsterdam, The Netherlands

Autotransporters (ATs) are often produced with an unusual signal peptide extension. The role of this extension in targeting of ATs to the Sec-translocon is subject to controversy. Using combined *in vitro* and *in vivo* approaches we show that the extension of the AT hemoglobin protease (Hbp) does not affect targeting of Hbp, suggesting that the extension is not involved in targeting pathway selection. Rather, the extension was found to be required for proper membrane assembly of Hbp and consequently for efficient secretion. The nature of the translocator of ATs in the outer membrane is another debated issue. Strategies will be discussed to trap intermediates of Hbp during translocation across the outer membrane with the intention to purify and characterize the translocator in action.

Sugars and spices: biogenesis and modular structure of the AIDA-I autotransporter

Marie-Ève Charbonneau & **Michael Mourez**

University of Montreal, 3200 Sicotte, Saint-Hyacinthe, Quebec, Canada (Email m.mourez@umontreal.ca)

The Adhesin Involved in Diffuse Adherence (AIDA-I) belongs to a family of *Escherichia coli* autotransporters recently named the Self-Associating AutoTransporters (SAAT), which comprises the Ag43 and TibA proteins. Remarkably, AIDA-I mediate adhesion to and invasion of epithelial cells, as well as biofilm formation and bacterial autoaggregation. We performed a mutational study to dissect the different functions of AIDA-I. Our results suggest the existence of a modular structure, where different domains are responsible for different functions. The biogenesis of AIDA-I reveals additional remarkable traits. First, the outer membrane translocation of its passenger domain is thought to be spontaneous. Second, AIDA-I can be glycosylated, and glycosylation influences its functions. Third, AIDA-I is proteolytically processed. In our study of AIDA-I biogenesis, we have observed that: (i) AIDA-I is O-glycosylated in the cytoplasm and glycosylation affects the folding and/or conformation of the protein; (ii) there are complex folding processes in the periplasm influencing outer membrane translocation; and (iii) the protein cleaves itself, but the resulting fragments remain associated and lack of cleavage does not influence the functions of AIDA-I in *in vitro* tests. Many of these results are true for other SAAT, suggesting that they are common traits of this family.

Do autotransporters require help to exit the cell?

Kim R. Hardie

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

The requirement for the Sec machinery for translocation of autotransporter proteins across the inner membrane was evident since their discovery due to the presence of an N-terminal signal peptide. Indication that some autotransporter proteins have specific requirements for this step has emerged recently through the observations that some have an unusually long signal peptide, and that some utilize SRP. The existence of a C-terminal domain which is proteolytically separated from the mature central domain of some autotransporters and is capable of forming a β-barrel in the outer membrane led to the hypothesis that these proteins directed their own transport across the outer membrane. However the implication of Omp85 (YaeT) in this step of the secretion process in *Neisseria* supports the hypothesis that autotransporters enlist the help of other proteins to achieve secretion. We have used a genetic approach aimed at identifying such accessory factors in *Escherichia coli* and *Pseudomonas aeruginosa*.

Serine protease autotransporters of *Enterobacteriaceae*: function and translocation

Fernando Ruiz-Perez & **James P. Nataro**

Center for Vaccine Development, School of Medicine, University of Maryland at Baltimore, Baltimore, MD 21201, USA

The autotransporter secretion system (AT) is the most common mechanism of outer membrane (OM) translocation. The mechanism comprises entry to the periplasm via the Sec apparatus, followed by formation of an outer membrane β-barrel, which hypothetically allows passage of the N-terminal passenger domain to the extracellular milieu. Among the largest families of AT proteins is the serine protease autotransporters of Enterobactericeae (SPATEs). Little is known of the roles of the SPATEs in pathogenesis. Our data suggest that SPATE proteins can be assigned to two main phylogenetic clusters. Cluster 1 comprises cytotoxins, several of which act by entering cells and cleaving cytoskeletal proteins. Cluster 2 includes several mucinases, which appear to promote colonization of the mucosa by cleaving mucin and releasing nutritive substrates. Our translocation studies focus on the roles of accessory proteins in AT translocation, specifically the major periplasmic chaperones (Skp, SurA, DegP, FkpA, PpiA and PpiD) and the essential outer membrane YaeT protein. Yeast 2-hybrid (Y2H) and/or overlay experiments suggested interactions between the EspP SPATE protein and the periplasmic SurA, Skp, DegP, and the outer membrane YaeT protein. These proteins each interacted with the EspP β domain. Surprisingly, we also found evidence for interaction of the SurA chaperone protein with the EspP passenger domain. Secretion of EspP was impaired in SurA and Skp mutants. Moreover, secretion of SPATE members was drastically reduced in a DegP mutant. Analysis of outer membrane protein profiles from all mutants confirmed the presence of the AT translocation domain at the same levels as the wild type; mutants yielded reduced levels of the EspP periplasmic intermediate, suggesting degradation of the passenger domain. Proscan analysis identified 12 putative SurA/DegP motifs ('aro-x-aro') on the EspP molecule. 4 potential SurA/DegP motifs were highly conserved throughout the SPATE family. Combined site directed mutagenesis on three conserved putative motifs showed drastically reduction of the EspP secretion, though individual mutations did not produce this phenotype.

A conserved glycine residue of trimeric autotransporter domains and the periplasmic protease DegP play a critical role in autotransport of *Yersinia* adhesin A

I. Autenrieth

Universitätsklinikum Tübingen, Germany

Abstract not received

Biogenesis of the *Escherichia coli* O157:H7 autotransporter EspP

Harris D. Bernstein[1], D. Eric Anderson[3], Travis J. Barnard[1,2], Susan K. Buchanan[2], Nathalie Dautin[1], Raffaele Ieva[1], Janine H. Peterson[1] & Kristen M. Skillman[1]

[1]Genetics and Biochemistry Branch; [2]Laboratory of Molecular Biology; [3]Mass Spectrometry Core Facility, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892 USA

To gain insight into the mechanism of protein secretion by the autotransporter pathway, we have been characterizing individual steps in the biogenesis of EspP, a model autotransporter produced by *E. coli* O157:H7. Our results show that a small segment of EspP that spans the passenger domain-β domain junction is incorporated into the pore formed by the β domain during its assembly in the periplasm. The observation that a folded polypeptide fused to the EspP passenger domain can be efficiently secreted even though it is too large to fit through the β domain pore, however, strongly suggests that the passenger domain is translocated across the outer membrane by an external factor (possibly YaeT/Omp85) rather than by the β domain. Further studies show that after the EspP passenger domain is translocated across the outer membrane, it is cleaved from the β domain in an unusual autocatalytic reaction that occurs inside the β domain pore. Finally, our data indicate that the β domain undergoes significant conformational changes that stabilize it and close the pore following the release of the passenger domain.

YaeT (Omp85) POTRA domain fold, structure and function

Timothy J. Knowles[1], Saeeda Bobat[1], Felician Dancea[1], Mark Jeeves[1], Darren McClelland[1], Tracy Palmer[2], Michael Overduin[1] & Ian R. Henderson[1]

[1]University of Birmingham; [2]University of Dundee

Membranes of Gram-negative bacteria, mitochondria and chloroplasts receive and fold β-barrel transmembrane proteins through the action of POTRA domains. In *Escherichia coli,* folding substrates are inserted into the outer membrane by the essential protein YaeT, a prototypic Omp85 protein. The NMR solution structure of a YaeT tandem of POTRA domains has been determined revealing novel domain articulation about tight linkers. Whilst small angle x-ray scattering of the N-terminus reveals the first insight into the positioning of the critical fifth domain together with novel domain-domain orientations distinct from the crystal structure. NMR titration studies show strands from YaeT's canonical folding substrate, PhoE, bind non-specifically along alternating sides of its mixed β-sheets providing an ideal platform for threading/folding nascent outer-membrane proteins.

The two-partner secretion pathway: structure and function of the transporter FhaC

Françoise Jacob-Dubuisson

Institut Pasteur de Lille, INSERM U629, 1 rue Calmette, 59019 Lille cedex, France

The secretion system of filamentous hemagglutinin (FHA) in *Bordetella* serves as a model for the Two-Partner Secretion (TPS) pathway. The structure of the FHA's specific transporter FhaC, a member of the Omp85/TpsB superfamily, has recently been determined by X-ray crystallography. FhaC is a 16-stranded β-barrel preceded by a periplasmic module composed of two POTRA domains involved in FHA recognition. The FhaC channel is obstructed by both an N-terminal α helix, H1, and an extracellular loop, L6, folded back into the barrel interior. L6 harbours a conserved motif that is the signature of the superfamily. Unlike H1, L6 is absolutely required for the function of FhaC, and short insertions or point mutations in L6 drastically affect the secretion of FHA and the channel properties of FhaC. We propose that initial interactions between FHA's conserved TPS domain and FhaC's POTRAs trigger a conformational change involving the displacement of L6 towards the surface. FHA crosses the outer membrane in an extended conformation through the FhaC channel and folds progressively into a long β helix at the cell surface.

Structural insights into serine protease autotransporter HBP from pathogenic *Escherichia coli*

Jeremy R.H. Tame

Yokohama City University, Suehiro 1-7-29, Tsurumi, Yokohama, Japan

Autotransporters (ATs) are a large family of virulence proteins secreted by Gram-negative bacteria associated with a variety of diseases. Hemoglobin protease (HBP) is a member of the subfamily called SPATEs, Serine Protease AutoTransporters from Enterobacteriaceae. All ATs appear to share similar export mechanisms, but SPATEs are distinguished by the presence of a trypsin-like serine protease domain. HBP is known to play a part in the onset of severe peritonitis, a common and potentially life-threatening condition. The crystal structure of HBP passenger has been solved and refined to 2.2 Angstrom resolution, allowing us to see almost the entire structure of this 1048 residue protein in atomic detail, with only a few small surface loops missing from the electron density map. Although different SPATEs have very different functions, and the precise role of HBP itself remains unknown, their structures are clearly highly related and share a number of architectural features.

Structural biology of trimeric autotransporters

G. Waksman

University College London

Abstract not received

Filamentous hemagglutinin: a paradigm for two-partner secretion and secretion-dependent protein folding

Joseph Mazar, Christopher Noel & **Peggy A. Cotter**

Dept of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, Santa Barbara, California, USA, 93106-9610, USA

Bordetella Filamentous Hemagglutinin (FHA), a large surface-associated and secreted protein, and FhaC, an outer membrane, channel forming, Omp85 family member, are prototypical of Two-Partner Secretion systems. Following Sec-dependent secretion across the cytoplasmic membrane, the N-terminal TPS domain of the FhaB proprotein interacts with FhaC to initiate translocation to the cell surface where SphB1-dependent cleavage forms the mature ~250 kDa FHA protein. The C-terminal ~130 kDa prodomain is presumably degraded in the periplasm. We have shown that the C-terminal (~500 aa) domain of mature FHA (which is predicted to be globular and not β-helical like the N-terminal ~2000 aa), is located distally on the cell surface and is required for FHA-dependent functions *in vitro* and *in vivo*. These data suggest a model in which the N-terminus of FhaB remains associated with FhaC, a hairpin forms initially within FhaC, and then the rest of FhaB is threaded through the pore, folding as it emerges on the surface. We found that the C-terminal FhaB prodomain is required for FHA-dependent phenotypes *in vitro* and *in vivo* and that it functions from within the cytoplasm to allow the C-terminus of mature FHA to fold into a functional conformation on the cell surface.

Secretion of *Bordetella* autotransporters

Rachel Fernandez

University of British Colombia, Vancouver, Canada

More than 30 autotransporters have been identified in the genomes of the sequenced strains of *Bordetella* species. Alignment of the β-barrel translocation units of these proteins results in 4 major groups, with 3

outliers. In general, within each group are found passengers of similar function. Group 1 autotransporters include pertactin, BrkA, Vag8 and tracheal colonization factor (TCF), and interestingly, autotransporters within this group are known or predicted to have either a β-helix fold (e.g. pertactin and BrkA), or to be in a 'coiled' conformation (eg. TCF). The ability of the passenger to be translocated to the cell surface depends on its folded (or unfolded) state and this impacts whether the final folded structure is attained concurrently with translocation or vectorially following translocation. We have examined the folding and secretion of the BrkA passenger and have identified regions, such as the 'junction' that affect its folded state. A biophysical characterization of the TCF passenger shows it to be a monomer that likely derives from a coiled structure. The TCF passenger does not have a junction, yet acquires a folded state as judged by limited proteolysis experiments. BapF is a *Bordetella bronchiseptica* autotransporter that does not fall into any of the 3 major groups. It appears to be acylated and this affords an opportunity to dissect periplasmic sorting between the lipoprotein and autotransporter biogenesis pathways.

Members of the *Bartonella quintana* (BQ) vomp family of four, trimeric autotransporter adhesins (TAA) are differentially expressed, required for virulence *in vivo*, and each confers distinct binding specificities

Peng Zhang, Joanna MacKichan & **Jane E. Koehler**

University of California-San Francisco, San Francisco, CA 94143-0654, USA

Background BQ is a Gram-negative human pathogen causing relapsing/prolonged bloodstream infection. BQ occupies two disparate niches, requiring different binding specificities for: the gastrointestinal epithelium of the body louse (28°C); and erythrocytes and endothelial cells in the mammalian bloodstream (37°C). BQ has four highly conserved, tandemly arranged TAA: the variably expressed outer membrane proteins (Vomp). We hypothesized that the Vomp are critical virulence determinants necessary for infection *in vivo*, with expression regulated by environmental cues.

Results By quantitative RT-PCR, 2-D-SDS-PAGE, and immunoblotting, we found that expression of each *vomp* is differentially regulated at the replication level by *vomp* deletion, and at the transcriptional level by temperature. We also developed the first strategy for in-frame deletion in BQ, finding the *vomp* null mutant is unable to infect an animal that is always infected by wildtype BQ.

Conclusions The Vomp are unusual TAA that permit adaptation to different niches by differential expression of individual *vomp*; deletion of all four *vomp* renders BQ avirulent *in vivo*.

Outer membrane secretion and folding of Type Va autotransporter proteins

Mirco Junker, Jonathan P. Renn & **Patricia L. Clark**

Dept of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

Bacterial pathogenesis requires the display and release of virulence proteins at the bacterial cell surface. Yet little is known regarding detailed mechanisms for outer membrane secretion in Gram-negative bacteria, particularly how secretion occurs efficiently in the absence of an external energy source such as ATP or a proton gradient. Of particular interest is the autotransporter secretion mechanism. Autotransporter proteins represent an abundant class of Gram-negative virulence proteins, and appear to operate autonomously: they require minimal interactions with other cellular components for efficient outer membrane secretion. Our laboratory has determined the *in vitro* folding and unfolding behavior of two unrelated autotransporter passenger domains, pertactin from *Bordetella pertussis* and Pet from *E. coli*. Both passenger domains include a long parallel β-helix domain, but differ in several other respects: Pet (100 kDa) is much larger than pertactin (60 kDa), and includes a larger β-helix as well as an N-terminal globular protease domain. Yet thermodynamic analysis revealed that both proteins contain a stable core structure located in the C-terminus of the passenger domain β-helix. Moreover, this stable passenger domain intermediate is also formed during the process of outer membrane secretion, suggesting that the energy released during passenger domain folding can contribute to efficient secretion.

Clinical Microbiology Group Session

Biological basis of infection control

MRSA as a public health threat

Hajo Grundmann

National Institute for Public Health & the Environment, Bilthoven, The Netherlands

Staphylococcus aureus is a Gram-positive bacterium that colonizes the skin and is frequently found in the anterior nares of about 30% of healthy humans. Depending on its intrinsic virulence or the ability of the host to contain its opportunistic behaviour, *S. aureus* can cause a wide range of pathology in man. The bacterium readily acquires resistance against all classes of antibiotics by one of two distinct mechanisms: mutation of an existing bacterial gene or horizontal transfer of a resistance gene from another bacterium. Several mobile genetic elements carrying exogenous antibiotic resistance genes, may mediate resistance acquisition. Of all resistance traits *S. aureus* has acquired since the introduction of antimicrobial chemotherapy in the 1930's, meticillin-resistance (meticillin-resistant *Staphylococcus aureus*, MRSA) is clinically the most important, since a single genetic element confers resistance to the most frequently prescribed class of antimicrobials –the β-lactam antibiotics, which include penicillins, cephalosporins, and carbapenems. The presentation will provide an overview of historical, current and future perspectives of MRSA as a public health threat.

Confronting and managing infection in the NHS

B.I. Duerden

Inspector of Microbiology and Infection Control, Department of Health, London

Reducing HCAI is a top priority for the NHS in England. It requires a partnership between clinicians responsible for the safe care of their patients, managers and boards providing the right environment for infection prevention and control, and government/DH setting standards, priorities and targets, monitoring and managing performance, and enacting legislation. The DH programme aims to change perceptions and behaviour to delivery safer patient care through: strengthened management; enhanced mandatory surveillance; improved clinical protocols, hand hygiene and aseptic practice; improved cleaning and decontamination; and mandatory training in IP&C for all staff. The Health Act 2006 introduced a statutory code of practice with compliance monitored by the Healthcare Commission. DH performance management focuses on delivery of key national targets for reducing MRSA bacteraemia and *Clostridium difficile* infection. It is supported by an improvement programme which promotes the implementation of the *Saving Lives* High Impact Interventions (care bundles) for care of intravenous lines, ventilated patients, urinary catheters, wounds, and patients with CDI together with guidance on antimicrobial stewardship, use of isolation facilities, screening admissions for MRSA carriage and a clinical dress code to support safe practice. Improvement teams visit Trusts to review their practice and procedures and to help develop and implement their action plans.

Control of MRSA in The Netherlands

M. Vos

Erasmus Medical Centre, Rotterdam, The Netherlands

Abstract not received

Prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) amongst residents and staff of nursing homes

Naomi S. Baldwin[1], Deirdre F. Gilpin[1], Michael M. Tunney[1], Paddy Kearney[2], Anne Gardiner[2] & Carmel M. Hughes[1]

[1]School of Pharmacy, Queen's University of Belfast, 97 Lisburn Road, Belfast; [2]Antrim Area Laboratory, United Hospitals Trust, 45 Bush Road, Antrim

It has been suggested that nursing homes can act as a reservoir for MRSA within the community and may contribute significantly to the spread of MRSA within hospitals when colonized residents are admitted for medical care. The aim of this study was, therefore, to determine the prevalence of MRSA amongst residents and staff in private nursing homes and to identify circulating strains.

Swabs from the anterior nares were taken from 1,111 nursing home residents and 553 staff in 45 private nursing homes and processed by inoculation onto cefoxitin-containing chromogenic agar. After 48 h incubation, positive colonies were confirmed as MRSA by multiplex PCR using primers to detect staphylococcal 16S, *nuc* and *mecA* genes. MRSA strains were further analysed by PFGE and compared with strains isolated in local hospitals.

The combined overall prevalence rates amongst residents and staff were 23% and 7.3%, respectively, with the strains isolated in the nursing homes similar to those isolated in hospital. The results show that MRSA is prevalent within nursing homes and that transfer of MRSA occurs between nursing homes and hospitals and vice-versa.

Rapid PCR testing of intensive care patients: effect on MRSA transmission

Richard Cunningham

Derriford Hospital, Plymouth PL6 8DH

This presentation will review the following questions;

- Does surveillance on Critical Care Units reduce MRSA transmission?
- If so, is rapid PCR testing more effective than conventional culture?
- What effect did it have on transmission in Plymouth?
- Did it deliver the savings the business case promised?

The presentation will outline recent studies on culture and PCR based surveillance, and discuss the pros and cons of each approach.

I will describe the Plymouth Critical Care Units, and the reduction in MRSA transmission and bacteraemia seen when culture based surveillance was replaced with PCR in 2005.

I will discuss why business cases for infection control have to assessed differently from other healthcare initiatives, and summarize the rationale and outcome of our business case.

References Huang *et al.* (2006) Impact of routine intensive care unit surveillance cultures and resultant barrier precautions on hospital-wide methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis* **43**, 971–978 / Harbarth *et al.* (2006) Evaluation of rapid screening and pre-emptive contact isolation for detecting and controlling methicillin-resistant *Staphylococcus aureus* in critical care: an interventional cohort study. *Crit Care* **10**, R25 / Cunningham *et al.* (2007) Effect on MRSA transmission of rapid PCR testing of patients admitted to critical care. *J Hosp Infect* 2007 **65**, 24–28.

Community-associated MRSA: current status and opportunities for infection control

Angela M. Kearns

Head of *Staphylococcus* Reference Laboratory, Health Protection Agency, Centre for Infections, Colindale, London NW9 5EQ

Panton-Valentine Leukocidin (PVL) is a potent exotoxin and a key marker of virulence in *Staphylococcus aureus*, most notably so-called Community-Associated MRSA (CA-MRSA). An epidemic of PVL-MSSA occurred in the 1950s and 1960s and was responsible for community- and hospital-acquired infections. Some 40 years later, the escalation in the number of reports of PVL-MRSA (i.e. CA-MRSA) cases world-wide suggests we are facing a recrudescence of PVL-related disease, predominantly affecting previously healthy individuals in the community.

Internationally, CA-MRSA have been associated with high morbidity and mortality. Most commonly patients present with pyogenic cutaneous lesions (e.g. boils, abscesses and furuncles); more rarely, life-threatening invasive infections such as necrotizing fasciitis, purpura fulminans, and necrotizing pneumonia with a high mortality rate have been reported. Whilst the majority of CA-MRSA infections are sporadic in occurrence, outbreaks among household contacts, military personnel, schoolchildren, close contact sporting groups, MSMs and so on highlight the transmissibility of CA-MRSA strains.

In summary, effective public health interventions are key to controlling this emerging global problem. The overall change in the pattern of MRSA-related disease and mainly community-based nature of transmission demand prompt diagnosis and case recognition allied to evidence-based therapeutic, management and infection control strategies.

Virulence of community-acquired MRSA

J. Etienne, G. Lina &F. Vandenesch

University of Lyon 1, Faculté Laennec, 69008 Lyon, France

The prevalent community-MRSA clones in the US (USA300 and USA400-MW2), and in Europe (ST80 clone) contain the Panton-Valentine leukocidin (PVL) genes (*lukPV*) genes, encoded onto phages (eg ϕSLT). Intravenous injection of PVL in rabbits results in granulocytopenia. Rabbit and human polymorphonuclear (PMN) cells are 10–100 times more sensitive than murine PMNs to the leukotoxic effects of PVL. PVL causes dermonecrotic lesions in rabbits and lethal necrotic lesions in the lungs of BALB/c mice via nasal instillation. In this model, comparing isogenic *S. aureus* strains lysogenized with either wild-type ϕSLT or mutated ϕSLT in which the *lukPV* operon was deleted, necrotizing pneumonia was observed with the wild-type ϕSLT only. Conversely, in a C57/black mice model of bacteraemia comparing the USA300 and MW2, with their PVL-negative derivatives, no difference in mortality was shown. The genome sequence of USA300 revealed the presence of the type I arginine catabolic mobile element (ACME). ACME may facilitate the transmission of USA300, but is not present in the European ST80 clone. Phenol-soluble modulins may also play a role on the infectivity of community-MRSA as they have leukocidal, pro-inflammatory and chemotactic activities. The virulence of community-acquired MRSA seems to reside in multiple pathogenicity determinants whose interactions are not clearly understood.

Epidemic community-acquired MRSA in a country with low levels of hospital-acquired MRSA

Robert Skov, MD

Statens Serum Institut, *Staphylococcus* Laboratory, Copenhagen, Denmark

Denmark has had a frequency of MRSA <1% for >30 years. Community-acquired MRSA was first recognized in Denmark in 1997, where cases of MRSA infections arose independently in a young adult and two families in a rural town. The latter lead to transmission at a kindergarten, a school, a factory and a farm. Since 1999 clinical and epidemiological information has been collected at the time of diagnosis on all new MRSA cases in Denmark. Between 1999 and 2006 the number of CA-MRSA infections increased from 11 to 175. Throughout the period 603 CA-MRSA infections out of a total of 2672 cases (22.6%) were found. Additionally, a number of imported non-hospital related cases were observed. CA-MRSA was mainly seen in children and younger adults and primarily caused skin and soft tissue infections (90%). Transmission between household members was the predominant identified mode of spread. Patients with family relations to high endemic countries were highly overrepresented. Typing showed that 88% of the isolates belonged to 5 clonal complexes CC80, CC8, CC30, CC5 and CC22. Increase of MRSA outside hospitals is of great concern as this not only gives rise to CA-MRSA infections but also inevitably will increase the risk of nosocomial MRSA infections. In response a new national guideline on prevention of MRSA including precautions in primary health care was launched in November 2006.

Immobilized bacteriophages as a method of ultra rapid screening and decontamination of meticillin-resistant *Staphylococcus aureus* (MRSA)

Janice Spencer, Fiona McColm, Luisa Verrecchia, Debra Smith, Emma Bell & Mike Mattey

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

Lytic bacteriophages are viruses that infect and lyse specific bacteria. This study has investigated the effects of chemically bonding (immobilizing) a *Staphylococcus aureus* bacteriophage (BVT01) to polymers including nylon. Bacteriophages can be chemically bonded to nylon where they remain active. There is no decline in the infectivity of the immobilized bacteriophages under differing temperature and humidity conditions where bacteriophages in suspension lose activity. Immobilized bacteriophages can be spread onto dry surfaces where they remain biologically active for up to 28 days.

Bacteriophage BVT01 can lyse many strains of *S. aureus* and is active against the major epidemiologically important MRSA strains in the UK i.e. EMRSA15 (NCTC13142) and EMRSA16 (NCTC13143).

Results have shown that bacteriophage BVT01 is active against 141 of 147 (96%), 27 of 29 (94%), and 31 of 32 (97%) of MRSA strains isolated from patients from 3 different hospitals in the UK and USA.

Immobilized bacteriophages expressing luciferase are being used to detect strains of MRSA. A prototype device and software have been developed as a method for rapid screening.

The same bacteriophage immobilized on nylon beads have been shown to decontaminate MRSA-infected surfaces including tiles and cotton.

Control of community-acquired MRSA in the community

R. Daum

University of Chicago, Chicago, USA

In the past decade the epidemiology of MRSA, infections caused by *Staphylococcus aureus* isolates resistant to meticillin and all available ß-lactams, has changed. No longer confined to health-care environments, novel MRSA strains have been identified circulating in the community that infect previously healthy individuals. They differ from their health-care associated counterparts in ways demonstrable by a variety of genetic testing methods. These novel community-associated MRSA strains (CA-MRSA) have been responsible for epidemic disease among children and adults in the US. Although skin and soft tissue infections (SSTIs) have been most frequent, invasive diseases such as necrotizing pneumonia, osteomyelitis, necrotizing fasciitis, and septic phlebitis of large pelvic veins masquerading as

right-sided endocarditis have also been described. Severe sepsis and the Waterhouse-Friderichsen syndrome have also been recognized with high mortality. The CA-MRSA epidemic has suggested that new risk factors have replaced the traditional ones defining association with the health-care environment. These have included household contacts of patients with CA-MRSA infections, men who have sex with men, children, soldiers, incarcerated persons, athletes, Native American populations and intravenous drug abusers. Preliminary data also suggest that the recurrence rate is high and individuals with SSTI are at substantial risk for recurrence. Strategies to address control of this CA-MRSA epidemic will require documentation of these new risk factors and the identification of new paradigms for their control.

Clostridium difficile

J.S. Brazier

Anaerobe Reference Laboratory, University Hospital of Wales, Cardiff

C. difficile infections are an increasing problem in UK hospitals despite attempts to control the problem. One arm of understanding the epidemiology of the disease is surveillance of the strains that are currently causing infections in England in addition to typing of isolates of *C. difficile* from outbreak investigations. The Anaerobe Reference Laboratory (ARL) has data on over 1,000 isolates from the Department of Health and Health Protection Agency surveillance programme which includes the PCR ribotypes and their antimicrobial susceptibility patterns. It has also tracked the spread of the 'hyper-virulent' strain Type 027 around the whole of the UK and abroad.

Clostridium difficile: the changing epidemiology in age distribution

S.N. Reddy[1], P. Kalima[2], A.J. Wroe[1], M.H.S. Collie[3], D.N. Anderson[3] & I.R. Poxton[1]

[1]Centre for Infectious Diseases, University of Edinburgh; [2]Medical Microbiology; 3Colorectal Surgery, Western General Hospital, Edinburgh

Background *Clostridium Difficile* Associated Disease (CDAD) is a health care and community associated disease. CDAD cases are increasing with increased prevalence in hospitals. Our aim was to review the age distribution of *C. difficile* incidence over a 7-year period (2000–2006 inclusive), within the in-patient population presenting to Lothian University Hospitals Trust.

Methods Patients, from the 5 hospitals within the trust, were identified via Medical Microbiology and Surgical Audit databases. Retrospective analysis of prospectively collected outcome data was then performed.

Results 3897 patients were diagnosed with CDAD over the 7-year period. A detailed breakdown of the number of cases per age-group each year is shown below.

Age	2000	2001	2002	2003	2004	2005	2006
1–10	0	0	0	0	1	2	11
11–20	0	3	8	4	1	6	17
21–30	5	1	11	6	24	14	21
31–40	1	3	18	16	27	32	30
41–50	1	7	29	36	49	37	40
51–60	7	9	32	34	54	76	102
61–70	13	24	67	90	119	142	136
71–80	25	57	120	185	177	201	286
81–90	30	57	132	183	182	216	322
91–101	16	14	46	58	54	81	87
Total	**98**	**175**	**463**	**612**	**688**	**808**	**1053**

Conclusions There has been an exponential increase in the number of patients diagnosed with CDAD each year. Whilst C. difficile primarily continues to affect the elderly population the number of positive cases identified in the younger age groups is also increasing.

Extended spectrum β-lactamase-producing bacteria

Sebastian G.B. Amyes

Centre for Infectious Diseases, University of Edinburgh, Edinburgh

The emergence of β-lactamases capable of conferring penicillin resistance encouraged a switch to the clinical use of the cephalosporins. However, in the early 1980s, both the ubiquitous TEM and SHV β-lactamases had mutated to allow access of these drugs to the active site. The success of the extended-spectrum β-lactamases (ESBLs) relied upon their ability to mutate and these new mutants to be selected in real-time. This reached a point where almost all the currently available cephalosporins can be hydrolysed. In the late 1980s, a new group of ESBLs emerged, the CTX-M ESBLs, derived from *Kluyvera ascorbata*. They confer high level resistance to many fast-penetrating cephalosporins and are have spread rapidly through clinical bacteria in recent years. There are now more that 300 ESBLs but the term ESBLs strictly speaking refers to increased cephalosporin-hydrolysing capability of enzymes previously devoid of this ability. However, these enzymes are not the only β-lactamases that are mutating in real-time as we are seeing similar patterns of β-lactamase expansion in the IMP, VIM and OXA β-lactamase responsible for carbapenem insusceptibility in non-fermenting Gram-negative bacteria. As reliance on carbapenems increases, we may find that these enzyme groups may be even more problematic that the traditional ESBLs.

Development of a rapid microarray for *Staphylococcus aureus* and MRSA

F.J. Hamilton, K.G. Wooldridge, D.P. Turner, R.P. Spence, V. Wright & R. James

Centre for Healthcare Associated Infections, University of Nottingham

Staphylococcus aureus is a Gram-positive coccus that has long been recognized as a major human pathogen. Meticillin-resistant *S. aureus* (MRSA) accounts for up to 40% of all *S. aureus* bacteraemia in the UK and has higher morbidity and mortality rates than meticillin-sensitive *S. aureus* (MSSA) infection (Brown et al. 2005). The current method of diagnosis for MRSA is culture and susceptibility testing, which is slow with turnaround times of up to 24 hours and provides limited information. Molecular techniques such as real time PCR have the potential to reduce turnaround time but are usually limited by the amount of information available. Microarray technology can address this problem as many genes can be detected simultaneously, however this is traditionally a time consuming technique.

A diagnostic microarray was developed using virulence, resistance and epidemiological markers. This microarray was optimized at each stage of the protocol to minimize the length of time taken for the whole process. Whole genome amplification (WGA) was used to amplify *S. aureus* DNA direct from blood culture bottles and Universal Linkage Labelling was used to label the DNA for the microarray. Genes incorporated into the microarray included species identification genes and genes encoding clinically relevant toxin, virulence and resistance markers. In addition some targets were included for detection of other major species of the Staphylococcus genus.

Reference Brown *et al.* (2005) Guidelines for the laboratory diagnosis and susceptibility testing of meticillin-resistant *Staphylococcus aureus* (MRSA). *J Antimicrob Chemother* **56**, 1000–1018.

Treatment of *Acinetobacter baumannii* infections

Matthew E. Falagas

Alfa Institute of Biomedical Sciences (AIBS), Athens, Greece

Infections due to *Acinetobacter baumannii* have become common in various settings and populations, especially critically ill patients. There has been a controversy regarding the mortality directly attributable to *A. baumannii* infection in patients with isolation of this micro-organism. However, recent data have shown that *A. baumannii*

infection is, indeed, associated with considerable mortality. Another development with major public health implications is the advancing antimicrobial resistance of *A. baumannii* isolates that may be intrinsic or acquired and is mediated with various mechanisms of resistance (production of β-lactamases or enzymes inactivating aminoglycosides, efflux pumps, lower permeability of the outer membrane, mutations in antibiotic targets, etc.). Broad-spectrum β-lactam antibiotics, mainly carbapenems, have been considered the first choice for the treatment of patients with *A. baumannii* infections. However, the worrisome pattern of increasing antimicrobial resistance of *A. baumannii* isolates has led to re-evaluation and use of intravenous and aerosolized polymyxins (mainly colistin) in various parts of the world. In addition, various agents with novel mechanisms of antibacterial action have been investigated, although there are no clinical data available regarding their effectiveness and safety.

Clinical Microbiology / Clinical Virology Groups Joint Session

How to pass the MRCPath Part 2: tips for the exam

How to pass the MRCPath Part 2: tips for the exam

Kate Gould

Dept of Microbiology, Freeman Hospital, Newcastle upon Tyne NE7 7DN

The MRCPath Practical examination has evolved over the years. Although no longer an 'exit' examination, it attempts to assess both basic practical skills and competency at Consultant level in clinical microbiology and infection control.

Laboratory management; including how to improve quality, how to how to introduce new technology into the laboratory

Tim G. Wreghitt

Regional Microbiologist – East of England, Health Protection Agency – Cambridge

Microbiology is a science-driven medical speciality which is constantly changing. The rapid development and roll out of molecular techniques is transforming how we deliver our clinical microbiology services, especially in virology. The two over-riding concerns of Trust managers are turnaround times (especially for HCAIs and Government imposed targets) and the cost of providing the service. These factors are being taken into account by Lord Carter in his review of Pathology Services and by Strategic Health Authorities in recommending changes in service configuration. Some tests, such as testing needlestick donors for blood-borne viruses are required urgently, since they affect patient management. Turnaround times can be reduced by the introduction of automation, seven day and extended hours working. IT connectivity between processing machines and the laboratory clinical computer, including barcoding for sample recognition, cuts down error rates, streamlines the process and reduces staffing costs. Virology and serology laboratories have been employing automated serology machines for some time. The introduction of automated sample processing, such as the Kiestra system in bacteriology laboratories will revolutionize clinical bacteriology practices in larger laboratories. The onward march of molecular testing, currently provided mainly in larger Trust laboratories, will expand rapidly when more commercial tests become available.

No other abstracts were received.

Clinical Virology Group Workshop

Rapid diagnostics – 'lab on a chip'

Overview of diagnostic micro-technologies

Gemma A. Cannon

National Virus Reference Laboratory, University College Dublin, Belfield

The recent advances and integration of engineering, chemistry, and biology has resulted in the miniaturization of existing techniques and the development of novel analytical techniques. Micro total analysis systems (μTAS) is the term used to describe such miniaturized systems that enable sample preparation, analysis and reporting in a single system. The three main techniques exploited in these systems include microarrays, microfluidics and bead-based arrays. Microfluidics refers to the controlled and precise handling of liquids at the sub-micro level. It is readily applicable to a number of existing techniques such as DNA isolation and amplification, immunoassays, DNA and protein separation and cell-based analysis. A typical microfluidic system comprises of a sample inlet, a reaction chamber and a waste reservoir. The term 'microarray' refers to a number of probes affixed at specific locations on a flat surface and allows for the detection of a number of parameters at a single time. A labelled target interacts with these probes and can be detected by a variety of methods. There are two main microarray formats, DNA and protein-based. Microarray technology is most commonly used in research for expression profiling, assessing genomic rearrangements and single nucleotide polymorphism identification, though recently developed miniaturized microarray formats lend to their use in diagnostics. Bead-based assays exploit the use of beads as a solid support, with each bead containing a number of probes. Again, the type of probe can be either DNA or protein in origin. The advantage of bead-based technologies over microarray technology is that multiplex analysis can be tailored to individual assay requirements. The benefits of miniaturized handling include; increased assay time and performance, along with associated decreases in cost, reagent consumption and sample requirement and preparation. It is anticipated that the future analytical systems will be a marriage of each of these techniques resulting in a streamlined high-throughput and rapid analyses. Further advances may include novel detection systems using nanoparticles, cantilever technology and electrochemical detection.

Bio-chip sensors for the rapid and sensitive detection of viral disease – an update

Andrew D. Livingston

UK Field Applications Scientist, Stratagene Europe (Agilent Technologies), Amsterdam, The Netherlands

Recent advances in DNA and protein microarray methodology and the emerging technology of cell-based sensors have massively increased the speed and sensitivity with which we can detect viral infections. The advantages of the multi-parameter microarray technologies could be combined with the speed and sensitivity of cell-based systems to give 'cell-omic' sensors.

Previous immunological and PCR-based pathogen detection systems have historically been hampered by numerous technical drawbacks due to their labour intensive and time-consuming nature. Over recent years the diagnostic world has seen an ever increasing focus towards microarray technologies designed to address such technical hurdles. Both DNA and protein microarray platforms have been designed to allow the parallel, sensitive and rapid detection of numerous pathogens. Such technologies are becoming evermore robust, however recent advances in cell-based sensors could soon see the development of array platforms which allow a high degree of sensitivity coupled with yet greater speed of detection.

A number of groups from around the world have demonstrated the ability to engineer highly specific cell-based sensors which allow pathogen detection in a matter of minutes. At the same time, the microarray platform has advanced from the original DNA and protein probe based design toward cell arrays. The combination of such complementary technologies opens numerous design possibilities for the development of bio-chip sensors for the rapid and sensitive detection of pathogens.

Pan-virus arrays for virus detection and characterization

Malcolm Banks

Deputy Head, Mammalian Virology Group, Veterinary Laboratories Agency, Weybridge, Surrey KT15 3NB

Virus isolation in cultured cells has been the mainstay of laboratory diagnosis of viral disease for many years. However, this approach has a number of shortcomings eg. lack of permissivity for the cell system used or masking by other viruses that may be present either as contributors to the disease pathogenesis or as bystanders.

The use of pan-PCRs against generic targets and the development of multiplex PCRs have improved the diagnostic situation relating to some fastidious viruses , but these do rely on representation of the agent amongst the panel of PCRs selected.

The advent of sequence-independent PCR primers allied to the development of micro-array printing and scanning facilities has enabled the development of a new generation of diagnostic tools that do not rely on any *a priori* knowledge or suspicion of the disease aetiology. Moreover, the use of pan-virus microarrays should facilitate elucidation of the aetiopathogenesis of complex, multi-agent diseases such as post-weaning multisystemic wasting syndrome (PMWS) of pigs.

Validation of thousands of oligonucleotides is an enormous task and so VLA has entered into a collaboration with several laboratories to design and validate a veterinary virus chip carrying 2884, 70-mer oligonucleotide probes from 308 viruses in 35 families.

This paper describes some of the problems and solutions encountered during this validation process, including development of genotyping arrays for notifiable disease agents such as European bat lyssaviruses and classical swine fever virus. Early results on the value of the arrays for the investigation of diseases of unknown aetiology (PMWS) will also be described.

Development of a microarray for the detection of exotic, endemic, zoonotic and emerging viruses in avian and other species

Paul Britton

Division of Microbiology, Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN

Early detection and identification of the aetiological agent(s) is a crucial factor in the control of a disease. However, diagnostic tests often rely on some prior knowledge of the causative pathogen. Recently diagnostic microarrays have been used for the identification of viruses and bacteria and have led to the identification of new viruses. A great advantage of using a microarray-based diagnostic test is that a single field or clinical sample can be analysed for the presence of multiple viruses in a single operation without prior knowledge of

the identity of the pathogens. I will describe a virus microarray we have developed in collaboration with three other laboratories consisting of over 2800 oligonucleotides derived from over 300 viruses from 35 virus families.

The oligonucleotides, comprising 70 nucleotides, were derived from sequence data, available both in the public domain or generated in-house, of either complete or partial virus genome sequences. The oligonucleotide probes were synthesized commercially and the microarrays spotted in-house using a previously published protocol. Virus-derived nucleic acid was isolated, randomly amplified, labelled and hybridized in the usual manner, but we have developed bespoke software to analyse the fluorescence data generated ('DetectiV') providing statistical support for virus identification.

Results have shown that the microarray is capable of discriminating different viruses following hybridization of labelled virus-derived nucleic acid to specific oligonucleotide probes. We have validated the microarray using the avian coronavirus, Infectious Bronchitis Virus (IBV), Foot and Mouth Disease Virus (FMDV) and other viruses as models to optimize probe design, sample labelling, hybridization and analysis for the array development. In addition, hybridization of labelled nucleic acids derived from different serotypes of FMDV and different topotypes within a serotype to the microarray have resulted in different hybridization profiles. These viral signatures are also detectable in 'real' samples from infected animals.

Microfluidic platforms for clinical diagnostics

Fiona Gilchrist

Stokes Institute, Dept of Mechanical and Aeronautical Engineering, University of Limerick, Limerick, Ireland

There is an urgent need for the development of rapid point of care microfluidic disposable chips for viral diagnoses of infections that utilize whole blood. Microfluidics is an attempt to miniaturize chemical and biological analysis devices in the laboratory. Current designs are often referred to as 'laboratory-on-a-chip' or micrototal analysis (μTAS). Microfluidic devices function by allowing a variety of chemical processes and interactions to occur as fluid flows within their miniature channels.

Our chip design is based upon a microfluidic platform that incorporates lateral flow. Conventional immunoassays are conducted in microwell plates with several disadvantages, including long assay times, difficult fluid handling techniques, and high sample and reagent consumption. These drawbacks have prevented immunoassay from being a point-of-care diagnostic tool. Our capillary driven flow chip will incorporate on-chip sample transport, reagent dispensing and mixing in a reaction chamber. Transport of sample and reagents and the resultant assay reaction will be achieved using the passive phenomena of capillarity and laminar diffusion, respectively. A sample preparation stage will be incorporated into the capillary driven flow chip involving microscale filtration to remove blood cell and platelets from the whole blood sample.

Development of multipathogen microarrays for diagnosis of infectious diseases

Nigel J. Silman

Operations Manager, Novel & Dangerous Pathogens Dept, Health Protection Agency, Centre for Emergency Preparedness & Response, Porton Down, Salisbury SP4 0JG

DNA microarrays are an attractive technology for the simultaneous detection and diagnosis of a range of viral and bacterial pathogens. Expression microarrays have been widely reported for studying the genome-wide response of a single organism to external environmental stimuli. The high discriminatory power of microarray technology has more recently been turned towards detection and diagnosis.

We have developed a multi-pathogen oligonucleotide microarray, which currently contains 2200 different gene probes. Both viral and bacterial pathogens with either RNA or DNA genomes are detected on the same chip. 16S rRNA probes designed against the hyper-variable regions give genus level identification and specific virulence gene probes allow absolute identification of bacterial pathogens. For detection of viral pathogens, a slightly different approach has been taken. Since there is no equivalent consensus gene such as 16S or 23S rRNA in viruses, probes have been designed (between 5 and 10 per virus) which target the regions of high discrimination within the viral genome. All probes have been extensively checked for potential cross-hybridization *in silico* by database searching.

Much of the development work has been undertaken using *Bacillus anthracis* as a model system, however, probes which detect *Francisella tularensis, Yersinia pestis* and many other Australia Group pathogens have been incorporated. Similarly, probes have been included to detect the viral pathogens on the Australia Group list, as well as a large number of respiratory and enteroviruses, orthopox viruses and parapox viruses. Data obtained indicate that the microarray has very high specificity and is capable of routinely detecting as little as 28pg of pathogen nucleic acid in a 2h assay.

The ability to run simultaneous molecular assays for a wide range of organisms is highly desirable in certain cases. The potential value to the discovery of newly emerging viruses will be discussed alongside the practical aspects of assay development.

Clinical Virology Group Session

Vaccines against viral infections from concept to practice

Developing vaccines against Ebola virus and other BSL-4 pathogens

Anthony Sanchez

Centers for Disease Control & Prevention, Atlanta, USA

The development of protective vaccines against biosafety level-4 (BSL-4) agents, primarily hemorrhagic fever viruses (HFVs), has been challenging for scientists for various reason. A significant factor that has hindered progress in this area has been the limited number of high containment facilities and staff capable and authorized to conduct the studies. Additionally, vaccines against certain HFVs, such as filoviruses (Ebola and Marburg viruses) and Lassa virus, have been difficult to produce, since simple 'killed' vaccines that primarily induce a humoral immunity against are not protective. It has been determined that the development of a strong cell-mediated immunity is required to prevent or clear infections caused by these viruses, and inducing this type of immunity has proven to be somewhat difficult.

Nevertheless, researchers have successfully applied recombinant DNA techniques to develop protective vaccines against Ebola virus infections. This has not only led to potential countermeasures against this biothreat, but has also provided important insights into vaccinology that can be applied to other infectious agents. The research behind this vaccine development will be presented, along with its implications for future vaccines and the impact of new BSL-4 facilities on these and related efforts

Development and implementation of HPV vaccines

S. Inglis

National Institute for Biological Standards & Control, South Mimms

Abstract not received

From laboratory development to licensing and field use of vaccines to control influenza in poultry

David E. Swayne

U.S. Department of Agriculture, Athens, Georgia, USA

Vaccines against avian influenza (AI) have had limited use in poultry until the 2003 when the H5N1 high pathogenicity (HP) AI began spread from China to multiple southeast Asian countries, and to Europe in 2005 and Africa in 2006. Over the past 40 years, AI vaccines have been primarily based on field outbreak AI strains that were grown in embryonating chicken eggs, chemically inactivated, emulsified in mineral oil adjuvant and injected into individual birds. Recently, recombinant viral vectored vaccine have been developed and licensed including fowlpox and avian paramyxovirus type 1 (ND) vectored vaccines with AI H5 gene inserts. Additional vectored technologies hold promise for usage in the future possibly including baculoviruses, herpesvirus of turkeys, infectious laryngotracheitis virus, adenoviruses, attenuated influenza A viruses, AI-ND virus chimeras and bacterial vectors such as salmonella. Advances in biotechnologies may overcome some existing limitations and result in vaccines that can be grown in tissue culture systems for more rapid vaccine production; provide optimized protection as the result of closer genetic relationship to field viruses through reverse genetics and gene insertions in vector systems; can be mass applied by aerosol, drinking water or in ovo administration; and provide easier strategies for identifying infected birds within vaccinated populations; i.e. DIVA. However, these new technologies will be licensed only after demonstration of purity, safety, efficacy and potency against AI viruses, and limitation on horizontal transmission in naïve poultry. There potential use in the field will also be determined on the requirement for low cost vaccines to be economically competitive.

Rinderpest vaccine strategy

P. Roeder

Food & Agriculture Organization of the United Nations, Rome, Italy

Abstract not received

Vaccination with live attenuated SIV confers potent protection from vigorous heterologous and homologous virus challenge

Neil Berry, Richard Stebbings, Debbie Ferguson, Claire Ham, Adrian Jenkins, Mark Page, Jim Stott & Neil Almond

Divisions of Retrovirology and Biotherapeutics, NIBSC, South Mimms, Herts

Despite concerted efforts we do not know what responses a broadly protective HIV/AIDS vaccine needs to elicit. In simian models, the most effective vaccines result from exposure to live, attenuated simian immunodeficiency virus (SIV). At NIBSC, we have demonstrated that vaccination with the minimally *nef*-attenuated SIVmac32H/C8 protects cynomolgus macaques (*Macaca fascicularis*) from detectable infection with homologous wild-type SIVmac32H/J5 within 3 weeks, even following CD8+ T cell depletion prior to challenge. We have extended these studies to evaluate the breadth of protection conferred by SIVmacC8 and its ability to resist potent virus challenges. A heterologous, antigenically distinct virus challenge (SIVsmE660) and an uncloned, highly potent and vigorously replicating homologous challenge virus (SIVmac251/32H/L28) were evaluated. High levels of protection were achieved against heterologous SIVsmE660 challenge with no evidence of superinfection in 5/8 vaccinates compared to 3/8 vaccinates solidly protected after challenge with SIVmac32H/L28. Where virus breakthrough was observed, levels of peak and steady-state viraemia were significantly lower in vaccinates compared to naïve challenge controls. Unexpectedly, clear evidence of recrudescence of the vaccine virus SIVmac32H/C8 upon re-challenge with SIVmac32H/L28, in both protected and superinfected macaques, was identified. Current efforts are focused on establishing the relative involvement of adaptive immune and other vaccine responses in conferring this potent protection.

Influenza HA and NA-pseudotyped retroviral vectors: applications to pandemic vaccine evaluation, sero-surveillance and antiviral drug screening

Nigel Temperton

MRC/UCL Centre for Medical Molecular Virology, University College London, W1T 4JF

The continuous rapid evolution of H5N1 influenza viruses has major implications for the sensitivity of serological assays and can limit the efficacy of avian and pandemic human vaccines and the susceptibility of these viruses to anti-virals. Retroviral pseudotypes bearing HA and NA envelope glycoproteins are ideally placed to address these problems and can be used for; 1. Sensitive, high-throughput, low-containment cell-based assays for neutralizing antibodies against influenza H5N1 HA. It is straightforward to update this HA neutralization assay to measure responses against newly emerging HA antigenic drift variants. Upon availability of the HA sequence of the emergent H5N1 virus, the

HA can be synthesized and retroviral pseudotypes prepared for use in neutralization assays. These assays can be used to address the cross-clade neutralizing potential of pandemic human vaccines and immuno-therapeutics (monoclonal antibodies etc.), and for sero-surveillance studies in new outbreak locations. 2. Sensitive assays for neutralizing antibodies against NA. 3. Sensitive assays for the evaluation of anti-HA and anti-NA drugs and for the study of drug resistance.

Persistence of varicella zoster virus viraemia in patients with herpes zoster

Mark Quinlivan[1], Karen Ayres[2], Mary Leedham-Green[1], Fiona Scott[1] & Judy Breuer[1]

[1]Centre for Infectious Disease, Institute of Cell & Molecular Science, Barts & the London; [2]Section of Applied Statistics, School of Biological Sciences, University of Reading

Herpes-zoster is caused by reactivation of Varicella-zoster virus and is often complicated by prolonged pain called post-herpetic neuralgia (PHN). Up to 78% of zoster patients become viraemic, however, the persistence of viraemia over time and its putative relationship to disease outcome are unknown, In order to investigate this, we recruited 65 patients with acute zoster and measured their viral loads at baseline, 4, 12 and 24 weeks. The severity of pain and its impact on activities of daily living were measured.. Overall, 89% of patients remained viraemic for up to 6 months. Baseline viral load was not associated with age, gender, immune status, rash age, presence of prodromal pain, being on antivirals or acute pain. Higher viral loads at 4 weeks, were found in males, subjects aged 50 years, immunocompromised patients and patients who were not on antiviral therapy, although none were significant. The rate of viral clearance from 4–12 weeks was faster in males (p=0.03) and patients aged less than 50 years but was not affected by being on antivirals. In four patients who still had PHN at 3 months viral load was higher than at baseline. In contrast patients whose pain had recovered had lower viral loads than at baseline. The data suggest that VZV viraemia persists following rash in most patients. Further studies are needed to explore whether the rate of clearance of viraemia is related to persistence of pain.

A novel serological assay for the detection of rabies virus and lyssavirus neutralizing antibodies

Edward Wright[1], Nigel Temperton[1], Denise Marston[2], Anthony Fooks[2] & Robin Weiss[1]

[1]MRC/UCL Centre for Medical Molecular Virology, University College London, 46 Cleveland Street, London; [2]Rabies and Wildlife Zoonoses Group, Veterinary Laboratories Agency, WHO Rabies Collaborating Centre, Addlestone

The use of retroviral pseudotypes as a vector for gene therapy is well documented. Recently, we have developed pseudotypes to determine neutralizing antibody titres for highly pathogenic viruses. This removes the need for high containment facilities and allows small volumes of serum to be used. G-protein sequences from the rabies isolate CVS-11 and European bat lyssaviruses were cloned and co-expressed with lentiviral gag-pol and GFP or luciferase reporter genes. The pseudotypes infected a number of target cell lines and produced titres almost equivalent to VSV-G protein pseudotypes. Neutralization assays using blinded sera and pseudotyped CVS-11 detected positive and negative samples with 100% specificity and sensitivity and correlated with the OIE FAVN (R^2 = 0.89). It is possible to detect cross-neutralizing antibodies to bat lyssaviruses pseudotypes.

Evaluation of Cationic Lipid DNA Complex (CLDC) in small animal models as a platform for both therapeutic treatment and vaccine development for alphavirus infections

Christopher H. Logue[1], Chris Bosio[2], Steven Dow[3], Kenneth E. Olson[2] & Ann M. Powers[1]

[1]Centres for Disease Control & Prevention, Fort Collins, CO, USA; [2]AIDL – Colorado State University, Fort Collins, CO, USA; [3]IDA – Colorado State University, Fort Collins, CO, USA

CLDC's have been shown to be protective against other arboviruses but the utility of these complexes is unknown against alphaviruses.We have undertaken experiments to examine the possible protective effects of CLDC's against encephalitic arboviral infection. In our initial studies mice were pre-inoculated intraperitoneally with 200 ul of CLDC's or diluent. Twenty four hours later, mice were subcutaneously challenged with a dose of VEEV or WEEV that is lethal in 4–6 days for all mice. Initial results demonstrated that CLDC's provided a protective effect for the pre-treated mice. Mice receiving the CDLC prior to infection showed no (or only mild signs of illness) while those that were mock-treated demonstrated significant and typical signs of illness. Additionally, serum from all of these mice has been tested for the presence of the cytokines interferon (IFN)-α, IFN-β, and IFN-γ and examined by immunohistopathology analysis of the brains. Mice that received CLDC treatment induced a high IFN-α response at 48 h indicating that the CLDC's were indeed priming the immune response. Further, the IFN-γ levels were slightly increased at 48 h but persisted to 120 h. We have demonstrated the utility of CLDC's as a therapeutic treatment for alphaviral infection. Expansion of these studies to include additional (earlier) timepoints, pre-, post, and co-infection cohorts, use of other encephalitic alphaviruses to determine if this protection is universal or species specific, and to test the ability of these complexes to protect if viral infection occurs via the aerosol route will be discussed.

Towards a safe conditional-live HIV-1 vaccine

Ben Berkhout, Bep Klaver, Monique Vink, Alex Harwig & Atze T. Das

Laboratory of Experimental Virology of the University of Amsterdam, The Netherlands

We previously reported the construction of an HIV-1 variant lacking a functional Tat-TAR axis, which was made replication-competent by introduction of the two components of the Tet-ON system: the doxycycline (dox)-activated rtTA transcriptional activator was inserted at the site of the nef gene and the tetO binding sites were inserted in the LTR promoter. This HIV-rtTA variant replicates in a dox-dependent manner, can be turned on and off at will, and the level of replication can be fine-tuned by adjustment of the dox-level. We have optimized this vaccine candidate by spontaneous virus evolution, which yielded greatly improved Tet-ON systems for use in human cells. In addition, we incorporated a second dependency: a variant Envelope protein that is dependent on the antiviral peptide T20. To allow vaccination experiments in macaques we developed a similar dox-dependent SIVmac239 variant. This SIV-rtTA variant will be tested in macaques in collaboration with Neil Almond (NIBSC) and Martin Cranage (University of London). The route towards this conditional-live vaccine candidate and our future plans will be discussed.

The epidemiology of West Nile virus infections in the Americas and the development of chimeric vaccines for horses and humans

E.P.J. Gibbs[1], M.T. Long[1] & T.P. Monath[2]

[1]College of Veterinary Medicine, University of Florida, Gainesville, FL, USA; [2]Kleiner Perkins Caufield & Byers, Pandemic & Biodefense Fund, 21 Finn Road, Harvard, MA, USA

Since its introduction to New York in 1999, West Nile virus (WNV) has spread widely to become endemic in the contiguous states of the USA, parts of Canada, and the Caribbean region. Several species of mosquito transmit the virus with wild birds serving as the reservoir. Since 1999, more than 25,000 equine cases of WNV encephalomyelitis, with an estimated 30–40% case fatality rate, have been reported in horses in

the USA. In 2007, the Centers for Disease Control reported 3,506 WNV infections in humans of which 1,172 were neuroinvasive. A live, attenuated, genetically engineered vaccine for use in horses and humans has been developed by replacing the prM-E genes of YF 17D vaccine virus with the corresponding genes of WNV. The vaccine developed for horses is now licensed in the USA and the human vaccine is in phase 2 clinical trials in young adult and elderly subjects.

Japanese encephalitis virus

T. Solomon

University of Liverpool

Abstract not received

HIV vaccine developments

D.R. Burton

The Scripps Research Institute, California, USA

Abstract not received

Genetics of vaccine responses – the example of smallpox

Ingileif Jónsdóttir

deCODE genetics, Reykjavik, Iceland

Genetic determinants of vaccine responses are only partly known, although HLA polymorphism is known to be of importance. Twin studies have shown significant genetic contribution to immune response to various paediatric vaccines. At deCODE genetics we have used a genealogical approach to study the heritability and genetics of responses to smallpox vaccination, with the aim to map and identify genes that contribut to lack of response or adverse events. Nationwide smallpox vaccinations were performed in schools until 1975 and outcomes recorded by school nurses in follow-up visits. We have entered into our database information on smallpox vaccination dates and outcomes for ~ 60.000 individuals in Reykjavik. Individuals that did not respond to repeated vaccination attempts have been identified. Absence from school after and prior to vaccination was registered to identify those that may have experienced adverse events We have demonstrated significant familial risk of non-response and adverse events to smallpox vaccination. This indicates that genetic factors contribute to both extreme phenotypes of the immune response. In order to map the genetic regions that segregate with the phenotypes of non-response or adverse events to smallpox vaccination we have performed genome wide microsatellite genotyping of non-responders and those with adverse events and their unaffected close relatives and performed linkage analysis. To identify genetic variants that contribute risk of non-response or adverse events genome wide association (GWA) studies based on genotyping 370.000 single nucleotide polymorphism (SNPs) are also under way. Using the GWA approach deCODE and others have identified many genetic risk factors for various complex diseases and phenotypes. Identification of genes that contribute to poor response or adverse events to vaccination will be useful for the design of improved vaccination strategies.

This study is part of Population Genetics Analysis Program: Immunity to Vaccines/Infections (NIH-NIAID-DAIT-BAA-04-18) funded by NIAID.

The development and implementation of rotavirus vaccines

Timo Vesikari

University of Tampere, Medical School, Tampere, Finland

Two live attenuated oral rotavirus vaccines were licensed in 2006. Rotarix™ (GSK) is a human rotavirus vaccine with G1P[8] serotype characteristics. RotaTeq™ (Merck) is a bovine-human reassortant vaccine expressing human G 1–4 and P[8] antigens. In prelicensure trials both vaccines showed low reactogenicity, high efficacy, and safety for intussusception (IS) (Ruíz-Palacios et al. NEJM 2006, Vesikari et al. NEJM 2006).

In Europe, efficacy of Rotarix™ against severe rotavirus gastroenteritis (RVGE) was 96% in the first and 86% in the second season (Vesikari et al. Lancet 2007). Vaccine efficacy for 2 years against severe G1 and non-G1 (including G2) RVGE was equal, but efficacy against all RVGE was better for G1 strains (90%) than non-G1 strains (73%).

Also in Europe, RotaTeq™ was 95% efficacious against severe RVGE in the first year, with efficacy demonstrated against all circulating RV serotypes including G9 not represented in the vaccine. In Finland, efficacy against RVGE of any severity was 72% for up to 2 rotavirus seasons (Vesikari ICP2007).

Both vaccines have now been distributed in millions of doses. Postmarketing data have not shown any gross signal for IS with either vaccine, but more data are required until final conclusions on safety for IS.

Environmental Microbiology Group Session

Microbial metabolism of nitrogen-, phosphorus- and sulfur-containing compounds: environmental challenges, possible solutions and future perspectives

Coupling of nitrogen regeneration and assimilation in the oceans

Ian Joint, Darren Clark & Andrew Rees

Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH

The availability of inorganic nitrogen compounds controls phytoplankton production in the oceans. It is important to have accurate estimates of production on global scales so that we can accurately model the global carbon budget in relation to climate change. Large regions of the oceans are extremely oligotrophic with nitrate and ammonium concentrations <10 nmol N l^{-1}. These low concentrations result from the activity of phytoplankton species – mostly cyanobacteria – that have extremely efficient uptake mechanisms. Rapid regeneration of ammonium and nitrate balances the assimilation by phytoplankton and bacterioplankton. Physical processes can also perturb the steady state and events such as upwelling can result in rapid phytoplankton growth and changes to assemblage structure. We have studied nitrification and NH_4^+ regeneration rates in the central oceanic gyres of the North and South Atlantic. Nitrogen cycling is extremely rapid and completely replaces the dissolved inorganic nitrogen pool within 1 day. Nitrification was more active than expected, suggesting that most of the NO_3^- pool in the surface oligotrophic Atlantic is derived from the regeneration of N. These findings increase the uncertainty of estimating new production and carbon export based on f-ratio.

The nitrite dependent anaerobic oxidation of methane and ammonium

Mike Jetten

Dept of Microbiology, IWWR, Radboud University Nijmegen, Huygens Building HG02.407, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands
(Email m.jetten@science.ru.nl)

Nitrite dependent anaerobic oxidation of methane (N-DAMO) and ammonium (anammox) are two recent discoveries in the nitrogen cycle catalysed by completely unrelated microbes of the NC10 and planctomycetes phylum, respectively. The two processes share some very interesting aspects. Both of them were once considered impossible and non-existent in nature, but have now been identified as important players in global carbon and nitrogen cycling despite their low growth rate and low growth yield. Although, the microbes responsible for both processes are only available in enrichment cultures, great progress has been made in unraveling their molecular ecology, genome assembly, cellular biology, and unusual lipid structures. Whereas most Prokaryotes consist of one compartment, anammox bacteria are compartmentalized by intracellular membranes. Finally processes of anammox and N-DAMO have an excellent potential for application in wastewater treatment as exemplified by 5 full scale operational anammox reactor systems worldwide.

Nitrate reduction in estuarine sediment; rate process measurements and quantification of key genes involved in nitrate reduction

Cindy J. Smith[1,2], Liang F. Dong[1], David. B. Nedwell[1] & A. Mark Osborn[2]

[1]Dept of Biological Sciences, University of Essex, Colchester CO4 3SQ; [2]Dept of Animal & Plant Sciences, University of Sheffield, Sheffield S10 2TN

Estuarine systems are the major conduit for the transfer of nitrate from agricultural and other terrestrial-anthropogenic sources into marine ecosystems. Within estuarine sediments, microbially driven processes (denitrification and anammox) result in the net removal of nitrogen, while others (dissimilatory nitrate reduction to ammonium) don't. The hypernutrified Colne Estuary, U.K, has a nitrate concentration gradient from the estuary head to the mouth. The fate of nitrate and nitrite from three sites along this gradient were investigated using rate process measurements combined with molecular analyses to determine the diversity, abundance and activity of the nitrate reducing bacterial communities in sediments by targeting key functional genes and their transcripts mediating nitrate and nitrite reduction. Rates of nitrate and nitrite reduction were greatest at the estuary head. This trend was in general reflected at the molecular level with highest genes and transcripts numbers also observed at the estuary head site.

The production and consumption of nitrous oxide by denitrifying and ammonifying bacteria

David Richardson

University of East Anglia, Norwich NR4 6PZ
(Email d.richardson@uea.ac.uk)

The most infamous greenhouse gases in the public eye are currently carbon dioxide and methane and there is much international focus on reducing emissions of these by countries signed up to the Kyoto Protocol. However, nitrous oxide (N_2O) now ranks alongside these as a cause for great concern. At present nitrous oxide is perhaps best known to the general public as laughing gas. However throughout the 20th century and continuing into the 21st century, nitrous oxide in the environment has increased by 50 parts per billion and this atmospheric loading is increasing further by 0.25% each year. Although it only accounts for around 9% of total greenhouse gas emissions it has a 300-fold greater global warming potential than carbon dioxide over the next 100 years and an atmospheric lifetime of 150 years. This is most definitely not a laughing matter and it is recognized in the Kyoto protocol that is important to begin to mitigate these releases. When faced with a shortage of oxygen many bacterial species, are able switch from using oxygen to using nitrates to support respiration in a processes known as denitrification and ammonification. Nitrous oxide can be released during these processes and efforts to improve the management of such emissions in the environment will benefit from the better understanding of the factors that influence the synthesis and activity of the enzymes involved.

Nitrification in biological wastewater treatment: Insights based on molecular approaches

Holger Daims[1], Sebastian Lücker[1], Frank Maixner[1], Roland Hatzenpichler[1], Marc Mussmann[1], Eva Spieck[2], Elena V. Lebedeva[3], Denis Le Paslier[4] & Michael Wagner[1]

[1]Dept of Microbial Ecology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [2]Universität Hamburg, Biozentrum Klein Flottbek, Mikrobiologie, Ohnhorststr. 18, D-22609 Hamburg, Germany; [3]Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow 117312, Russia; [4]Genoscope, 2 rue Gaston Crémieux CP 5706, 91057 Evry, France

Nitrification is a key process of the biogeochemical nitrogen cycle and of wastewater treatment. It is catalysed by chemolithoautotrophic ammonia-oxidizing bacteria (AOB) and archaea (AOA) and by nitrite-oxidizing bacteria (NOB). Molecular techniques have revealed that many nitrifiers in wastewater treatment plants (WWTPs) are uncultured and mainly uncharacterized microbes.

One group of such organisms is the genus *Nitrospira*, whose representatives are the dominant NOB in WWTPs and other ecosystems. Recently the genome of '*Candidatus* Nitrospira defluvii', which is not available in pure culture, was fully sequenced by environmental genomics. The ongoing genome analysis has revealed details of key pathways in *Nitrospira*, including carbon fixation, nitrite oxidation, and the use of organic carbon sources (mixotrophy). In parallel, the metagenome of the whole microbial community of a nitrifying WWTP was partially sequenced (322,361 BAC ends) and screened for genome pieces of *Nitrospira*. The results show a fascinating extent of genomic rearrangements and recombination among closely related *Nitrospira*.

AOB are abundant in most nitrifying WWTPs, whereas the importance of AOA for wastewater treatment is unclear. A combination of several molecular tools revealed that AOA are abundant *in situ* in specific industrial WWTPs. Interestingly, AOA and *Nitrospira* also occur and nitrify in geothermal hot springs.

Biological phosphate removal

Mark C.M. van Loosdrecht

Delft University of Technology, The Netherlands
(Email M.C.M.vanLoosdrecht@TUDelft.nl)

Biological phosphate removal is a widely used process for removal and recovery of phosphate from wastewater. It is based on the activity of a specific group of micro-organisms that can make up 50% of the population in a wastewater treatment plant but as yet has not been cultivated in pure culture despite 40 years of research. The presentation will discuss the state of the art and history of development in the light of interactions between process engineers and microbiologists. The phosphate accumulating bacteria and their competitors have a unique physiology that is adapted to life in strongly dynamic systems. These organisms can only grow in the presence of oxygen or nitrate, but can accumulate their substrates inside the cell in the absence of an electron donor. These dynamic conditions are exploited in wastewater treatment systems to accumulate these organsism in the system. In the presentation several open research topics will be discussed.

References Van Loosdrecht *et al.* (1997) Biological phosphate removal processes. *Appl Microbiol Biotechnol* **48**, 289–296 / Mino *et al.* (1998) Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Res* **32**, 3193–3207 / Oehmen *et al.* (2007) Advances in enhanced biological phosphorus removal – From micro to macro scale. *Water Res* **41**, 2271–2300.

Meeting phosphorus consents – the role for biological phosphorus removal in Severn Trent Water

P. Vale

Severn Trent Water Ltd, Wastewater Strategy, 2297 Coventry Road, Birmingham B48 7JX (Email peter.vale@severntrent.co.uk)

The UK water industry is faced with a large increase in the number of sewage treatment works (STW's) that are required to meet a phosphorus (P) consent. In Severn Trent there will be around 100 works requiring P removal by the end of AMP4 (2010). Biological P removal is becoming an increasingly attractive option due to an anticipated rise in the cost of iron precipitants, and the requirement to meet low effluent iron standards. However, the treatment efficiency of an enhanced biological phosphorus removal (EBPR) process depends not only on the design and configuration of the activated sludge plant (ASP), but is also strongly dependent on the influent wastewater characteristics. In order to ensure that the use of EBPR is maximized, and thus the reliance on iron salts reduced, Severn Trent Water has developed an approach whereby:

- wastewaters are screened to assess how amenable they are to an EBPR process,
- for large works, generally > 100,000 PE, pilot trials are commissioned to assess the most suitable configuration,
- for medium sized activated sludge plants, an economic retrofit solution has been developed,
- for smaller works oxidation ditches are configured to remove N and P, and
- to widen the viability of EBPR further, designs to enhance EBPR performance where wastewaters are particularly weak are being developed.

Phosphorus accumulating organisms reveal their secrets: a genome-level understanding of enhanced biological phosphorus removal

Katherine D. McMahon, Shaomei He, Jason Flowers, Hector Garcia Martin, Victor Kunin, Euan Burton, Vincent J. J. Martin & Philip Hugenholtz

Dept of Civil and Environmental Engineering, 1415 Engineering Drive, University of Wisconsin – Madison, Madison, WI, 53706, USA

Genome-enabled analyses of microbial communities have generated tremendous insight into the structure and functioning of these complex assemblages. The recent metagenomic sequencing of multiple enhanced biological phosphorus removal (EBPR) sludges has created the opportunity to study the expression of genes and pathways involved in this metabolism. Results of (meta)transcriptomic and (meta)proteomic analyses during normal operation and following system perturbed will be presented. A comparative genomics approach will be used to explore ecophysiological differences between closely related phylotypes frequently enriched in laboratory-scale bioreactors used to study EBPR. The role of phage in controlling community dynamics and population evolution will also be discussed. This multidisciplinary collaboration between engineers, microbiologists, and bioinformaticists has made great strides towards unraveling the basis for this remarkable metabolism that is used so widely for phosphorus removal from wastewaters.

Polyphosphate and medium chain length polyhydroxyalkanoate (mclPHA) accumulation under aerobic growth conditions

Kevin O'Connor

University College Dublin, Ireland

Polyhydroxyalkanoate (PHA) is a biodegradable polymer accumulated by bacteria as an intracellular carbon storage material generally in response to inorganic nutrient limitation in the presence of excess carbon. It has been the target of intense research as a new generation of environmentally friendly polymers. There are two types of PHA accumulated by bacteria. Short chain PHA which contains a short side chain (-CH_3 or C_2H_5) e.g. Polyhydroxybutyrate (PHB) and medium chain length PHA containing side chains with greater than 3 carbons ($CH_2CH_2CH_3$). Several studies have shown that polyphosphate and PHB are accumulated in an alternating cycle of anaerobic (PHB) and aerobic (polyphosphate) growth in waste waster treatment sludges. We have recently discovered that medium chain length PHA accumulating *Pseudomonas* species accumulate polyphosphate (polyP) and medium chain length polyhydroxyalkanoate (mcl-PHA) concurrently. It would

also appear that that polyphosphate is not the rate limiting step for mcl-PHA accumulation in these *Pseudomonas* strains. Polyphosphate and mcl-PHA accumulation as well as enzyme activities over the growth cycle will be discussed.

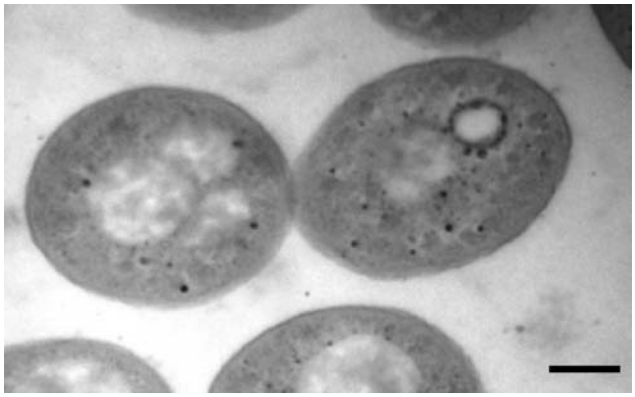

Fig. 1. Transmission electron micrograph of *Pseudomonas putida* CA-3 accumulating polyphosphate and mcl-PHA under aerobic growth conditions. PHA appears as white/grey granules and polyphosphate as black spots. Bar, 200 nm.

Inorganic polyphosphate: a universal biopolymer of multiple functions

Narayana N. Rao

Dept of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305 USA

Inorganic polyphosphate (Poly P) is a polymer of tens to hundreds of phosphate residues linked by 'high-energy' phosphoanhydride bonds. Found in abundance in all cells in nature, Poly P is essential for growth of cells, their responses to stresses and stringencies, and the virulence of pathogens. Poly P kinase 1 (PPK1), is the major enzyme implicated in Poly P synthesis, and is highly conserved in many bacterial species, including some major pathogens. For example, mutants of *P. aeruginosa* lacking PPK1 are defective in motility, quorum sensing, biofilm formation, and virulence. PPK1 also plays a role in cytokinesis and cell division of *Dictyostelium discoideum*, sporulation and biofilm formation of *Bacillus cereus*, and development and survival of *Myxococcus xanthus*. Structural studies reveal a unique ATP-binding site of PPK1. Another widely conserved enzyme is PPK2 with distinctive kinetic properties. These enzymes, absent in yeast and animals, are novel and attractive targets for treatment of many microbial diseases. A unique form of PPK2 has been found in *Dictyostelium discoideum* (DdPPK2); this enzyme transforms itself into an actin-like fiber concurrent with the synthesis, step by step, of a Poly P chain made from ATP. Homologs of DdPPK2 are found in pathogenic protozoa and in the alga, *Chlamydomonas*.

Differential protein expression during aerobic and anaerobic phases of the enhanced biological phosphorus removal process

Margaret Wexler[1], David Richardson[1] & Philip L. Bond[2]

[1]School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ; [2]Advanced Wastewater Management Center, University of Queensland, Brisbane, Queensland 4072, Australia

Enhanced biological phosphorus removal (EBPR) is a wastewater treatment process that operates for P removal. In EBPR systems, activated sludge cycles through anaerobic and aerobic phases, resulting in microbial intracellular accumulation of phosphorus, and its removal from effluent wastewater. Metabolic details of transformations that occur during these alternating phases are not well understood. Our research aims to understand further the biochemistry of EBPR.

We compared proteins expressed by the mixed microbial community of a sequencing batch reactor run for high performance EBPR, during aerobic and anaerobic phases, using spectrometry-based proteomics and radiolabelling of proteins. The majority of differentially expressed proteins were assigned to the polyphosphate-accumulating organism '*Candidatus* Accumulibacter phosphatis', an uncultured polyphosphate-accumulating organism associated with EBPR. These results and associated molecular studies will be discussed in relation to EBPR biochemical models.

New ways to break an old bond: the role of the carbon-phosphorus hydrolases in biogeochemical phosphorus cycling

John P. Quinn, Anna N. Kulakova, Natalie A. Cooley & John W. McGrath.

School of Biological Sciences and Questor Centre, Queen's University of Belfast, 97, Lisburn Road, Belfast BT9 7BL

Phosphonates are organophosphorus molecules that contain the highly stable C-P bond, rather than the more common, and more labile, C-O-P phosphate ester bond. They have ancient origins and their biosynthesis is widespread amongst more primitive organisms. However the importance of phosphonates in the contemporary biosphere is increasingly recognized *e.g.* as a nutrient source in the oceans, in which productivity is frequently P-limited. The microbial degradation of phosphonates was originally thought to occur only under conditions of phosphate starvation, mediated by the poorly characterized C-P lyase multienzyme system, under Pho regulon control. More recent studies have demonstrated the Pho-independent, substrate-inducible, mineralization by environmental bacteria of three of the most widely distributed biogenic phosphonates: 2-aminoethylphosphonic acid (ciliatine), phosphonoacetic acid, and 2-amino-3-phosphonopropionic acid (phosphonoalanine). Whole-genome and metagenome sequence analysis indicates that the genes encoding these newly described C-P hydrolases are distributed widely amongst prokaryotes. Since they are able to function under conditions in which C-P lyases are inactive, the three enzymes may play a hitherto-unrecognized role in phosphonate breakdown in the environment and hence make a significant contribution to global biogeochemical P-cycling.

Biodegradation of linear alkylbenzenesulfonates: microbiology, biochemistry and genetics

Alasdair M. Cook, David Schleheck & Hans-Peter E. Kohler

Dept of Biology, The University, Konstanz, Germany, and EAWAG, Dübendorf, Switzerland

The major synthetic surfactant in use worldwide is linear alkylbenzenesulfonate (LAS), which comprises 20 mainly chiral congeners that are subject to biodegradation by microbial communities. Our first heterotrophic communities comprised two tiers of bacteria. The first tier is represented by *Parvibaculum lavamentivorans* DS-1, whose genome has been sequenced, and which attacks all LAS congeners to yield 50 largely chiral sulfophenyl(di)carboxylates that are degraded in the second tier, whose components, e.g. *Comamonas testosteroni* KF-1 (also sequenced), have narrow substrate ranges. Strain KF-1 degraded the racemates of 3-(4-sulfophenyl)butyrate (SPB) sequentially, and via *ortho* cleavage of 4-sulfocatechol. A cell suspension of the organism degraded SPB with transient release of an unknown intermediate, which was identified as 4-sulfoacetophenone. An 18-reaction degradative pathway for chiral SPB was proposed, initiated by generation and manipulation of CoA-esters to acetyl-CoA and sulfoacetophenone. The latter should undergo a Baeyer-Villiger monooxygenation. We found and separated the enzyme. RT-PCR tentatively indicated the genetic locus involved. Sulfophenol-3-monooxygenase and the ring cleavage were measured, but not yet identified. Despite considerable literature on the desulfonation segment, the genes involved in strain KF-1 are still unknown. The sulfite dehydrogenase gene(s) was identified. Variants of this pathway are needed to degrade 48 more sulfophenyl(di)carboxylates.

The marine phosphonate barrel: investigating a great untapped reservoir

Jack A. Gilbert

Plymouth Marine Laboratory, Prospect place, Plymouth
(Email jagi@pml.ac.uk)

Marine phosphonates represent a potential source of bio-available organic phosphorous. Phosphonoacetate is an organophosphonate of both biotic and xenobiotic origin and is mineralized by phosphonoacetate hydrolase, encoded by the *phnA* gene. During this study, water was collected from two sites in the Western English Channel, located inside and outside of a mixed phytoplankton bloom. mRNA from each site was analysed to determine the diversity of the expression of Phosphonoacetate hydrolase transcripts. Both sites showed a phylogenetically diverse set of phnA genes from various different phylogenetic bacterial groups. We also investigated the prevalence of phosphonoacetate hydrolase from strains of marine bacteria associated with marine invertebrates. These results indicate a significant untapped reservoir of active phosphonate utilizing microbes within these environments, indicating that marine phosphonates probably constitute a far more important phosphorous source than was previously considered.

Desulfurization of organosulfur compounds in soil and rhizosphere environments

Achim Schmalenberger, Emma Fellows & **Michael A. Kertesz**

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

Sulfur is an important nutrient element for both microbes and plants. In agricultural soils most of the sulfur is bound to the soil organic matter, and spectroscopic studies (XANES) reveal that it is largely present as sulfate esters or sulfonates. Using model substrates, we have shown that the sulfonatase enzymes in bacteria that assimilate sulfonate-sulfur are reduced-flavin-dependent monooxygenases (SsuD), and that the AsfA reductase protein is essential for desulfurization of aromatic sulfonates. AsfA may also play an important role in mobilization of sulfonates as part of the soil sulfur cycle. We have explored bacterial diversity in rhizospheres of field-grown wheat plants grown with and without sulfate fertilization, and in grassland rhizospheres. Genetic profiling of 16S rRNA gene fragments from the wheat rhizospheres revealed that fertilizer sulfate treatment was correlated with changes in both the overall bacterial community structure, and that of the betaproteobacteria. Community analysis at the functional gene level (*asfA*) showed that 40% of clones in *asfAB* clone libraries were affiliated to the genus *Variovorax*, and cultivation-dependent studies also yielded *Variovorax* and related Comamonadaceae as the dominant desulfonating bacteria. These taxa therefore seem to be important in organosulfur cycling in wheat rhizospheres, though other desulfonating strains have been isolated from grasses.

The climate-changing gas dimethyl sulfide: a product that is made in surprising ways by some surprising bacteria

Andrew W.B. Johnston, Andrew R.J. Curson, Jonathan D. Todd, Rachel Rogers, Nefeli Nikolaidou-Katsaridou, Matt Sullivan & Sun Lei

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

Several hundred million tons of dimethyl sulfide (DMS) are made annually in the oceans, ~10% of being liberated into the atmosphere. In addition to this massive transformation of carbon and sulfur DMS affects climate, its oxidation products inducing cloud formation over the oceans. And, it's a component of the 'smell of the seaside'!

DMS is formed by microbial catabolism of dimethylsulfonio-propionate (DMSP) an abundant anti-stress molecule made by phytoplankton, seaweeds and some land plants.

We identified '*ddd*' gene clusters for DMSP-dependent DMS production in several bacteria. Different lineages use wholly different enzymatic mechanisms for this process and some wholly unexpected 'terrestrial' bacteria (*eg* some rhizobia) make DMS. The *ddd* clusters have similarities, but also striking differences regarding (*e.g.*) DMSP transporters and regulators in different lineages. The *ddd* genes seem prone to horizontal gene transfer, and some individual bacteria have multiple ways of making DMS.

The implications of this remarkable diversity in different bacteria will be discussed.

DMSP degradation in marine roseobacters: genomics and transcriptomics

Helmut Bürgmann, Erinn C. Howard, Wenying Ye, Feng Sun, Shulei Sun & Mary-Ann Moran

Dept of Marine Sciences, University of Georgia, Athens, GA 30602-3636, USA

Marine roseobacters are an abundant alphaproteobacterial group involved in the turnover of dimethylsulfoniopropionate (DMSP), and thus play an important role for the marine sulfur cycle and the release of the climatically active dimethylsulfide from the world's oceans. The availability of full genome sequences for a number of these organisms, among them *Silicibacter pomeroyi,* together with other genetic and molecular tools have allowed for advances in the study of DMSP related pathways, genes, and gene transcription in these organisms. Identification of dmdA, the gene responsible for the first step in DMSP demethylation in *S. pomeroyi* was an important step in improving our understanding of this pathway. This glycine cleavage T-family protein with DMSP methyltransferase activity identified marine bacterioplankton in the Roseobacter and SAR11 taxa as primary mediators of DMSP demethylation to methylmercaptopropionate.

Studies on the specific transcriptional response of *S. pomeroyi* to DMSP using microarrays shed new light on ecological strategies of the organism, and provided new hypothesis for genes involved in the DMSP degradation pathway. These studies not only improved our understanding of the genetics and regulation of DMSP degradation in roseobacters, but also provide a foundation for an improved understanding of their ecology and role in marine sulfur cycling.

New insights into microbial degradation of dimethylsulfide

Hendrik Schäfer[1], Josh Neufeld[2], Colin Murrell[2] & Rich Boden[2]

[1]Warwick HRI, Wellesbourne, Warwick CV35 9EF; [2]Biological Sciences, University of Warwick, Coventry CV4 7AL

Dimethylsulfide (DMS) is an organosulfur trace gas that accounts for the majority of biogenic atmospheric sulfur input. Its atmospheric oxidation products backscatter heat radiation and promote cloud formation, giving DMS a climate-cooling role. Most DMS produced from dimethylsulfoniopropionate in the oceans is not emitted to the atmosphere but degraded by micro-organisms in the ocean surface using various metabolic pathways and key enzymes. Little information has been available about the identities and activities of microbial populations that are controlling the amount of DMS that is available for sea-to-air transfer. Using enrichment and isolation and ^{13}C-DMS stable isotope probing, we have identified *Methylophaga* spp. as key populations of marine DMS degradation and investigated the DMS metabolism of a *Methylophaga* isolate. Progress has also been made in identifying enzymes and genes of biochemically distinct DMS degradation pathways in *Methylophaga* sp. DMS010, *Hyphomicrobium* species and other marine DMS-degrading bacteria, paving the way to cultivation-independent identification of DMS-degrading populations in natural samples and the marine metagenome dataset based on functional genetic markers.

Fermentation & Bioprocessing Group Session

Commercial industrial bioprocess development

Technical and regulatory challenges encountered in the manufacture and marketing of a therapeutic drug; a case study

Derek Rutherford

Centre for Emergency Preparedness and Response, Health Protection Agency, Porton Down

Taking a drug from research and development through process optimization to large scale manufacture & sale presents significant technical & regulatory challenges. Regulatory authorities worldwide expect a manufacturer to demonstrate a robust manufacturing process capable of consistently producing an effective and safe drug in a compliant manner

Manufacturing equipment must adhere to strict validation requirements to verify appropriate levels of performance. PLC control systems are playing a greater part in equipment operation and adherence to the requirements of Good Automated Manufacturing Practice (GAMP) have presented the industry with an additional challenge.

In a global marketplace with ever increasing competition companies must ensure they maintain their supply to market as any interruption could lead to a loss of market share which, once lost, can be extremely difficult to regain.

This presentation aims to outline the key technical and regulatory criteria which must be met to achieve compliance using the manufacture of a current therapeutic drug as a model.

Raid upstream process development using statistical experimental design

Jim Mills

Xenova Biomanufacturing, 310 Cambridge Science Park, Milton Road, Cambridge CB4 0WG

Statistical experimental design, or Design of Experiments (DoE), has been used for a number of years within the process industries. However, the applications of this powerful technique have been somewhat limited within the field of bioprocess development. The limited applications in which it has been used have included microbial medium development and formulation development. It has also only had very limited application in eukaryotic cell culture development. This presentation will describe how DoE can be applied to all stages of the product development cycle. Specific examples will be used to illustrate how DoE can be utilized to enable the rapid development of robust prokaryotic and eukarotic upstream processes.

Harnessing fungal cell factories for enzyme production

M.G. Tuohy

National University of Ireland – Galway, Ireland

Abstract not received

Commercial production of tens of thousands of tonnes of diverse enzyme products

S.M. Stocks

Novozymes A/S, Denmark (Website www.novozymes.com)

Novozymes A/S is the largest producer of food, feed or technical enzymes world wide, producing tens of thousands of tons of solid or liquid enzyme preparations each year. The enzyme sector is highly competitive and mature, yet continues to grow with new applications and challenges emerging constantly. Compared to pharmaceutical GMP, food/feed GMP often permits optimization, leading to a dynamic research – development – production environment. GRAS (generally regarded as safe) strains are used by Novozymes for production of a range of native & GM products; the organisms are typically excretors of the proteins, growing well in a range of media under a range of temperatures and pH's, with robust performance at scales up to at least 160m^3. Some hosts or strains are filamentous and produce a viscous broth creating challenges for mass transfer, while bacilli tend to have a lesser viscosity, creating different challenges. The presentation aims to give a general insight into the business of microbial enzyme production for food and feed and for technical applications such as laundry, or cellulose degradation (for fuel ethanol).

Production of fuel ethanol at Suedzucker AG

Michael Klingeberg

SUEDZUCKER AG Mannheim/Ochsenfurt, Research, Development & Services Dept, Wormser Str. 11, D-67283 Obrigheim/Pfalz; Germany (Email michael.klingeberg@suedzucker.de)

Bioethanol and biodiesel as first-generation biofuels are currently the only widely available biofuels. In Brazil the production of ethanol is based on sugar cane, while in the USA the production is mainly based on corn, a starch containing raw material. On the other hand in Europe diverse raw materials such as beet molasses, potatoes, fruit and cereals are used in the small distilleries, while cereals have become established in the larger factories which have been built up in the last 2–3 years. Here the main focus is wheat and to a lesser extent rye, barley, corn and triticale are processed.

The SUEDZUCKER-group has built up an ethanol factory in Zeitz, Germany, which is able to process 700,000 metric tonnes of starch containing crops such as wheat, corn, barley or triticale in order to convert it to 260,000 m^3 of ethanol. As by-product the protein rich *distillers dried grains with solubles* (DDGS, Protigrain®) is produced and sold as livestock feed. An annex factory for the conversion of sucrose containing thick juice to another 60,000 m^3 of ethanol is going to start in spring 2008. Further, a factory in Wanze, Belgium, with an annual capacity of 300,000 m^3 ethanol is under construction. In Wanze the protein fraction of the wheat will be extracted and the remaining bran will be used as an energy source for the plant. Recently, a bioethanol factory located in Pischelsdorf, Austria, has been put into operation. This plant which will process mainly wheat, corn and thick juice has got a capacity of 240,000 m^3 ethanol/year. Another production side with a smaller capacity is located in Eppeville, France, where raw ethanol is produced from molasses and thick juice.

The presentation will give an overview about the ethanol production process in the factories and the work which has been done in the R&D department concerning the evaluation of different yeast strains, starch hydrolysing and viscosity reducing enzymes.

Optimizing industrial amino acid production applying metabolic engineering and systems biology tools

R. Takors, B. Bathe, M. Rieping, R. Kelle & H. Huthmacher

EVONIK DEGUSSA GmbH, Health & Nutrition, Kantstr. 2, 33790 Halle / Westfalia, Germany

As a global market leader in speciality chemicals, EVONIK DEGUSSA is the only company in the world that provides all four important amino acids for animal nutrition: DL-methionine, L-lysine (Biolys™), L-threonine and L-tryptophan. Furthermore, other amino acids such

as L-valine, L-isoleucine, and L-proline – which are used as precursors for fine chemicals and pharmaceuticals as well as for nutrition applications – are produced too.

Amino acid production via fermentation plays an important role at EVONIK DEGUSSA. Consequently detailed R&D activities for further optimizing production strains and processes are performed typically concentrating on *E. coli* or *C. glutamicum*. These approaches make use of modern lab-scale 'omics' technologies as well as of 'classical' engineering disciplines to ensure a successful technology transfer from the lab into production.

This contribution illustrates some of the process developments exemplarily concentrating on efforts for L-lysine, L-valine or L-threonine. Considering L-lysine, examples will be given for unravelling gene functions of the biosynthetic pathway and for illustrating regulatory networks in *C. glutamicum* based on a systems biology approach. Metabolome studies will be presented that allowed the identification of metabolic engineering targets in Valin synthesis.

Scale-up of *Saccharomyces cerevisiae* fermentation for the manufacture of recombinant human albumin

A. Wigley, **D. Wilkinson** & D. Mead

Novozymes Biopharma UK Ltd, 59 Castle Boulevard, Nottingham NG7 1FD

A robust fermentation process for the large scale manufacture of recombinant human albumin for pharmaceutical use is described. It is essential to operate the fermentation in a reproducible and consistent manner because as a secreted product it is potentially exposed to biological or chemical post-translational modification which could result in poor product quality. A process for the production of recombinant human albumin in the yeast *Saccharomyces cerevisiae* offers a number of advantages. The yeast and mammalian secretion processes are sufficiently similar that correctly folded and processed albumin is obtained in the culture supernatant. The extensive knowledge of *S. cerevisiae* genetics has permitted the development of a stable, plasmid based expression system. This case study demonstrates that yeast episomal plasmids can be wholly suitable for industrial use P2P if they are designed as whole 2 µm vectors in a cir° background exemplified by the disintegration vector system. A simple high intensity fed-batch fermentation process has been developed using automatic feed control, which has been applied at the manufacturing scale. Animal derived products are avoided and the production costs are typical of a microbial fermentation based process. Endotoxin removal by ultrafiltration of the medium was shown to be unnecessary. Automation was part of the design from the laboratory development stage, with fermentation and down stream planned as an integrated system avoiding intermediate steps. The resulting process has been successfully scaled-up , most recently to a total working volume of 27,000 litres. The process is reliable for the industrial scale production of pharmaceutical grade recombinant human albumin in *S cerevisiae*. The robustness of the system has been demonstrated by a process simulation study of genetic stability. No loss of plasmid, product yield or quality was observed even after more than 200 generations of growth.

The *Streptomyces* matrix... hijacking natural biosynthetic paths to produce commercial products

F. Burke

Eli Lilly & Company Ltd, Speke

Streptomyces are developmentally complex prokaryotes and are used extensively for their capacity to produce complex bioactive molecules of wide diversity. So-called 'Classical Fermentation' is now a mature technology and in many instances has formed the background from which techniques and standards for the more recent biotechnology products have been derived.

This presentation will discuss aspects of specific molecules synthesized by large-scale fermentation using *Streptomyces* together with some of the current commercial, regulatory and environmental challenges of operating large scale *Streptomyces* fermentations.

Food & Beverages Group Session

Transmission of viruses through the food chain

Establishing networks for the surveillance of food-borne norovirus outbreaks

M. Koopmans

National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Abstract not received

Shellfish-borne virus transmission: progresses and challenges

Albert Bosch[1], M. Isabel Costafreda[1], Julien Schaeffer[2], Sylvain Parnaudeau[2], Françoise S. Le Guyader[2] & Rosa M. Pintó[1]

[1]Enteric Virus Laboratory, Dept of Microbiology, University of Barcelona, Barcelona, Spain; [2]Laboratory of Microbiology, Ifremer, Nantes, France

Although over one hundred different enteric viruses may be found as bivalve mollusk contaminants most viral outbreaks are restricted to norovirus (NoV), and hepatitis A virus (HAV) making them the main targets for virus detection in shellfish.

Molecular data have demonstrated relatively conserved regions at the 3′-end of ORF1 and the 5′-end of ORF2 regions of NoV, being the ORF1/ORF2 junction employed as a target for real-time amplification. Real-time procedures based on the amplification of a fragment of the highly conserved 5′ non-coding region have also been successfully developed for HAV quantitative detection in shellfish. To avoid any false negative result due to inhibitors, NoV and HAV RNA internal controls have been developed. Each sample is then analysed for the different NoV genogroups as well HAV and also co-amplified with each internal control to evaluate the RT-PCR efficiency. Standard reagents, such as the MC_0 Mengo virus strain and ssRNA internal controls have been employed as controls of nucleic acids extraction and RT-PCR, respectively. Quality control and quality assurance issues have been implemented through the use of standardized molecular procedures that may enable its inclusion in regulatory standards for viruses in bivalves.

Impact of food- and water-borne spread on the diversity of enteric viruses

Miren Iturriza-Gómara

Enteric Virus Unit, Virus Reference Dept, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5HT

In recent years viruses have been recognized increasingly as an important cause of foodborne infections. More than 160 enteric viruses are excreted in the faeces of infected individuals, and some may also be present in the vomitus. Food and water can become directly contaminated with faecal material, through the use of sewage sludge in agriculture, sewage pollution of shellfish culture beds, or may be contaminated by infected food-handlers. Several groups of enteric viruses have been reported to be transmitted via food and water. Some of theses viruses cause gastroenteritis: rotaviruses, astroviruses,enteric adenoviruses and human caliciviruses, which include noroviruses and sapoviruses. Other enteric viruses often associated with food and waterborne transmission but which cause systemic disease are enteroviruses and hepatitis A virus. Most enteric viruses have RNA genomes, and are a highly diverse group of viruses, with multiple types co-circulating at any one time in the population. In addition, immunity to enteric viruses is often type-specific with little cross-protection, and it is not-sterilizing, therefore asymptomatic carriage of enteric viruses is common. These factors facilitate the conditions by which genome exchanges among related viruses can occur either through recombination, which is widely recognized for noroviruses and enteroviruses, or reassortment as identified for rotaviruses.

Detection of food-borne viruses in food based on PCR technique: development of a European standard

Jane Sellwood on behalf of UK British Standards Institute (BSI)

Environmental Virology Unit, Health Protection Agency, Microbiology Laboratory, Royal Berkshire Hospital, Reading

Noroviruses and Hepatitis A virus transmitted by shellfish and fresh produce such as raspberries are a major cause of food-borne outbreaks of viral gastroenteritis. The methods to process this range food for virological investigation are in development and the methods for the detection of norovirus and HAV are not yet optimized or consistent. Appropriate Quality Assurance for these systems is challenging.

The European Commission has a mandate with CEN (European Committee for Standardization) regarding the establishment and validation of standard methods for food stuffs. The approach has been to validate methods to process bottled water, hard surface swabs, lettuce, spring onions, raspberries and shellfish; to evaluate and validate nucleic acid extraction methods. Virus detection is done using one-step TaqMan qRT-PCR incorporating process controls, amplification controls and quantitation estimation.

There has been good progress towards a single ‘horizontal’ method agreed by experts from across Europe. Large scale validation trials are to begin in the near future.

Monitoring and control of food-borne viruses in the food supply chain

N. Cook

Central Science Laboratory, York

Abstract not received

Possible zoonotic transmission of hepatitis E virus

Malcolm Banks

Deputy Head, Mammalian Virology Group, Veterinary Laboratories Agency, Weybridge, Surrey KT15 3NB

Until the last few years hepatitis E in developed countries was almost always associated with foreign travel to developing regions where water sanitation is poor and the disease is endemic.

In recent years there have been increasing numbers of reports of sporadic cases of hepatitis E from developed regions, in the absence of an association with foreign travel.

The observation that the viruses detected from these autochthonously acquired cases were genetically diverse from the endemic region human strains and showed a high sequence homology to hepatitis E virus (HEV) strains derived from pigs from the same geographic region, aroused suspicion in relation to possible zoonotic transmission.

In addition to pigs, several other mammals have been implicated as potential reservoirs of zoonotic transmission of hepatitis E.

Ingestion of poorly cooked pig and deer meat have been documented as direct evidence of foodborne zoonotic transmission and antibodies to HEV have been detected in pigs, cattle, rodents, and other species. However, despite this evidence, in the great majority of autochthonously acquired hepatitis E cases, the route of transmission of the virus is unknown.

This paper examines the evidence for and against zoonotic transmission of HEV, and suggests ways in which we might fill the knowledge gaps.

Comparative behaviour of human noroviruses and feline Calicivus vaccine strain F9 under different inactivation conditions

A. Knight[1], H. Schnerr[1], J. Haines[1], M. Scott[1], J. Topping[1], M.J. Carter[2], M.M. Carter[2], K. Bellamy[3], D. Brown[4], J. Gray[4] & C. Gallimore[4]

[1]Leatherhead Food International, Randalls Road, Leatherhead, Surrey KT22 7RY; [2]University of Surrey, Guildford, Surrey GU2 7XH; [3]Unilever Central Resources Ltd, Colworth Park, Sharnbrook, Bedford MK44 1LQ; [4]Health Protection Agency, Centre for Infections, Colindale Avenue, London NW9 5EQ

Current understanding of the inactivation of human noroviruses (NoV) by treatments relevant to food processing are derived from a variety of sources. There is limited information concerning human NoV itself and a wealth of data using related viruses. These may be grouped into two classes: viruses related to human NoV and used as surrogates eg feline calicivirus (FCV) and more recently murine norovirus (MNV); and viruses spreading by similar routes and used as indicators (mainly hepatitis A and polioviruses). Although surrogate virus systems are useful in providing indicative data they cannot, as yet, either predict the behaviour of human NoVs or reflect any differences that may exist among the diverse genogroups and genotypes of NoV. Furthermore, assays that address NoV survival by means of PCR cannot reflect viability/functionality of the sequences detected. For this reason we have developed methods to investigate the inactivation of human NoVs based on capsid activity (receptor binding and RNA protection) in combination with RT-qPCR. We have used these methods to investigate the stability of human NoV and FCV under a range of conditions relevant to food processing and environmental dissemination.

Using samples containing low RNA copy numbers (10^4–10^5 copies) we have found little change in RNA copy number over the range 60–85 ^{o}C. In contrast, the ability of the capsid to protect the RNA was significantly impaired resulting in complete exposure to RNAse over the same temperature range. Our results demonstrate that the FCV capsid is more temperature-sensitive than that of either Genogroup I or Genogroup II NoVs from clinical samples. Additional data will be presented comparing receptor binding and capsid stability of human NoVs from different genogroups and genotypes with that of FCV under different inactivation conditions. The relevance of the current FCV and MNV models for predicting the safety of food processing conditions will be discussed.

From household to country – the evolution, diversity and transmission of feline calicivirus with comparisons to the noroviruses

K.P. Coyne, S Dawson, R Christley, RM Gaskell & **A.D. Radford**

University of Liverpool, Faculty of Veterinary Science, Leahurst Campus, Chester High Road, Neston, South Wirral, CH64 7TE

Calicivirus diversity is a known fact that we are all having to deal with. Its consequences include low immunological cross-reactivity, difficult vaccine design and diagnostic assays that under-perform. Here we will review what is known about feline calicivirus evolution and diversity on different ecological scales from the household to the community to the country. Within households, FCV persists in individuals by one of two mechanisms – long-term infection with a single variant of virus and cyclical reinfection with different variants. Although true long-term persistence appears to be a relatively rare event (~10% of cats), it is likely to be critical to the development of early strain diversity. Two measures of evolution rate can be measured; for a virus progressively evolving within an individual ($1.32–2.64\times10^{-2}$ substitutions per nucleotide per year, i.e. no transmission) and for a strain circulating within a population ($3.84–4.56\times10^{-2}$ substitutions per nucleotide per year, i.e. including transmission). These high prevalence populations allow for inter-strain recombination by a similar mechanism to that reported for noroviruses, and may lead to the evolution of new highly virulent pathotypes. At the community level, multiple strains co-circulate, often with overlapping geographical footprints, and can persist for many months. At the UK national level, many strains are present. However, there is little evidence of widespread strain movement or for the existence of highly prevelant pathotypes. The high prevalence of FCV has made it a useful model organism to study the evolution of caliciviruses in their natural host populations.

Role of food and domestic animals in the transmission of Nipah virus

M. Jahangir Hossain[1], Emily S. Gurley[1], Shakila Banu[1], Rasheda Khan[1], Nazmun Nahar[1], Joel M. Montgomery[3], Darin S. Carroll[3], Jonathan H. Epstein[4], Peter Daszak[4], Robert F. Breiman[3], Mahmudur Rahman[2] & Stephen P. Luby[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh; [2]Institute of Epidemiology, Disease Control and Research, Ministry of Health and Family Welfare, Government of Bangladesh; [3]Centers for Disease Control and Prevention, Atlanta; [4]The Consortium for Conservation Medicine, New York, USA

Background Nipah virus (NiV) is an emerging pathogen causing severe encephalitis and respiratory involvement resulting in 40–75% case fatality ratios in humans. NiV first emerged in Malaysia and Singapore in 1997–1998 and seven outbreaks recognized in Bangladesh since 2001. Fruits bats are the natural reservoir for NiV. Food and domestic animals play an important role in NIV transmission from bats to humans.

Findings A large porcine outbreak in Malaysia and Singapore resulted in largest NiV outbreak in humans to date. Pigs on farms became infected presumably through eating bat-bitten fruits, developed encephalitis and respiratory symptoms, and transmitted the virus to other farms and humans. Contact with sick dogs and chickens was also associated with NiV infection in Malaysia. In Bangladesh, NiV infection has been associated with contact with sick cows, pig herds and a sick goat. During one outbreak in Bangladesh, NiV was transmitted to humans through consumption of fresh date palm sap presumably contaminated with bat secretions. Consumption of fresh date palm juice and bat-bitten fruit are common in Bangladesh and present an ongoing risk for transmission of NiV from bats to humans.

Conclusion To prevent NiV transmission from bats to humans, efforts should focus on restricting bat-bitten fruit consumption in animals and humans, restricting human contact with sick animals, and protecting date palm sap from contamination with bat secretions.

Bushmeat and viruses: Ebola, SARS and HIV

Robin A. Weiss

Division of Infection and Immunity, UCL, 46 Cleveland Street, London W1T 4JF

Many human infections by viruses originally came from animals. Viral diseases like measles and smallpox probably evolved to become human-to-human infections from a zoonotic origin when we domesticated ruminants for food and beasts of burden. Thus viruses that we consider to be age-old infections actually have a very recent human history on the evolutionary time-scale. We may be witnessing a similar food-borne zoonotic transfer of novel virus infections today, but from bushmeat. One can argue that any potentially zoonotic virus infections that are enzootic in domesticated species such as goats, cattle and camels have had ample opportunity to jump host species to humans in the past, whereas the growing predilection for exotic foods is increasing the risk of food-borne viral infections acquired from wild animal species. This concept will be discussed with particular reference to HIV, Ebola virus and SARS coronavirus.

Microbial Infection / Physiology, Biochemistry & Molecular Genetics Groups Joint Session

The horizontal gene pool: the mobilome and virulence

Impact of horizontal transfer events: the genomics prospective

Hervé Tettelin

Institute for Genome Sciences, Dept of Microbiology & Immunology, University of Maryland School of Medicine, Baltimore MD, USA

Until recently, whole genome sequencing projects targeted a 'representative' strain of any given species. To understand species diversity, we sequenced the genome of eight group B *Streptococcus* strains. New genes were discovered with each genome, and mathematical modeling predicts that new genes will be discovered after sequencing many more genomes. Similar analysis led to the same conclusion in other species. Therefore, a bacterial species can be described by its *pan-genome* composed of a *core genome* containing genes present in all strains and a *dispensable genome* containing genes present in a subset of strains. Given that the number of dispensable genes is vast, the pan-genome of a bacterial species is often much larger than any single genome.

Lateral gene transfer (LGT) can contribute to the pan-genome. It is known to occur a lot among bacteria. Recently, we demonstrated LGT events between bacteria and multicellular eukaryotes. We examined eukaryotic genomes for LGT events from *Wolbachia* to their hosts. We characterized transfers in 4 insect and 4 nematode species that range from the entire *Wolbachia* genome to short insertions. We also showed that some inserted *Wolbachia* genes are transcribed. Such LGT events potentially provide a mechanism for acquisition of new genes and functions.

The transportation infrastructure: a mobile genetic elements perspective

A.O. Summers

University of Georgia, USA

Mobile genetic elements are the agents of intercellular (plasmids and 'phages) and intracellular (transposons and integrons) gene transfer in prokaryotes. The sequencing and bioinformatic analysis of these agents has lagged behind that of the chromosomes on which they operate continuously. Fortunately, in the last 5 years improvements in sequence data acquisition has improved and sequences of individual plasmids number nearly 1000 with about a third that many bacteriophages and a larger number of transposons and insertion sequences. Databases for all these agents are increasingly well staffed and are engaging members of their expert communities to assist in development of ontologies and in annotation. The potential information pool borne by mobile elements throughout the Prokaryota is unimagineably large and varied and will be a challenge for bioinformatics for some time to come. This presentation will cover the basic biology of these entities as well as recent advances and resources for investigators.

Incorporation and excision of virulence genes into enterobacterial genomes

U. Dobrindt

University of Würzburg, Germany

Abstract not received

Integrating conjugative elements of the SXT/R391 family

Vincent Burrus

Département de biologie, Faculté des sciences, Université de Sherbrooke, 2500 boul. Université, Sherbrooke, QC J1K 2R1, Canada

In the bestiary of mobile elements, Integrating Conjugative Elements (ICE) resemble chimeras that result from the collision of temperate bacteriophages with conjugative plasmids. ICEs integrate into and replicate with the host chromosome. Upon activation, they excise themselves as plasmid-like molecules that serve as substrates for conjugative transfer. ICEs were long believed to be restricted to *Bacteroides* and Gram-positive bacteria, where they were first identified and called conjugative transposons. ICEs are now recognized as major drivers of horizontal gene transfer among proteobacteria. ICEs range from 10 kb to 610 kb and besides ensuring their own mobility, most encode additional functions such as symbiosis establishment, quorum sensing, degradation pathways, production of antimicrobial peptides, or resistance to bacteriophage infection, antibiotics or heavy metals.

The SXT/R391 family regroups more than 30 ICEs identified in clinical and environmental vibrios. These ICEs share a highly conserved backbone of genes necessary for transfer, integration and activation in response to DNA damages. Yet, they also exhibit considerable variations due to the presence of transposons, integrons and hotspots for insertions. While the function of most genes inserted in these hotspots is unknown, ongoing investigations suggest that some of them could restrain the motility of *Vibrio cholerae* and enhance biofilm formation.

The evolutionary history of Group B *Streptococcus* (GBS), a bacterial genome shaped by DNA conjugation

Mathieu Brochet[1], Elisabeth Couvé[1], Christophe Rusniok[1], Patrick Trieu-Cuot[2], Frank Kunst[1] & Philippe Glaser[1]

[1]Genomics of Microbial Pathogens, URA-CNRS2171; [2]Unité Biologie des Bactéries Pathogènes à Gram-Positif, URA-CNRS2172 Institut Pasteur, 28 Rue du Dr Roux, 75724 Paris cedex 15, France

Bacterial populations are subject to complex processes of genome diversification that involve horizontal DNA transfer events, mediated by three main mechanisms: transformation, transduction and conjugation. It has been previously observed that the resulting horizontal exchanges lead to bacterial genomes being pocked by small chromosomal replacements from other related lineages. We used single-nucleotide polymorphism patterns among related isolates as a powerful tool to study DNA transfer events. By this means, we analysed the genetic flux among 8 genome sequences representative of the diversity of clinical isolates of GBS, the leading cause of neonatal infections. This allowed us to reconstruct the evolutionary history of these strains in which a specific ecotype has recently emerged and subsequently diversified by transfer of large DNA fragments. This scenario was supported by the demonstration of conjugative transfers of large chromosomal DNA fragments between different GBS isolates.

Genome scale analysis of avian host adaptation by *Staphylococcus aureus*

Bethan V. Lowder[1], Nouri L. Ben Zakour[1], Caitriona M. Guinane[1], Toby Johnson[2] & J. Ross Fitzgerald[1]

[1]Laboratory for Bacterial Evolution and Pathogenesis, Centre for Infectious Diseases, The Chancellor's Building, University of Edinburgh, Edinburgh EH16 4SB; [2]Departement de Genetique Medicale, Université de Lausanne, Switzerland

Staphylococcus aureus is an important human and animal pathogen. Population genetic studies have shown that most strains of *S. aureus* are restricted to a single host species. To investigate the diversity of

strains infecting poultry we carried out multi locus sequence typing of 35 poultry isolates from 5 countries in 4 continents. The majority of isolates belonged to a successful human lineage but had phenotypic differences to human strains providing evidence for host-adaptation. Our data suggest that a host jump from humans to poultry has recently occurred followed by wide geographical dissemination. The rapid development and globalization of the poultry farming industry over the last 50 years has provided increased opportunities for transmission. In order to investigate the avian host adaptation of *S. aureus,* we have determined the genome sequence of a representative poultry-specific strain, and carried out comparative analysis with several human strains. These analyses have revealed putative host-specific virulence factors and mobile genetic elements unique to avian strains which may be important for host adaptation.

Conjugative transposons; a myriad of modules

Adam Roberts

UCL Eastman Dental Institute, London

Conjugative transposons are an extremely diverse group of mobile genetic elements that have the ability to excise from and insert into a host genome and to transfer by conjugation to another host cell. They encode a variety of different proteins which catalyse these reactions. Their gene products also confer a range of accessory functions, the most commonly investigated of which is antibiotic resistance. During this overview the various different families of conjugative transposons will be introduced and their properties and structures described. The talk will then focus on the most extensively investigated family of conjugative transposons: the Tn*916* family. Tn*916*-like elements have been found in, or introduced into, over 30 different genera of bacteria and are the most common conjugative transposons yet discovered. The have an extremely broad host range and usually confer tetracycline resistance upon their host. Tn*916* is composed of modules involved in conjugation, the excision and insertion reactions, transcriptional and translational regulation and the various accessory functions. This modular architecture will be described and discussed using examples of elements currently under investigation to illustrate differences between different elements.

Integrons

D. Mazel

Institut Pasteur, Paris, France

Abstract not received

The evolution of class 1 integrons and rise of the antibiotic resistance epidemic

M.R. Gillings[1], Y. Boucher[2], M. Labbate[2], H.W. Stokes[2] & **A.J. Holmes**[3]

[1]Dept of Biological Sciences; [2]Dept of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, NSW 2109, Australia; [3]The School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia

Class 1 integrons are central players in the worldwide problem of antibiotic resistance. They are embedded in various mobile DNA elements, where they acquire and express diverse resistance genes. We now show that class 1 integrons are also commonly found on chromosomes of non-pathogenic soil or freshwater *Betaproteobacteria*. The pool of Betaproteobacterial class 1 integrons exhibits characteristics consistent with their including the immediate ancestors of class 1 integrons found in pathogens. These include *intI* sequence diversity, absence of genes for antibiotic resistance, and shared sequence breakpoints for boundaries with adjoining genetic elements. We conclude that a betaproteobacterial species was the source of all clinical-type class 1 integrons. Furthermore, betaproteobacterial class 1 integrons show phylogenetic signatures consistent with significant levels of lateral transfer within this bacterial group. We postulate that the apparent predisposition of class 1 integrons for mobilization, their presence in a bacterial group that is ubiquitous and relatively abundant in water supplies and their inherent capacity to acquire and express resistance genes carried by gene cassettes were all significant factors in their pre-eminence in the integron-borne antibiotic resistance epidemic.

SaPIs and their role in the pathogenic profile of *Staphylococcus aureus*

José R. Penadés

Insituto Valenciano de Investigaciones Agrarias, Spain
(Email jpenades@ivia.es)

The typical bacterial genome can be considered to contain two major genetic domains – a standard and broadly conserved set of genes that are responsible for the basic cellular processes of growth and multiplication, and a second facultative set that is concerned with adaptations to environmental contingencies. In recent years, it has become evident that the facultative genome includes a large variety of mobile genetic elements (MGEs), including phages, pathogencity islands, plasmids or transposons, and that this 'mobile genome' may exceed 10% of the total. In *Staphylococcus aureus*, the vast majority of strains carry one or more phages and one or more pathogenicity islands (SaPIs), so that the phages and SaPIs, as well as their mobility, constitute a significant feature of staphylococcal virulence.

SaPIs are a family of related 15–17 kb mobile genetic elements that commonly carry genes for superantigen toxins and other virulence factors. Concomitantly, SaPIs are largely responsible for the spread of these factors. The key feature of their mobility and spread is the induction by certain phages of their excision, replication and efficient encapsidation into specific small-headed phage-like infectious particles. As a consequence of their high transfer frequency, the SaPIs are very widely distributed, with many *S. aureus* strains containing two or more of them. All SaPIs are integrated at specific chromosomal sites, are flanked by short direct repeats, which represent *att* site cores, and encode specific integrases that recognize these sites and are required for integration and excision.

The occurrence of similar or identical SaPIs in unrelated strains strongly suggests that transfer occurs under natural circumstances as well as in the laboratory. The understanding of SaPI biology, genetics and dissemination will greatly contribute to the epidemiological tracking and control of superantigen and other SaPI-mediated diseases.

Shiga toxin encoding phages: drivers of pathogen evolution

H.E. Allison

Microbiology Research Group, School of Biological Sciences, University of Liverpool, Crown Street, Liverpool, Merseyside L69 7ZB

Shigatoxigenic *Escherichia coli* (STEC) first emerged as deadly human pathogens in 1982. In that year, only one serotype was associated with human morbidity and mortality; however, there are now multiple STEC serotypes associated with severe human disease. Additionally, over 500 *E. coli* serotypes have been reported capable of producing Shiga toxin (Stx) along with several other members of the *Enterobacteriaceae* and even a strain of *Acinetobacter haemolyticus*. Though pathogenic STEC possess a multitude of virulence factors, the ability to produce Stx is arguably the most important as it can lead to life threatening human disease. The genes encoding Stx lie within a bicistronic operon and are carried by temperate lambdoid phages known as Stx-phages, which are all less related to one another than their name implies due, at least partly, to genetic mosaicism. This considerable genetic diversity has been further revealed by genome sequencing and multi-loci characterization and includes: a large reservoir of expression regulators, integrases with various specificities, non-Stx-related virulence factors,

recombination systems, host recognition strategies and overall phage morphology. The emergence of STEC and Stx-producing pathogens has not been due to a single bacteriophage infection, but has, and remains, the result of different Stx-phage infections driven by a population of heterogeneous and dynamic Stx-phages.

Phenotypic and genetic variations in populations of *Pseudomonas aeruginosa* during pulmonary exacerbations in cystic fibrosis

Joanne L. Fothergill, Martin J. Walshaw, Tyrone L. Pitt & Craig Winstanley

Division of Medical Microbiology, University of Liverpool, Liverpool L69 3GA

Chronic respiratory infection by *Pseudomonas aeruginosa* contributes significantly to the morbidity and mortality associated with cystic fibrosis (CF). We analysed fluctuations within populations of the *P. aeruginosa* Liverpool Epidemic Strain (LES) during pulmonary exacerbation. Three sequential sputum samples were collected from each patient: (1) on presentation with exacerbation at the CF unit, (2) 3 days into intravenous antibiotic treatment and (3) at the end of therapy. From each sample, 40 LES isolates were analysed using a series of phenotypic and genotypic tests. We found significant changes in the phenotypic and genotypic properties of the LES populations during the sampling period. Variations in morphotype distribution, pyocyanin and LasA production, antibiotic resistance profiles, and levels of auxotrophy and hypermutability were observed. We used PCR assays to screen for the presence of novel regions of the LES genome, including 4 prophages and a genomic island. There were significant changes in the prevalence of the novel genomic island. Using PCR assays we were able to detect 3 of the prophages in filter-sterilized, DNase I-treated sputum.

Natural transformation in the *Pasteurellaceae*

Paul R. Langford

Dept of Paediatrics, Imperial College London, St Mary's Campus, London W2 1PG

Many members of the *Pasteurellaceae* family undergo natural transformation i.e. the active uptake and heritable integration of extracellular DNA. Naturally competent *Pasteurellaceae*, of which *Haemophilus influenzae* is the best studied, preferentially take up DNA containing specific uptake signal sequences (USSs) typically 9–10 bp. There is a significant over-representation of USSs in the sequenced genomes of the *Pasteurellaceae*. There are two related USSs types known: Hin and Apl present in *H. influenzae* and *Actinobacillus pleuropneumoniae* respectively. Natural transformation can be induced in *H. influenzae* under starvation conditions, a microarray analysis of which identified a postulated competence regulon, characterized by a promoter-associated 22 bp competence regulatory element (CRE). The CRE regulon contains 25 genes in 13 transcription units and is dependent on the gene *sxy*. In the porcine pathogen *A. pleuropneumoniae* there is significant variation in the ability of the 16 serovar reference strains to undergo natural transformation, a high level only being found in the serovar 15 reference strain. Over-expression of APP Sxy in the serovar 1 reference strain did not result in increased levels of natural transformation. It is unclear as to whether USS-dependent DNA uptake has evolved for survival advantage via acquisition of new genetic traits and/or nutrients.

The X-state (competence for genetic transformation), a substitute for SOS in *Streptococcus pneumoniae*

J.-P. Claverys

CNRS Toulouse, France

Bacterial transformation, originally discovered in the human pathogen *Streptococcus pneumoniae* (the Pneumococcus), relies on a process that is inherent to the species and constitutes a bacterium-programmed mechanism for genetic exchange. It allows the uptake and integration of exogenous DNA into the genome. Pneumococcal transformation is believed to play a central role in genetic plasticity and the adaptation of this pathogen to host defense.

Competence for genetic transformation is a transient physiological state that requires transcriptional activation of the *com* regulon (105–124 genes). This regulon is induced when a competence-stimulating peptide (CSP; encoded by *comC*) which has accumulated in the medium stimulates its receptor, the membrane-bound histidine kinase ComD. ComD then activates the response regulator ComE, which turns on expression of the early *com* genes, including *comCDE* and *comX*. The latter encodes an alternative sigma factor (σ^X) specific for late *com* genes.

Although often cited as an example of quorum-sensing, competence induction does not rely simply on passive CSP accumulation. Instead, CSP can increase temporarily in response to environmental changes. These observations are consistent with a role of CSP as an alarmone and the proposal that the X-state (competence) constitutes a general stress response, an SOS substitute in *S. pneumoniae*.

Regulatory integration of horizontally transferred genes in bacteria

Charles J. Dorman

Dept of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin, 2, Ireland

Horizontal transmission of genetic material is thought to play a significant role in the evolution of bacteria, allowing them to acquire novel traits that may improve their competitiveness. Much research has been carried out on the major mechanisms of horizontal transfer, principally conjugation, transduction and transformation. Recently, there has been increased interest in the molecular mechanisms by which newly acquired genes can be integrated into the existing regulatory circuits of the new bacterial host. In the Gram-negative bacteria *Escherichia coli* and *Salmonella enterica,* a role has been proposed for the nucleoid-associated protein H-NS as a general repressor of horizontally acquired genes containing A+T-rich DNA sequences. This presentation will address the problem of how H-NS-mediated repression of transcription can be counteracted in ways that benefit both the bacterium and the laterally transmitted DNA sequences. It will be shown that H-NS antirepression can be achieved by a multitude of mechanisms.

Restriction and anti-restriction

David T.F. Dryden

School of Chemistry, Univ. of Edinburgh, The King's Buildings, Edinburgh EH9 3JJ (Email david.dryden@ed.ac.uk)

In this presentation I will survey the known types of DNA restriction and modification (R/M) systems found in bacteria and the various mechanisms they use to protect the host cell from infection by foreign DNA. In addition to the well known DNA cleavage and DNA methylation function of these systems, many restriction enzymes are capable of additional functions such as binding to multiple DNA sequences simultaneously or coupling the hydrolysis of ATP with extensive translocation of DNA using molecular motors. R/M systems present a formidable barrier to DNA mobility and have forced the evolution of numerous anti-restriction capabilities by phage and mobile genetic elements. Of particular interest is the synthesis of antirestriction-proteins which physically resemble the DNA duplex recognized by the type I class of R/M systems. These DNA mimics effectively inhibit the restriction enzyme. I will present data on the phage T7 ocr antirestriction protein and the ArdA and ArdB proteins from conjugative transposons.

References DNA mimicry by proteins and the control of enzymatic activity on DNA. *Trends Biotechnol* (2006) **24**, 378–382 / The biology of restriction and anti-restriction. *Curr Opin Microbiol* (2005) **8**, 466–472.

Physiology, Biochemistry & Molecular Genetics Group Session

Prokaryotic cell biology

FtsZ and division control in prokaryotes

Joe Lutkenhaus

Dept of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS 66160, USA

Cytokinesis in bacteria is mediated by a cytokinetic ring, termed the Z ring. The Z ring is composed of FtsZ filaments, which form a scaffold for recruitment of other cell division proteins. In *Escherichia coli*, the position of the Z ring is largely determined by the Min system. This system is composed of three proteins which establish a gradient of an inhibitor of Z ring assembly which has a minimum at midcell. The effector of the Min system, MinC controls the scaffolding function of FtsZ by antagonizing the mechanical integrity of FtsZ structures. MinC is a modular protein whose two domains ($MinC_C$ and $MinC_N$) synergize to inhibit FtsZ function. $MinC_C$ interacts directly with FtsZ polymers and this interaction accounts for the efficient targeting of MinC to Z rings. $MinC_C$ also prevents lateral interactions between FtsZ filaments, an activity which seems to be unique among cytoskeletal proteins. $MinC_N$ contributes to MinC activity by weakening the longitudinal bonds between FtsZ molecules leading to a loss of polymer rigidity and consequent polymer shortening. It seems likely that control over the scaffolding activity of FtsZ represents a universal regulatory mechanism to spatially regulate bacterial cytokinesis.

Spatial regulation of cell divison in *Caulobacter crescentus*

Martin Thanbichler

Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Straße, D-35043 Marburg, Germany

A fundamental problem in cell biology is the proper temporal and spatial regulation of cell division. Our work has revealed the existence of a novel mechanism that is responsible for positioning of the cell division plane in *C. crescentus*. At its heart lies the Walker ATPase MipZ, which forms a dynamic complex with the chromosome partitioning protein ParB at the chromosomal origin of replication. Upon initiation of DNA replication, the two newly synthesized origin regions are immediately re-decorated with the MipZ/ParB complex and subsequently positioned at the two opposite cell poles. This generates an intracellular gradient of MipZ, with its concentration being highest at the cell poles and lowest at the cell center. Our analyses showed that MipZ has the ability to inhibit the polymerization of the bacterial tubulin homologue FtsZ and, thereby, to prevent the assembly of the FtsZ ring, which forms the foundation of the ring-shaped cell division apparatus. As a consequence, cytokinesis is limited to the midcell region and, furthermore, only initiates once chromosome segregation has started. MipZ is highly conserved among alphaproteobacteria, which suggests that it represents the prototype of a new and widespread class of bacterial cell division regulators.

Control of cell wall synthesis in *Bacillus subtilis*

D. Claessen

University of Newcastle – corresponding address: University of Groningen, The Netherlands

The characteristic shape of bacterial cells is mainly determined by the cell wall, the synthesis of which is orchestrated by penicillin binding proteins (PBPs). Rod-shaped bacteria have two distinct modes of cell wall synthesis, involved in cell elongation and cell division, which are believed to employ different sets of PBPs. A long held question has been how these different modes of growth are co-ordinated in space and time. We have now identified that the cell division protein, EzrA, and a newly discovered protein, GpsB, act as key players in the elongation-division cycle of *Bacillus subtilis*. Mutations in these genes have a synthetic lethal phenotype with defects in both cell division and cell elongation. They also have an unusual bulging phenotype apparently due to abnormal wall synthesis in mature cell poles, which are usually inert. We show that this lethality is mainly due to deranged localization of PBP1 protein. EzrA and GpsB are key determinants in the localization cycle of PBP1, in which EzrA controls the recruitment of PBP1 to division sites, and GpsB ensures its removal after completion of cell pole maturation.

Cell polarization and chromosome capturing in *Caulobacter crescentus*

G. Ebersbach & **C. Jacobs-Wagner**

Dept of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA

Bacteria rely on cell polarization for many important processes including chromosome segregation, cell division, cell cycle signaling and polar morphogenesis. We have evidence that bacteria, similarly to eukaryotic cells, use underlying cell polarity programs to govern and link polarization events involved in seemingly distinct processes. We have discovered a polar factor (TipS) with a broad role in cell polarization in the Gram-negative bacterium *Caulobacter crescentus*. TipS displays a polar localization, which is achieved, at least in part, by inheritance, self-attraction and general affinity for bacterial cell poles. During chromosome segregation, polar TipS captures the migrating chromosomal origin via an interaction with the chromosome partitioning protein ParB bound to a centromeric-like sequence near the origin. TipS-mediated anchoring of the chromosomal origin at the poles in turn sets up cell division by stabilizing the accumulation of a cell division inhibitor at opposite poles. Furthermore, TipS is required for the polar localization of components involved in polar morphogenesis and cell cycle signaling.

Relationship between chromosome structure and Z ring placement

Rebecca Rashid, Christopher Rodrigues, Shigeki Moriya & Elizabeth Harry

The Institute for the Biotechnology of Infectious Diseases, University of Technology, Sydney (UTS), Australia

The earliest known event in cell division is formation of the Z ring precisely at midcell between segregated chromosomes. Generally, when DNA replication is blocked in outgrown spores, Z rings form acentrally, beside the nucleoid. However, we have identified conditions of replication inhibition that allow a central Z ring to form over the unreplicated nucleoid. The question remains, does central Z ring formation in these cases reflect a direct link between DNA replication and Z ring positioning, or is it due to relief of nucleoid occlusion? We co-visualized the nucleoid and Z ring position in two temperature-sensitive DNA replication initiation mutants, one that allows central Z rings over the nucleoid and one that doesn't. Central Z rings formed over unreplicated DNA if it had a bilobed shape, but only next to nucleoids that had a single lobe. This suggests that, even in the absence of DNA replication and chromosome segregation, a change in DNA conformation to a bilobed structure relieves nucleoid occlusion sufficiently to allow midcell Z ring formation.

Control of polarized growth and hyphal branching in *Streptomyces coelicolor*

Sheng-bing Wang, Antje Marie Wollkopf & **Klas Flärdh**

Dept of Cell and Organism Biology, Lund University, Lund, Sweden

Streptomycetes grow in an extremely polarized fashion. They form branched hyphae that elongate through tip extension, i.e. incorporation of new cell wall material at one cell pole only – the hyphal tip. This mode of growth is independent of the bacterial actin MreB that is inolved in organizing cell wall assembly during elongation of many other rod-shaped bacteria. Instead, the essential protein DivIVA is a determinant of polar growth and branching in *Streptomyces*. DivIVA is localized in a large assembly at each growing tip in *Streptomyces coelicolor*, and has a strong impact on tip extension and cell shape determination. During hyphal branching, cell polarity has to be re-oriented and a new site of cell wall assembly established in the lateral hyphal wall, from where the branch emerges. Time-lapse imaging of growing hyphae show that branching is preceded by the appearance of a focus of DivIVA-EGFP at the site before any visible lateral outgrowth. This presentation will discuss the link between DivIVA and the machinery for cell wall assembly, and how the localization of DivIVA may be regulated.

Localization of bacterial chemosensory and motility proteins

J.P. Armitage

University of Oxford

The chemosensory proteins of *Escherichia coli* form a large quaternary complex. The complex is essential for the sensitivity and gain of the chemosensory system. The mechanisms involved in localization have been the subject of a number of recent studies.

Most bacterial species have not one chemosensory pathway, but several chemotaxis pathways. How do multiple homologues regulate specific behaviour? The phototroph *Rhodobacter sphaeroides* has 3 chemosensory pathways. Using fluorescent fusions to the chemosensory genes we showed that the proteins of one pathway localize to the cell poles with transmembrane chemoreceptors while the proteins of the other pathway localize to a cytoplasmic cluster. For optimal chemotaxis on division each daughter cell needs to inherit a complete chemosensory pathway. We have identified a protein partitioning system, related to plasmid partitioning mechanisms, that ensure each daughter cell inherits complete chemosensory pathways.

The flagella of *E. coli* appear randomly localized. In other species they may be localized at the poles. The flagellar motor has a central rotor complex moving against a ring of anchored stator proteins. Fluorescent fusions to the rotor and stator proteins show that newly synthesized complete flagellar rotors move randomly in the cell membrane, eventually becoming anchored and forming functionally rotating motors. Proteins in rotating motors also show dynamic turnover. Localization and protein dynamics will be discussed.

Structural insight into the mechanism of peptidoglycan synthesis

Andrew L. Lovering, Liza De Castro & Natalie C.J. Strynadka

Center of Blood Research, University of British Columbia, Canada

The bacterial cell wall is essential to bacterial survival and has historically been an excellent target for the development of antimicrobials. Peptidoglycan is the major component of the cell wall and the final stages of its synthesis are catalysed by the two activities of bifunctional PBP (penicillin-binding protein) enzymes. The first activity, that of the glycosyltransferase (GT) domain of the enzyme, resides in the membrane and is poorly characterized. The GT reaction results in the polymerization of a membrane-bound lipid II monomer into soluble sugar/peptide chains. The secondary activity, that of the transpeptidase (TP) domain is well characterized, and is the target of the β-lactam group of antibiotics. The TP reaction cross-links the nascent sugar chain via its peptide crossbridges, forming the peptidoglycan mesh.

The GT reaction has excellent potential for the development of novel antibiotics. It is also of great interest in the field of bacterial physiology, and may serve as a prototype for the study of other lipid glycosyltransferases. The first structure of this enzyme (Lovering *et al.*, Science 315 1402-, [2007]) initiated structure-based hypotheses as to the exact mechanism of the reaction, and we present recent data from our laboratory that highlights a remarkable interplay between the GT and TP activities, and also details the role of conserved active site features. We describe a fuller model for the separate stages of the reaction (initiation, processive polymerization and termination), and postulate how specific inhibitors may be designed.

Distinct 'tissues' in the filamentous bacterium, *Streptomyces coelicolor*

G. Kelemen

University of East Anglia, Norwich

Abstract not received

Collagen-like proteins from the enterohaemorrhagic *Escherichia coli* strain O157:H7

Neelanjana Ghosh, Ian Roberts & Jordi Bella

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

The genome of the enterohaemorrhagic *Escherichia coli* strain O157:H7 contains eight putative collagen-like genes that are missing in non-pathogenic strains. To determine if these proteins behave as collagens and to help establish if they contribute to O157:H7 virulence we have cloned one of these genes, EclA, and analysed it biophysically. Analytical ultracentrifugtion and light-scattering showed that EclA is trimeric in solution, with molecular weight of 132 kDa. EclA forms characteristic dumbbell-shaped structures visible by rotary shadowing electron microscopy. The 'stalk' region adopts a collagen triple-helical conformation as demonstrated by CD spectroscopy, while the CD of full-length EclA is dominated by α-helical structure from the N-terminal domain. Thermal denaturation of EclA shows two transitions: the first one at 42°C corresponds to loss of triple-helical structure; the second one at 52°C corresponds to loss of α-helical structure. The first transition is much higher than the equivalent seen for mammalian collagens. The results show that EclA behaves like an unexpectedly stable collagen protein. While the function of these proteins needs to be elucidated, mRNA transcripts of collagen-like genes were detected by RT-PCR both in stationary and exponentially growing cells, suggesting protein expression and functional significance.

Death of the replication factory?

Rodrigo Reyes-Lamothe & **David J. Sherratt**

Dept of Biochemistry, University of Oxford, Oxford OX1 3 QU

By tracking the progression of sister replication forks with respect to genetic loci in live *Escherichia coli*, we show that at replication initiation replisomes assemble at the replication origin, irrespective of whether it is positioned normally at midcell, or aberrantly close to a pole. Within 5 min of replication initiation, sister replisomes, marked by protein fusions to multiple replisome components, move to opposite cell halves, migrating outwards as replication proceeds, and then returning to midcell as replication termination approaches. Prior to termination, the sister replisome foci are replaced by a single focus, which disappears as replication completes. DNA polymerase is maintained at stalled replication forks. Over short time-scales, replisomes are more dynamic and less constrained than genetic loci. We conclude that independent replication forks follow the path of the

compacted chromosomal DNA, with no structure other than DNA anchoring the replisome to any particular cellular region. The data are not consistent with a stationary replication factory, containing sister forks, into which parental DNA enters prior to replication, with sister chromosomes exiting after replication.

Maintenance of stalled replication forks in UV-irradiated *Escherichia coli* cells

Christian Rudolph, Amy Upton & Robert G. Lloyd

Institute of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham NG9 2UH

Stalled DNA replication forks impose a serious threat to genomic integrity and cell viability. What happens to stalled forks depends on the nature of the obstacle, and in many cases is unknown. To gain further understanding of how arrested forks are restarted, we examined DNA synthesis, chromosomal segregation and cellular division in UV-irradiated *Escherichia coli* cells. Our data indicate that DNA synthesis is delayed for a considerable period. Restart depends on factors which load the replicative helicase, indicating that the replisome may have dissociated. Our studies confirm that RecFOR proteins, which are known to load RecA recombinase, promote efficient restart. However, they are also needed for damage induced DNA synthesis and productive replication initiated at *oriC*. All modes of replication recover in *recFOR* mutants but only after a significant delay, during which nascent DNA strands are extensively degraded. However, degradation is also observed in *recFOR*$^+$ cells when restart is blocked by other means, suggesting that RecA loading is not sufficient for fork protection. In conclusion our data suggest that stalled forks are not specifically stabilized in bacteria, but may be protected by the simple expedient of promoting restart. This contrasts with the active stabilization of arrested forks in eukaryotic cells.

Centromere pairing by a plasmid encoded type I ParB protein

Simon Ringgaard[1] & Kenn Gerdes[2]

[1]Dept of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark; [2]Institute for Cell and Molecular Biosciences, The Medical School, University of Newcastle, Framlington Place, Newcastle NE2 4HH

Bacterial plasmids often rely on active segregation by partition systems (*par* systems) to guarantee stable inheritance in a growing bacterial population. The partition locus, *par2*, of *Escherichia coli* plasmid pB171 encodes two *trans*-acting proteins, ParA and ParB, and two *cis*-acting centromere-like sites, *parC1* and *parC2*. ParB dimers bind co-operatively to direct repeats in *parC1* and *parC2* in formation of the partition-complex. Walker-type ATPase ParA forms ATP dependent filaments *in vitro* and oscillates in helical structures *in vivo*. Oscillating ParA interacts with the ParB/*parC* complex and positions plasmids regularly over the nucleoid. We obtain solid evidence that ParB can pair *parC1*- and *parC2*-encoding DNA fragments *in vitro*. Electron microscopy demonstrates that ParB binds specifically at *parC* and mediates binary pairing of DNA fragments at the *parC* centromere site. In addition, ParB mediates the formation of higher order complexes consisting of several DNA fragments joined by ParB at *parC*. N-terminus truncated versions of ParB still dimerize and possess specific DNA binding activity, however, are incompetent in pairing. Thus, the N-terminus of ParB is a requirement for ParB-mediated centromere pairing but not DNA binding. These observations suggest that centromere pairing is an important step in plasmid partitioning mediated by the common type I loci.

Subcellular localization of transcription factors

Peter J. Lewis

School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW 2308, Australia

Despite the fact that bacteria only contain a single RNA polymerase to carry out all classes of transcription, examination of fluorescently labelled RNA polymerase in both Gram-positive and -negative bacteria has shown that transcription is crudely segregated in the nucleoid into regions where mRNA transcription predominates and regions where rRNA synthesis predominates. Subcellular sites where rRNA synthesis predominates are characterized by a concentration of RNA polymerase into regions termed transcription foci. As the rate of cell growth increases, transcription foci increase in both intensity and frequency concomitant with the demand for rRNA.

The dynamic localization, and cellular levels of transcription elongation factors have been determined in the model Gram-positive organism *Bacillus subtlis*. We have also determined the stoichiometries of transcription factors with RNA polymerase in both m- and rRNA elongation complexes. Our results indicate transcription elongation complexes are highly dynamic and are differentially segregated within the nucleoid according to their functions.

Investigating *Streptomyces coelicolor* via fluorescence microscopy

Khoa D. Nguyen & Justin R. Nodwell

Dept of Biochemistry & Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, ON L8N 3Z5, Canada

Streptomyces coelicolor is a sporulating, filamentous, Gram-positive bacterium. Unlike many bacteria, this organism's life cycle resembles that of filamentous fungi and involves substantial cell-type specialization. Interestingly, the normally essential process of cell division is dispensable in this organism. However, cell division is required for septation of the multigenomic aerial hyphae to produce unigenomic spores. To better understand cell division and its regulation in *S. coelicolor*, I have established several fluorescent proteins (FPs) for use in this organism, including mRFP, CyPet and YPet. I have constructed FP vectors and demonstrated that they are functional and efficient markers to detect protein localization. More recently, I have established a two-colour fluorescence reporter system that can be used to study co-localization and protein-protein interactions within cells. I plan to use co-localization of FP fusions and FRET between CyPet and YPet to investigate the assembly of protein complexes within the different cell types. My principle interest in this area is the cell division apparatus in *S. coelicolor*, where relatively little is understood.

Systematics & Evolution Group Session

supported by the Environmental Microbiology Group

Cyanobacteria, architects of our environment: who are they and what do they do?

Diversity and classification: methodology (morphology and molecular), cultivation and preservation issues

J.G. Day

Scottish Association for Marine Science, Dunstaffnage PA37 IQA (Email jgd@sams.ac.uk)

Cyanobacteria are amongst the most ancient organisms on earth with fossil records dating back >3.5 billion years. They can truly be considered to be architects of our environment, because of their photosynthetic and nitrogen fixation capacities. These will be discussed in more detail by other speakers and I will focus on their biodiversity, cultivation/long-term *ex situ* conservation.

Cyanobacteria are virtually ubiquitous in the euphotic environment; furthermore, some have the capacity to live in extreme/unusual niches e.g. endolithically, or in symbiosis. They are prokaryotes, lacking membrane bound organelles, and can vary from 0.4–100 µm in diameter. They range in colour from blue-green to red and can be unicellular, colonial or filamentous. Traditionally they have been considered to be 'plants' with their taxonomy based on gross morphology. More recently they have been included under the International Code of Nomenclature of Bacteria; however, most have not yet been validly published under this code. Today, any meaningful taxonomy depends on the use of both phenotypic and genotypic characters.

Changes in phenotype on cultivation, e.g. colour, or gas vacuole production, are relatively common. Cryopreservation, storage <130°C, is the only realistic conservation option that insures genotypic and phenotypic stability. Some major culture collections have cryopreserved 50–90% of their cyanobacterial holdings.

Evolution, species concept and taxonomy

Annick Wilmotte

Center for Protein Engineering, Institute of Chemistry B6, University of Liège, 4000 Liège, Belgium

The RDP database now includes more than 12000 cyanobacterial 16S rRNA sequences. Only 3500 are from isolates, of which about 1900 are longer than 1200 bp.

The present talk will give an overview of the information contained in the trees made with such strains' sequences, concerning the evolution and taxonomy of the cyanobacterial phylum. A variety of situations exist, with monophyletic (ex. *Microcystis, Planktothrix*) and polyphyletic (ex. *Synechococcus, Leptolyngbya*) 'genera' present. The 16S rRNA is however quite conserved and alternative taxonomic markers were used with success to give additional information in several taxa.

A basic question is the concept of the species, a classical debate among bacteriologists. In the case of cyanobacteria, it is further complicated by the existence of two codes of nomenclature, that have different requirements. A consensus nomenclature accepted by both botanists and bacteriologists is highly necessary.

This review intends togive a synthesis of the molecular data that was published in the last decennia concerning the molecular diversity of cyanobacteria on the basis of 16S rRNA gene sequences. Sequences of strains that are longer than 1000 bp are used to build a tree, that will serve as a guide to present what we know about the genotypic relationships of cyanobacterial taxa.

Population genetics of cyanobacteria

Paul K. Hayes

School of Biological Sciences, University of Bristol

Quantitative descriptions of the genetic structure of cyanobacterial populations are few and far between, and yet the type of information that they provide is of fundamental importance if we are to understand what has made these organisms so successful over such a prolonged period of evolutionary time. This contribution will present an overview of our current understanding of cyanobacterial population genetics, describing both the techniques that have been used and the results that have been obtained. In essence it seems that many populations of planktonic cyanobacteria have an epidemic structure, i.e. one where many genotypes are present but where very few genotypes dominate at any given point in space or time. The significance of this finding will be discussed in terms of total the genetic repertoire available within a cyanobacterial species and in terms of the resultant phenotypic diversity. How the observed population structures are generated and how interactions with the environment drive the succession from one genotype to another will also be discussed.

Phages and photosynthesis in the oceans

Nicholas H. Mann

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

Cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are abundant in the world's oceans and make a major contribution to global primary production. The phages that infect these organisms exert an impact on cyanobacterial photosynthesis in a variety of ways and in consequence influence the global carbon cycle. Phages control cyanobacterial population structures and cause the release of fixed carbon into the dissolved organic matter pool. However, there is increasingly clear evidence that a significant fraction of marine cyanobacterial photosynthesis is carried out by phage-infected cells and that key components of the photosynthetic apparatus may be phage-encoded. Phages may also participate in the horizontal transfer of photosynthetic genes between strains.

A genomic perspective on niche adaptation mechanisms in marine picocyanobacteria

Dave Scanlan

Dept of Biological Sciences, University of Warwick, Coventry

Marine cyanobacteria are the most abundant photosynthetic organisms on Earth with only two genera numerically dominating most oceanic waters, *Prochlorococcus* and *Synechococcus*. Both genera are genetically diverse e.g. the marine *Synechococcus* lineage contains at least ten genetically distinct clades and includes isolates with a wide range of pigmentation.

The availability of over a dozen complete or high-draft picocyanobacterial genomes is enabling the description of the 'core', genes common to all members, and the 'accessory' or 'dispensable' genome, genes present in a subset of strains, which together constitute the pan genome of a species.

By comparing the available *Synechococcus* genomes, along with representative *Prochlorococcus,* our goals were to explore the relationship between ribotype and genomic diversity and to estimate the importance and relative contribution of vertical inheritance, gene gain and gene loss in the core and accessory gene complements. Analysis of the functional genes associated with the accessory genomes and recently acquired genome islands provided insights into the biotic and abiotic factors influencing niche adaptation i.e. that account for vertical, horizontal and seasonal distributions of genetically distinct lineages.

Our data supports hypotheses for phosphorus, metals, light quantity and quality, and habitat 'stability' as defining factors of ocean niches which have clear imprints on the genomes of their *Synechococcus* inhabitants.

Toxic cyanobacterial blooms: research and risk management

Geoffrey A. Codd

Division of Molecular & Environmental Microbiology, College of Life Sciences, University of Dundee, Dundee DD1 4HN

The recognition of cyanobacteria as the initial source of the biosphere's oxygen and as major global contributors to primary productivity is overshadowed today

by their increasing adverse impacts on water resources and safety. Affected human activities include drinking, aquaculture, irrigation, industrial processes, recreation and tourism. All can be constrained if cyanobacterial populations exceed thresholds which may relate to e.g. biomass, turbidity, odour, taste, and not least, potent cyanotoxins. Climate change may exacerbate the established effects of eutrophication and over abstraction on cyanobacterial mass developments via e.g. reduced flows, droughts, increases in geographical spread due to temperature rise and extended bloom growth seasons in temperate latitudes.

Cyanotoxin types, their concentrations and compartmentation vary widely but appear to occur globally. They can be found: (i) at concentrations able to cause acute illness/death via ingestion; (ii) singly or as multiple variants and classes; (iii) at higher trophic levels in food chains; (iv) alongside other health hazards in waterbodies and derived products. Cyanotoxin risk management closely depends upon molecular and environmental research on the toxins, their toxicity assessment, analytical method development and case studies. International progress is reviewed here, including the introduction of guideline and legislative tools, and uncertainties and gaps are identified.

Marine cyanobacterial mats

L.J. Stal

Netherlands Institute of Ecology, Yerseke, The Netherlands

Abstract not received

Cyanobacteria in extreme environments

Antonio Quesada

Dpt. Biología, Universidad Autónoma de Madrid, Spain

Cyanobacteria are widely distributed on Earth. They are typically aquatic organisms inhabiting almost every kind of marine and freshwater ecosystems. However, cyanobacteria also inhabit sub-aerophytic environments, as soils, barks or rocks. They are one of the most important groups of primary producers in extreme environments as cold and hot deserts, extremely saline or highly alkaline environments, becoming in some cases the highest biomass in the complete ecosystems, as it is the case in non-marine aquatic ecosystems from polar regions.

Cyanobacteria associated to the lithic substrate (endo, hipo or epilithic organisms) are the only biota found in some of the most extreme ecosystems on Earth, which are similar to some ecosystems present on Mars. However, the knowledge about the physiology, ecology and diversity of these organisms is quite fractionary. We will show in this presentation the most recent advances in the knowledge of cyanobacteria in extreme environments, insisting also in the methodological approaches leading to the most recent advances on ecology, physiology and diversity of these peculiar ecosystems.

We will also discuss evolutionary aspects of cyanobacteria in extreme ecosystems, trying to explain the adaptation strategy to these marginal ecosystems.

Ion sensing mechanisms conserved between cyanobacteria and mammals

A. Hammer, P.M. Holliday, P.D. Townsend, R.W. Hodgson & **M.J. Cann**

School of Biological and Biomedical Sciences, Durham University, South Road, Durham DH1 3LE

All prokaryotes and eukaryotes must possess signalling mechanisms to sense and adapt to fluctuations in the major inorganic components of the cellular environment. As the nature of inorganic stress has not significantly changed over evolutionary time we investigated whether sensing mechanisms have also remained unchanged. Analysis of select cyanobacterial and mammalian models revealed that signalling mechanisms responsive to carbon dioxide and sodium ion have been conserved since the divergence of the last common ancestor for these organsisms, about 3.8 billion years ago. This remarkable conservation of biochemistry reveals that select evolutionarily diverse organisms may use fundamentally similar mechanisms for sensing inorganic molecules and ions.

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

Dilara Sharif, Ed Dudley, Chris Smith & John Gallon

Biochemistry Research Group, School of the Environment and Society, Swansea University, Singleton Park, Swansea SA2 8PP

Cyanobacteria are a group of photosynthetic organisms that have both beneficial and harmful effects in the environment. Dense growth of these organisms has been found to be associated with blooms and in biofilms. It is of particular interest to examine mechanisms such organisms have, in order to overcome problems of stress and survival, associated with growth at a high cell density. Recently, quorum-sensing, via homoserine lactones, has been demonstrated in a group of proteobacteria, in which it can function to communicate increased cell density. However, to date there is no evidence of such processes operating in the cyanobacteria. Our research provides the first evidence that a C8-homoserine lactone (C8-HSL) is released by a cyanobacteria. A number of proteins displaying altered expression levels in response to treatment with C8-HSL were identified, suggesting that the cyanobacterium responds to the homoserine lactone through changes in gene expression.

Cell development in cyanobacteria living in a perpetual plant symbiosis (Azolla sp.)

B. Bergman

Dept of Botany, Stockholm University, SE-10691 Stockholm, Sweden (Email bergmanb@botan.su.se)

A limited number of cyanobacteria form stable nitrogen-fixing symbioses with diverse eukaryotes. The small free-floating water-fern *Azolla* carries a nitrogen-fixing cyanobacterium (cyanobiont) in all its leaves and the symbiosis shows a pronounced intimacy between the partners, also including bacteria (bactobionts). It is the only perpetual N_2-fixing symbiosis, i.e. the cyanobiont is vertically transmitted in sporocarps between plant generations; and the cyanobiont seems incapable of independent growth. The cyanobiont in *Azolla* undergoes

several cell developmental processes during the reproduction of the symbiosis, including differentiation of hormogonia, heterocysts and akinetes. The data demonstrate that the vertical transmission of the cyanobiont between the *Azolla* generations requires complex developmental and adaptive mechanisms of the cyanobacterium, pointing to a highly co-ordinated co-evolution, potentially on its way to become (the first) nitrogen-fixing plant. The genome of the cyanobiont is currently being sequenced (DOE/JGI, USA).

Cyanobacteria shaping the earth's environment

Stjepko Golubic

University of Boston Biological Science Center, USA

Cyanobacteria are an ancient group of micro-organisms with a critical historic role in shaping of our planet, but were until recently unjustly ignored and marginalized. They attracted less attention then prokaryotic pathogens, and were considered less important than eukaryotic algae and plants. The recent advances, especially in molecular genetics, could document: that oxygenic photosynthesis has its monophyletic origin within cyanobacteria; that this invention, which changed the redox condition of the planet, could not be re-invented, but was laterally transferred and incorporated into eukaryotic organization; that the evolution of this group continued to diversify beyond expectation both in free-living forms and as endosymbioses. The role of photosynthesis as the main source of Earth's atmospheric oxygen has been postulated, as no other mechanism could explain this planetary anomaly. The impact of cyanobacteria in shaping see floor, organizing sediments, constructing reefs and modifying landscapes has been learned from the study of ancient stromatolites, and cyanobacterial contribution was confirmed by recognition of their mineralized fossil remains. Their role as primary producers, nitrogen fixers and principle constructors of oxidation-reduction interfaces promoted other metabolic systems that diversified among chemolithotrophic and heterotrophic organisms. Together, they continue to modify the environment by the processes of bio-sedimentation and bio-erosion.

Ecology and evolution: insights from marine genomes

Martin Ostrowski

Dept of Biological Sciences, University of Warwick, Coventry

Cluster sequencing of marine picocyanobacteria and their associated phage has revealed a higher than expected genetic diversity and genomic flexibility than could have been imagined just a few years ago. Comparative genome analysis has enabled us to better understand the processes driving genome divergence and niche adaptation. Despite the relatively high proportion of gene content influenced by horizontal gene transfer phenotypic and molecular markers can be correlated with environmental data to define clusters of ecologically distinct groups. Comparisons of gene content and nucleotide identity, from classical markers to genome-wide estimates, have refined a functional species concept for marine picocyanobacteria. The ongoing challenge is to incorporate these insights into the framework for continued research aimed at understanding how environmental gradients and biological factors dictate the geographical distribution and evolution of these important primary producers.

Genomics and secondary compounds

Adam M. Burja

Fermentation and Metabolic Engineering Group, Ocean Nutrition Canada Ltd, 101 Research Drive, Dartmouth, Nova Scotia, B2Y 4T6, Canada

The genomic and now the post-genomic era, has brought with it the ability to unlock complex interactions in micro-organisms and biological systems. To date, research has mainly focused on a few representative pathogenic species, leaving the vast majority of the microbial community relatively overlooked. Amongst these are the cyanobacteria, ancient micro-organisms that represent one of the oldest known oxygenic photosynthetic groups and having relatively small genomes (on average 4.1 MB), which are known for their ability to produce biochemically active natural products. Compounds of natural origin have long been an important contributor to drug discovery programs. In recent years, however, screening of secondary metabolites, particularly microbial compounds has fallen out of favour with big pharma, with recent trends suggesting it is becoming increasingly difficult to obtain new leads. By analysing both journal publications and patent applications, we found that cyanobacteria represent a relatively untapped, potentially more productive microbial source with a far greater multiplicity of biological activities. This presentation will firstly provide a background to the nature of cyanobacterial secondary compounds, followed by examples whereby this production ability is harnessed in order to develop many diverse products, covering the pharmaceutical, nutraceutical, bioremediatory and energy production arena.

The genus *Cyanothece*, unicellular diazotrophic cyanobacteria that produce O_2, fix N_2 and that have highly versatile metabolic systems

Jörg Toepel & **Louis A. Sherman**

Purdue University, Dept of Biological Sciences, West Lafayette, IN 47907, USA

Cyanothece sp. ATCC 51142 is a unicellular, diazotrophic cyanobacterium with a versatile metabolism and very pronounced diurnal rhythms. When grown under 12h light-dark (LD) periods, it performs photosynthesis during the day and N_2 fixation and respiration at night. During this process, carbohydrate and amino acids are compartmentalized in granules in the light and dark, respectively. This strain also grows exceedingly well heterotrophically (on glycerol). The excellent synchrony of a culture under LD diazotrophic conditions permits analysis of cellular morphology, mRNA levels, proteomics and metabolomics as a function of time. In addition, genome sequencing of *Cyanothece* sp. ATCC 51142 is complete and that of 6 other *Cyanothece* sp. is in progress. *Cyanothece* sp. ATCC 51142 has 2 chromosomes—a 4.9 Mb circular chromosome and a 0.43 Mb chromosome containing a total of 5,300 genes. This strain has the capability of producing high-energy compounds, such as ethanol and hydrogen and *Cyanothece* sp. PCC 7822 can produce these compounds as well as rather large quantities of parahydroxyalkanotes (PHAs).

We are particularly interested in the regulation of these metabolic processes and the way in which these organisms respond to environmental cues such as light, the lack of combined nitrogen and changing O_2 levels. We have performed a series of microarray experiments on *Cyanothece* sp. ATCC 51142 and can demonstrate the relationship of the metabolic synchrony with gene expression. The growth under light-dark and continuous light conditions provides a great deal of information on this regulation and on how *Cyanothece* can perform N_2 fixation simultaneously with photosynthesis—a very clever trick that demonstrates the environmental superiority of cyanobacteria!

The genome of *Arthrospira* sp. PCC8005: an insight into the role of repeated sequences in cyanobacterial genomes

Nicolas Morin[1,7], Nicole Tandeau de Marsac[2], Valérie Barbe[3], Carole Dossat[3], Sophie Mangenot[3], Cheng-Cai Zhang[4], Tatiana Vallaeys[5], Christel Paillé[6], Paul Janssen[1], Annick Wilmotte[7], Larissa Hendrickx[1] & Max Mergeay[1]

[1]Laboratory of Molecular and Cellular Biology, Belgium Nuclear Research Center (SCK•CEN), Mol, Belgium; [2]Institut Pasteur, Unité des Cyanobactéries, CNRS, Paris, France; [3]Genoscope, French National Sequencing Center, Evry, France; [4]Laboratoire de Chimie Bactérienne, IBSM CNRS, Marseille, France; [5]Microbiology Dept, INRA-INA-PG, Thiverval Grignon, France; [6]European Space Agency, ESTEC, Noordwijk, Netherlands; [7]Centre for Protein Engineering, Institute of Chemistry B6, Sart Tilman, University of Liège, Liège, Belgium

Due to its performant oxygen production, along with its valuable nutritional properties, the cyanobacterium *Arthrospira* sp. PCC8005 has been selected as oxygen producer and complementary food source in the bioregenerative life-support system MELiSSA of the European Space Agency. In order to study the effects of long-term culturing under space flight conditions from the genomic up to the metabolic level, the genome of *Arthrospira* sp. PCC8005 was submitted to the French National Sequencing Center, Genoscope. Sofar, three genomic DNA libraries (having an average of 3 kb, 10 kb and 25 kb inserts, respectively) were sequenced resulting in a first assembly of 21 supercontigs representing circa 5.9 million bp.

The first bioinformatics studies performed on the sequence showed a surprisingly high amount of repeated sequences, covering 18–20% of the actual assembly. The nature of these repeats appears to be extremely diverse. Small repeated patterns were detected, including highly iterated palindromes already described in many cyanobacterial species, or tandem-like repeats. Interestingly, larger regions of the genome are repeated, varying in size from 1 to a few kb while their similarity to each other reaches 95% or more. Among these large repeated sequences, an important proportion of mobile genetic elements has been identified.

In this presentation a first view is offered on the current status of the genome sequence of *Arthrospira* sp PCC8005, including a detailed catalogue of the repetitions detected through the annotation process, and a discussion on their function and potential role along the evolution of this particular cyanobacterium.

Acknowledgements The presented project was financially supported by the European Space Agency and the Belgian Science Policy (Belspo) (MELISSA/BIORAT Genetic stability study – phase 2 (MELGEN 2) TEC-MCT/2006/3501/In/CL). We are grateful to C. Lasseur and F. Marty, both from ESTEC/ESA, for their constant support and advice. We gratefully acknowledge the Consortium National de Recherche en Genomique – Genoscope, Evry, for its contribution to the construction of the genome sequence *Arthrospira* sp. PCC8005.

Virus Group Session

Virus modulation of host defences

Pattern recognition and the red queen: antagonistic host virus relationships between retroviruses, TRIM5 and Cyclophilin A

Greg J. Towers

University College London

TRIM5 is an important species-specific anti-retroviral protein mediating innate immune activity in mammals. Cyclophilin A (CypA) is a peptidyl prolyl isomerase recruited to incoming capsids from divergent lentiviruses. Blocking CypA activity often reduces the sensitivity of retrovirus to restriction by TRIM5. We propose that CypA alters the shape of incoming viral capsids, through cis/trans isomerization of exposed prolines, altering the sensitivity of the capsid to pattern recognition by TRIM5. The owl monkey TRIM5 gene has been modified by replacement of the viral binding domain with CypA, encoded by a retrotransposed cDNA. This leads to an antiviral TRIMCyp fusion molecule that restricts retroviruses that recruit CypA. We have recently identified a second example of fusion of CypA to TRIM5, by retrotransposition of a CypA cDNA, in Old World monkeys. Remarkably, the two TRIMCyps have arisen independently and have different antiviral specificities. In rhesus macaques TRIMCyp exists as a single allele of the TRIM5 locus, amongst at least six further TRIM5 encoding alleles. The recruitment of CypA to incoming virions appears to have provided powerful selection pressure for the fixation of TRIMCyp alleles in primates.

Intrinsic cellular repressors of cytomegalovirus – repression through chromatin structure

John Sinclair

University of Cambridge

The eukaryotic cell has an arsenal of adaptive and innate anti-viral responses which must be overcome by the virus to permit efficient viral gene expression, DNA replication and subsequent virus production and dissemination. During human cytomegalovirus (HCMV) infection, as with infection with many DNA viruses, viral genomes associate with a cellular subnuclear structure known as nuclear domain 10 (ND10) and recent studies have shown that individual protein components of these ND10, such as hDaxx or promyelocytic leukemia protein (PML), mediate an intrinsic anti-viral response against HCMV. This ND10-mediated anti-viral response appears to act by silencing expression of HCMV major immediate early (IE) gene expression, a class of viral genes essential for the subsequent activation of the full lytic cycle.

We will show that cellular hDaxx in ND10 functions to repress transcription of viral IE genes immediately upon infection via the establishment of an inhibitory chromatin structure around the viral major immediate early promoter (MIEP) on the viral genome. However, we will also show that disruption of ND10, mediated by viral IE gene expression itself as infection proceeds, results in modification of hDaxx to an activator of the MIEP.

Innate recognition of viral infection

Caetano Reis e Sousa

Immunobiology Laboratory, Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London WC2A 3PX

Virus infection elicits potent responses in all cells intended to contain virus spread before intervention by the adaptive immune system. The sensors involved in coupling recognition of viruses to the induction of the innate response genes have only recently been uncovered. One pathway for sensing infection by RNA viruses involves recognition of viral genomes or virally infected cells in endosomal compartments and utilizes members of the toll-like receptor (TLRs) family, including TLR9, 7, or 3. Viral genomes can additionally be recognized in the cytosol by DExD/H-box helicases such as RIG-I, which are activated by RNAs bearing 5′ phosphates. The emerging properties of virus sensing proteins suggest that the body detects virus invasion by recognizing the unusual presence of atypical nucleic acids in particular sub-cellular compartments.

The role of rotavirus NSP4 in host entry

W. Yang & **M.A. McCrae**

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

The hurdle of entry to an organism is a major component of defence against infection. Rotaviruses are major enteric pathogens whose primary site of replication is currently recognized to be gut epithelia, however viral pathology and disease symptoms have been found to precede detectable virus replication. In addition rotavirus viremia indicative of viral penetration of the gut mucosa has been reported. The virus encoded NSP4 protein has been shown to have cytotoxic activity when applied extracellularly to cells. Consequently it has been earmarked as being responsible for the early cellular pathology in the gut whose cytotoxicity is currently believed to spread by secretion of the protein from virus-infected cells. Using real time confocal microscopy we have shown that NSP4 expression alone triggers the formation of cytoplasmic extrusions and the spread of the protein through direct cell-cell spread of the protein rather than extracellular spread. The results obtained will be presented and their possible significance to both viral pathology and overcoming the entry defence of the host will be discussed.

Resistance of human respiratory syncytial virus to neutralization by anti-F murine monoclonal antibodies, palivizumab and human serum

Sarah Welsh, Edna L.M. Gias, Soren U. Nielsen & Geoffrey L. Toms

School of Clinical Medical Sciences, The Medical School, University of Newcastle, Newcastle upon Tyne

Fresh isolates of human respiratory syncytial virus (hRSV) exhibit marked resistance to neutralization by anti-fusion protein monoclonal antibodies. Resistance is transient on propagation of the viruses in cell culture and this has hitherto hindered analysis of the mechanisms involved. In this study, clonal analysis of early passage, resistant virus stocks yielded predominantly resistant clones whilst late passage, susceptible stocks yielded clones which were predominantly susceptible. Replication rates of susceptible clones were markedly higher than those of resistant clones indicating that this phenomenon is the result of a quasi-species shift in the virus population with the emergence and selection of rapidly replicating, neutralization susceptible variants in cell culture. The clones derived were phenotypically stable allowing further analysis of the mechanisms involved. Resistant clones were co-resistant and susceptible clones co-susceptible to neutralization and fusion inhibition by murine anti-F monoclonal antibodies and to neutralization by palivizumab and human sera. Sequence analysis revealed no consistent change in the F gene or G-F intragenic sequences between resistant and susceptible clones. The phenotype was preserved for density gradient purified virions from each clone and F gene content per genome was similar

for both resistant and susceptible phenotypes. Paradoxically, UV inactivated resistant virions bound neutralizing anti-F antibodies markedly more efficiently than susceptible virions. Also UV inactivated resistant but not susceptible virions inhibited the infectivity of a susceptible clone of virus. This data suggests the hypothesis that resistant virus binds more avidly to cell surface receptors and, thereby is more resistant to neutralization.

Triggering and control of innate immune defenses against RNA virus infection

Michael Gale Jr

Dept of Immunology, University of Washington School of Medicine, Seattle, WA, USA

Innate immune defenses are essential for resistance to viruses and can be triggered through the actions of the cytoplasmic helicases RIG-I and MDA5 during infection by RNA viruses. We have evaluated innate immune signaling requirements of RIG-I, MDA5, and their IPS-1 signaling adaptor protein, by human pathogenic viruses of the *Flaviviridae, Hepaciviridae*, *Orthomyxoviridae, Paramyxoviridae*, and *Reoviridae*. In cultured cells, IPS-1 was essential for innate immune signaling of downstream IRF-3 activation and interferon-stimulated gene expression but the requirement for RIG-I and MDA5 was variable. While RIG-I or MDA5 were individually dispensable for signaling triggered by reovirus, West Nile virus, and Dengue virus, RIG-I was essential for signaling by hepatitis C virus (HCV), influenza A virus (FluA), influenza B virus, and human respiratory syncytial virus. Functional genomics analyses identified shared and unique biosets of cellular genes triggered during infection by these viruses whose expression was strictly dependent on RIG-I and are involved in processes of innate or adaptive immunity, apoptosis, cytokine signaling, and inflammation. Our studies show that HCV and FluA infection each impart control of the RIG-I pathway, and that differential regulation of innate immunity through viral triggering and control of RIG-I signaling is a major determinant of infection outcome.

Rinderpest virus C protein blocks type I interferon induction

Emma L. Boxer, Sambit K. Nanda & Michael D. Baron

Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Surrey GU24 0NF

The innate immune response is the first line of defence against viral disease, characterized by production of various cytokines, including interferons (IFNs). Rinderpest virus (RPV), a member of the genus *Morbillivirus*, causes a highly contagious viral disease that affects even-toed ungulates. The aim of this study was to investigate whether RPV is capable of blocking the induction of type I interferon and, if so, determine the viral protein responsible and its mechanism of action. Using a luciferase reporter assay, virulent RPV was found to actively block activation of the IFN-β promoter. Further assays, using both knockout viruses and transient expression of viral proteins, showed that it is the non-structural C protein which is responsible for conferring this ability. The C protein blocks induction of interferon through both mda5 (dsRNA) and RIG-I (NDV) pathways. The RPV V protein appears to have no role in this function. The C protein may act within the nucleus, downstream of activation of the various transcription factors.

Broad-scale screening for poxvirus modulators of type I interferon

K.R. Buttigieg[1], S.M. Laidlaw[1], C. Ross[2], S. Goodbourn[2] & M.A. Skinner[1]

[1]Dept of Virology, Imperial College London, W2 1PG; [2]Division of Basic Medical Sciences, St George's, University of London

Multiple modulators of type I interferon are encoded by mammalian poxviruses. Fowlpox virus (FWPV) is resistant to chicken interferon (chIFN) but genome divergence and the scarcity of avian interferon reagents has made it difficult to identify the genes responsible. We therefore used two broad-scale genetic approaches: (i) production of a library of recombinant Modified Vaccinia Ankara (MVA) viruses, each chimaeric for about 8 kbp of FWPV genome, screened for increased resistance to low dose chIFN, and (ii) production of an FWPV gene knock-out library, screened for loss of the ability to block induction of the chicken β-IFN promoter in infected avian cells.

Thus far, two chimaeras (of 60 spanning the 266 kbp FWPV genome), each containing a small cluster of FWPV genes, have demonstrated significantly increased resistance to chIFN and three knock-out mutants (out of fifty non-essential mutants) have demonstrated significantly decreased ability to block induction of the chicken β-IFN promoter.

Subversion of dsDNA detection pathways by the human papillomavirus E7 protein

Oliver Watherston, G. Eric Blair & **Andrew Macdonald**

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

DNA is a potent activator of immune responses upon viral infection. It is detected by TLR9 and the cytosolic protein DAI, which activate transcription of IFNβ. Viruses have evolved numerous mechanisms to evade the IFN response, a factor governing the development of virally transformed cells into tumours. Papillomaviruses (HPV) are able to successfully evade the immune response, however, the ability of HPV to inhibit IFN is poorly characterized. Here we address the role of the multifunctional E7 oncoprotein in subverting IFN signalling, and provide direct evidence that E7 is an inhibitor of IFNβ transcription. Furthermore, this is the first demonstration of a viral inhibitor of the novel DAI signalling pathway. Using a proteomics approach we established that E7 functions upstream of the IRF3 transcription factor and identified novel E7-interacting partners that may mediate subversion of IFNβ signalling. We propose that E7 is a novel inhibitor of IFNβ signalling that targets the newly identified DAI signalling pathway to facilitate a chronic viral infection.

Induction and suppression of the interferon response by highly pathogenic RNA viruses

Friedemann Weber

Dept of Virology, University of Freiburg, D-79008 Freiburg, Germany

Type I interferons (IFN-α/β) are an essential part of the innate immune defense. They are synthesized and secreted by infected cells and stimulate expression of potent antiviral proteins. A rapid production of IFN is important for the host to ward off virus infections in a timely manner. To achieve this, viral hallmark molecules are recognized by specific receptors, thus triggering a signaling chain which culminates in transactivation of the IFN-β promoter. Viruses, in turn, were forced to evolve strategies to counter the IFN response which would otherwise bring their replication to a halt. In our group we are studying the IFN system interaction of highly pathogenic RNA viruses such as SARS-coronavirus and members of the *Bunyaviridae* family. In particular, we investigate how these pathogens are recognized by intracellular receptors and how they manage to escape the IFN induction machinery. The results obtained so far illustrate that viruses make use of an astonishing range of both passive and active mechanisms in order to delay and block the antiviral IFN response.

MCMV IE1 protein and modulation of TNF *in vitro* and *in vivo*

Sara Rodriguez-Martin[1], Andrew D. Livingston[1], Astrid Krmpotic[3], Stipan Jonjic[3], Martin Messerle[4], Ana Angulo[2] & Peter Ghazal[1]

[1]Division of Pathway Medicine, University of Edinburgh Medical School, The Chancellor's Building, Little France Crescent, Edinburgh EH16 4SB; [2]Institut d'Investigacions Biome`diques August Pi i Sunyer, 08036 Barcelona, Spain; [3]Dept of Histology and Embryology, Medical Faculty, University of Rijeka, 51000 Rijeka, Croatia; [4]Dept of Virology, Hannover Medical School, 30625, Hannover, Germany

MCMV IE1 protein has been described as a transactivator of viral gene expression, however its primary function still remains elusive, especially in regard to the modulation of innate immunity in infected BMMΦ.

Results From microarray studies, we show that IE1 selectively regulates the expression of genes associated with an inflammatory response in BMMΦ. In particular, using *ie1*-deletion mutants, parental and revertant MCMV, we show that the lack of IE1 results in the increased production of TNF at both RNA and protein level in infected BMMΦ. Further, in BALB/c and C57B/6 mice experiments, IE1 mutant virus exhibits an attenuated phenotype which is correlated with an enhanced TNF production. Using TNF−/− mice the IE1 mutant virus growth is partially restored in an organ-specific manner.

Conclusions MCMV IE1 suppresses the production of TNF *in vivo* and *in vitro*, blocking the antiviral effect that TNF exerts to control viral infection. In the natural host, TNF is responsible, at least in part, in the control of viral growth in an organ-specific manner.

The role of the influenza A virus PA protein in host-cell shutoff

Anne Lo[1], Sarah Linton[2], Romy Dalton[1], Louise Bell[1], Michael M.-T. Lee[3], John McCauley[4], Laurence Tiley[3], Wendy Barclay[2] & Paul Digard[1]

[1]Division of Virology, Dept of Pathology, University of Cambridge, Tennis Court Road, CB2 1QP; [2]Division of Investigative Science, Imperial College, St Mary's Hospital, London W2 1PG; [3]Clinical Veterinary Medicine, University of Cambridge, Madingley Road. Cambridge CB3 0ES; [4]NIMR, Mill Hill, London NW7 1AA

PA is one of the three subunits of the influenza A virus polymerase. It has also been reported that when expressed from a plasmid, PA from some but not all virus strains interferes with the expression of co-transfected genes. We found that PA acts as a general inhibitor of RNA Pol II-mediated gene expression, reducing the accumulation of both protein and mRNA of a variety of target genes. Fluorescent *in situ* hybridization analysis also showed that PA induced nuclear retention of the reporter gene mRNAs. This activity suggests the hypothesis that PA plays a role in host-cell shutoff. Supporting this, reporter gene assays showed that PA antagonized IFN, TNF and dsRNA induced gene expression. Furthermore, using a panel of otherwise isogenic viruses, we found that PA genes with higher levels of gene expression inhibitory activity conferred a higher degree of host-cell shutoff. We propose that PA acts as a virulence factor through blocking cellular gene expression.

Influenza A viruses and PI3K signalling

B.G. Hale, D. Jackson, M.J. Killip, H. Xiao & R.E. Randall

Centre for Biomolecular Sciences, University of St Andrews

In infected cells the non-structural (NS1) protein of influenza A virus modulates multiple virus and host activities. One such function of NS1 is the stimulation of phosphoinositide 3-kinase (PI3K), a cellular enzyme involved in the control of gene transcription, protein synthesis, cell survival, and cytokine production. During infection, NS1 binds directly to PI3K via the inter-SH2 domain of p85b, a subunit of heterodimeric (p85:p110) PI3K. Binding of NS1 to the inter-SH2 domain likely masks a specific regulatory element in p85b that normally contributes to repression of p110 catalytic activity. Thus, activation of PI3K early during infection is concomitant with NS1 expression. Here, we report on our ongoing studies concerned with the mechanism, regulation, and biological significance of NS1-mediated PI3K activation. We highlight virus strain-specific differences, and discuss potential consequences of these for both virus and host.

Investigating the role of the novel influenza PB1-F2 protein in pathogenesis

Wendy Howard[1], Anna Hayman[1], Alison Whiteley[1], Brandon Londt[2], Jill Banks[2], Tracy Hussell[1] & Wendy Barclay[1]

1Imperial College London, W2 1PG; [2]VLA, Weybridge KT15 3NB

The influenza PB1-F2 protein was only discovered in 2001 (Chen *et al.*) *In vitro* studies suggest PB1-F2 induces apoptosis of immune cells. Studies in mice have shown that PB1-F2 increases disease and susceptibility to secondary bacterial infections (McAuley *et al.*, 2007). We aim to investigate the role of PB1-F2 in pathogenesis using reverse genetics in a mouse and an avian model. We developed an avian reverse genetics system for the H5N1 virus A/Turkey/England/50-92/91 and rescued recombinant PB1-F2 knock-out viruses and viruses containing PB1-F2 truncated at 57 amino acids. The latter mutant reflects current H1N1 strains that exhibit especially low pathogenicity. Inoculation of chickens revealed an overall decrease in pathogenicity compared to the original wild type isolate. However, all birds harboured virus enabling us to use this model to look at differences in the immune response over a 10 day period. Studies are ongoing. The same panel of PB1-F2 mutants were generated in a PR8 virus background for use in mice. Mice infected with PB1-F2 knock-out and 57 amino acid truncated viruses exhibited significantly decreased weight loss compared to PR8 wild type-infected mice. This was not linked to lymphopoenia or differences in apoptotic monocytes/dendritic cells.

Poxvirus immune evasion is linked to host tropism

G. McFadden

University of Florida, USA

Abstract not received

Anti-immunology: suppression of antigen presentation, apoptosis and lymphocyte activities by adenovirus E3 proteins

Hans-Gerhard Burgert

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

The early transcription unit E3 of human adenoviruses (Ads) contains a cluster of 5–9 genes that are non-essential for virus replication in cell culture. The majority are membrane proteins exhibiting immunomodulatory functions. For example, the E3/19K glycoprotein blocks the transport of newly synthesized MHC class I molecules to the cell surface, thereby interfering with antigen presentation to cytotoxic T lymphocytes (CTL). Recently, we discovered an additional function for E3/19K showing that it also down-regulates MHC class I chain–like molecules (MICA/B) that serve as ligands for the activating receptor NKG2D on Natural killer (NK) cells. Thus, E3/19K appears to inhibit activation of several types of cytotoxic lymphocytes. Two other E3 proteins, E3/10.4K and E3/14.5K, form a complex named RID that reroutes apoptosis receptors to lysosomes where they are degraded. Consequently, RID may contribute to protection of cells from CTL and NK cell lysis.

Interestingly, Ads of different subgenera express different sets of E3 proteins which may play a role in the pathogenesis of different Ad serotypes. Characterization of the subgenus D-specific E3 membrane protein E3/49K revealed two novel features for E3 proteins: E3/49K is secreted and can directly bind to and affect lymphocytes. Together, E3 proteins seem to be devoted to protect infected cells from the lytic attack by the immune system.

Molecular mechanisms of HIV immune evasion

K.L. Collins

University of Michigan, USA

HIV establishes a chronic infection, which leads inexorably to the development of AIDS. To evade the host immune response, the HIV

Nef protein limits presentation of viral antigens by host MHC-I proteins. Nef reduces the expression of MHC-I through a physical interaction between Nef and specific amino acid residues in the MHC-I cytoplasmic tail. Once bound, Nef domains stabilize the recruitment of the cellular trafficking factor (AP-1) to a tyrosine residue in the MHC-I cytoplasmic tail. Our work indicates that Nef stabilizes the interaction of this tyrosine with the natural tyrosine-binding pocket of AP-1. The MHC-I/Nef/AP-1 complex is then targeted into the endolysosomal pathway and MHC-I is degraded at an accelerated rate. In addition, we have determined that a second trafficking signal in Nef (a dileucine motif, which is necessary for Nef to disrupt the trafficking of CD4) can affect MHC-I trafficking only when Nef is fused to the MHC-I cytoplasmic, but not when Nef binds MHC-I naturally. These data support the concept that Nef takes on notably different structural forms in different contexts, revealing or obscuring trafficking signals as needed. A greater understanding of the surfaces involved in these interactions will facilitate the development of pharmaceuticals, which may someday help combat AIDS.

AIDS – an accident of primate lentiviral evolution?

Frank Kirchhoff

Institute of Virolgy, University of Ulm, Germany

Although all primate lentiviruses are referred to as human and simian 'immunodeficiency' viruses, HIV and SIV, respectively, SIVs do generally not cause disease in their natural monkey hosts. The exact mechanisms underlying nonpathogenic SIV infection are unclear. However, a consistent difference between pathogenic and nonpathogenic primate lentiviral infections is that high levels of chronic immune activation, associated with accelerated T cell turnover and apoptosis, are observed in HIV-1-infected humans and SIVmac-infected macaques but not in naturally SIV-infected primates. Increased immune activation is also a key predictor of progression to AIDS in HIV-1-infected humans. Recently, it has been shown that most primate lentiviral Nef proteins efficiently downmodulate CD3, a key component of the T cell receptor (TCR), from the surface of virally infected T cells to suppress their activation and apoptosis. Only Nef alleles from HIV-1 and its immediate SIV precursors fail to perform this function. Further analyses support that the ability of Nef to remove TCR-CD3 from the cell surface helps the natural hosts of SIV to maintain normal $CD4^+$ T cell counts despite high levels of viral replication. Thus, the evolutionary loss of this protective Nef function may have predisposed the precursor of HIV-1 for greater pathogenicity in humans.

Short silencing RNA networks

David Baulcombe

Dept of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA

RNA silencing was first implicated as a defense system against plant viruses. However, from genetic analysis and through the use of high throughput sequencing, it is now clear that this process pervades many aspects of regulation at the RNA and DNA level. I will propose that the short silencing RNAs serve roles as negative switches, as mediators of systems robustness through positive and negative feedback and as initiators of epigenetic mechanisms. Current understanding of silencing RNA mechanisms reveals the potential for complex interaction networks in which the silencing RNAs determine the specificity of the interactions between initiators, nodes and endpoints. These small RNA networks provide novel opportunities for genetic manipulation of important traits in crop plants and as an analytic tool of the genetic and epigenetic regulatory states in developmental stages, in response to stresses and in different genotypes.

Immune evasion by herpesviruses – masters of disguise

Maaike Ressing[1], Andrew Hislop[2], Daniëlle Horst[1], Marieke Verweij[1], Danijela Koppers-Lalic[1], Daphne van Leeuwen[1], Victoria Pudney[2], Nathan Croft[2], Eric Reits[4], Jacques Neefjes[3], Alan Rickinson[2] & **Emmanuel Wiertz**[1]

[1]Leiden University Medical Center, Leiden, The Netherlands; [2]CRUK Institute for Cancer Studies, University of Birmingham, Birmingham; [3]Academic Medical Center, Amsterdam, The Netherlands; [4]Netherlands Cancer Institute, Amsterdam, The Netherlands

Herpesviruses cause a lifelong infection of their host, despite the existence of a vigorous anti-viral immune response. Evasion of host immunity is facilitated by viral proteins specifically interfering with the detection and elimination of the virus by the adaptive and innate immune response. The various immune evasion strategies employed by herpesviruses will be reviewed. A detailed report will be presented on the identification of two new families of herpesvirus immune evasion proteins, acting as inhibitors of cytotoxic T-cell recognition. These are the UL49.5 proteins, encoded by a subgroup of varicelloviruses, and the BNLF2a proteins of Epstein-Barr virus (EBV) and related lymphocryptoviruses of Old World primates. They all act through inhibition of the Transporter associated with Antigen Processing (TAP), thereby compromising MHC class I-restricted antigen presentation to $CD8^+$ cytotoxic T-lymphocytes. The mechanisms of TAP inhibition are completely different from those of other herpesvirus-encoded TAP-inhibitors: the HSV-1 and -2 encoded ICP47, HCMV US6, and the murine γ-herpesvirus 68 mK3. The fact that TAP-inhibitors are encoded by α-, β- and γ-herpesviruses, but are unrelated in sequence and mechanism of action, presents a striking example of functional convergent evolution. Their existence testifies to the strength of evolutionary pressure exerted on herpesviruses by $CD8^+$ T-cells.

Epstein–Barr virus lytic cycle genes targeting the HLA class I antigen processing pathway to evade CD8+ T cell responses

Jianmin Zuo, Nathan Croft, Wendy Thomas, Andrew Hislop & **Martin Rowe**

Division of Cancer Studies, University of Birmingham, Edgbaston, Birmingham B15 2TT

Background Epstein–Barr virus (EBV) persists in memory B cells of healthy infected individuals as a largely latent infection that is invisible to virus-specific T cell responses. Activation into lytic cycle leads to the expression of about 80 viral genes, potentially exposing the virus-producing cells to T cell responses. There is accumulating evidence, however, that EBV has mechanisms to evade these responses.

Methods We have focussed on the identification of EBV genes that might interfere with the HLA class I antigen presentation. Individual genes were expressed in target cells, and the effects on cell surface HLA expression and on T cell recognition were monitored.

Results To date, we have identified: BGLF5, affecting protein synthesis; BNLF2a targeting peptide transport by TAP complexes; and a third gene targeting the antigen presentation pathway by an as yet unidentified mechanism.

Conclusion As with other herpesviruses, EBV has multiple mechanisms working together to modulate antigen presentation and protect from $CD8^+$ T cell responses.

HPV proteins interfere with immune molecules

M. Cortese, P. Cordano & **M.S. Campo**

Division of Pathological Sciences, Institute of Comparative medicine, University of Glasgow, Garscube Campus, Glasgow G61 1QH

HPV persistence is a prerequisite for successful infection and neoplastic progression of mucosal or cutaneous lesions. Persistence is due to several factors: the non-lytic nature of infection, the wholly epithelial virus cycle, and the down-regulation of immune molecules by the viral

(onco)proteins. We have shown that mucosal HPV-16 E5 down-regulates surface MHC I by retaining the complex in the Golgi apparatus, thus potentially contributing to immunoevasion. The N-terminal domain of HPV16-E5 is necessary and sufficient for this re-localization. By mutating each of the four LL motifs in this domain, we show that the second LL motif is responsible for MHC I down-regulation and localization in the Golgi apparatus. Cutaneous HPVs, involved in Non-Melanoma Skin Cancer, do not have an E5 gene, however. To determine if also HPVs can interfere with MHC I, we investigated E6 and E7 of HPV-38. Expression of E6E7 leads to a decrease of MHC I on the cell surface. This down-regulation is associated with a reduction of expression of MHC I heavy chain, of the chaperone TAP and of the STAT-1 downstream effector IRF-1. The down-regulation of these proteins is ultimately due to the inhibition of STAT-1 expression. These effects are primarily the result of E6 expression.

Thus, HPVs can employ different proteins and different mechanisms to circumvent the host immune response.

KSHV vOX2 and its cellular counterpart, CD200, inhibit antigen-specific T cell responses

Rachel Colman, Karen Misstear, Andrew Hislop, Heather Long, Rahim Rezaee, Alan Rickinson & David Blackbourn

CR UK Institute for Cancer Studies, University of Birmingham, Birmingham B15 2TT

Background & objectives The KSHV lytic protein vOX2 shares 36% identity with human cellular CD200. An engineered soluble derivative of vOX2 inhibited neutrophil activity *in vitro* and had anti-inflammatory activity *in vivo*, suggesting the protein inhibits innate immune responses. In the present study we determined whether adaptive immune responses are modulated by native, membrane-bound vOX2 and CD200.

Methods vOX2 and CD200 were expressed on antigen presenting cells (apc) by retroviral transduction and membrane expression was confirmed by flow cytometry and IFA. Apc were loaded with peptides and incubated with appropriately HLA-matched, antigen specific CD4+ or CD8+ T cells. T cell activation was measured by IFN-γ release.

Results In comparison to apc transduced with the negative-control vector, expression of vOX2 and CD200 inhibited CD4 and CD8 T cell responses up to 50%.

Conclusion These data are the first to show CD200 and vOX2 inhibit antigen-specific cytotoxic T-lymphocyte activity. They suggest vOX2 suppresses adaptive immune responses against KSHV lytically infected cells, facilitating virus replication.

CMV and T cell: obsession and deception

A. Hill

Oregon Health and Science University, USA

Abstract not received

The T cell immune response to human cytomegalovirus

Paul Moss

CRUK Institute for Cancer Studies, University of Birmingham

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that infects up to 70% of the population during their lifetime. The virus undergoes latency at a number of sites including salivary gland, renal epithelium and cells of the monocyte-dendritic cell lineage.

HCMV infection triggers a brisk T cell immune response and studies in the last 10 years have revealed that the cellular immune response to HCMV is the largest that has been recorded to any infectious agent. Moreover, the immune response increases with age and the CMV-specific CD8+ T cell response may comprise up to 40% of the CD8+ T cell pool in healthy donors aged over 65 years. The CMV-specific CD4+ T cell response is somewhat smaller but can still represent at least 10% of the total population.

Both populations have similar characteristic features in that they have cytotoxic activity, typically express a common CD57+CD28– phenotype and are comprised of large oligoclonal expansions. The relative importance of the two populations in the control of viral replication remains unclear but evidence is emerging to suggest that CD4+ response may be required for viral clearance from specific sites.

The phenotypic and functional features of the CMV-specific immune response in both healthy donors and patients with immunosuppression will be discussed.

Virus Group Session

Control of virus gene expression

DNA viruses

Repressors of transcription in herpesvirus latency and oncogenicity

Martin J. Allday

Imperial College London

Repression of gene expression and epigenetic silencing by a combination of viral and cellular factors is probably central to the establishment of latency and persistence by all herpesviruses. Here I will consider these processes in the biology of two oncogenic herpesviruses: the alphaherpesvirus Marek's Disease virus and the gammaherpesvirus Epstein-Barr virus (EBV). Both establish latent infection in lymphocytes and induce lymphomas. MDV produces rapidly fatal T-lymphoma in chickens and EBV is associated with several types of B-lymphoma in humans and non-human primates.

MDV encodes a nuclear protein Meq and EBV encodes nuclear antigens EBNAs 3A, 3B and 3C. All these proteins are expressed in latency and can act as transcriptional repressors. Meq, EBNA3A and EBNA3C bind to common cellular factors, including the highly conserved co-repressor CtBP that co-ordinates histone modifications associated with repression and epigenetic gene silencing.

Using bacterial artificial chromosome (BAC) clones, we have introduced deletions and specific mutations into these various viral repressors and used the recombinant MDV and EBV to probe their functions in latency and the transformation of lymphocytes. These results will be discussed.

Post-transcriptional regulation of human papillomavirus type 16 late gene expression in response to epithelial differentiation

S.A. Cumming[1], T. Cheumin[2], S.G. Milligan[1], S. Mole[1] & **S.V. Graham**[1]

[1]Institute of Comparative Medicine, Faculty of Biomedical and Life Sciences, University of Glasgow, Garscube Estate, Glasgow G61 1QH; [2]Dept of Microbiology, Faculty of Science, Silkaporn University, Nakorn Pathom, Thailand 73000

Expression of the L1 and L2 capsid proteins of human papillomavirus type 16 (HPV16) is tightly restricted to cells of the granular layer of stratified epithelia. Late gene expression, including expression of the capsid proteins is regulated at a post-transcriptional level in response to epithelial differentiation. Mapping of HPV16 late mRNAs revealed multiple promoters and polyadenylation sites and extensive alternative splicing. We demonstrate that HPV16 infection upregulates key cellular RNA processing factors in an epithelial differentiation stage-specific manner. The viral transcription factor E2 is involved in transcriptional regulation of some of these alternative splicing factors.

The 3′ untranslated region of all HPV16 late transcripts contain a 79 nt late regulatory element (LRE) that has a dominant role in controlling production of translatable mRNAs. This is a complex *cis*-acting element that represses gene expression in undifferentiated epithelial cells. Transcripts containing the LRE are inherently unstable *in vivo*. HuR, a key protein that can control mRNA stability in the cytoplasm binds the 3′ U-rich portion of the LRE directly. Overexpression and siRNA knock down experiments demonstrate that HuR is a major regulator of capsid protein production.

Regulation of herpesvirus mRNA nuclear export

Adrian Whitehouse

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

In contrast to the majority of mammalian genes, analysis of herpesvirus genomes has highlighted that most lytically expressed viral genes lack introns. Herpesviruses replicate in the nucleus of the host cell, and therefore require their intronless mRNAs to be exported out of the nucleus to allow viral mRNA translation in the cytoplasm. This raises an intriguing question concerning the mechanism by which the herpesvirus intronless mRNAs are exported out of the nucleus in the absence of splicing. To circumvent this problem, and to facilitate viral mRNA export, Kaposi's sarcoma associated herpesvirus encodes the functionally conserved ORF57 protein. Here we describe how an intronless KSHV transcript is made export competent by the formation of a viral ribonucleoprotein particle (vRNP). Specifically, we show that ORF57 binds intronless viral mRNAs and then recruits the complete human TREX complex. The formation of the ORF57-hTREX complex is mediated by a direct interaction between KSHV ORF57 and Aly. Importantly, UAP56 is required to bridge the interaction between Aly and the human THO-complex. In addition, we show that a point mutation in ORF57 which disrupts the ORF57-Aly interaction leads to a failure in hTREX recruitment. Strikingly, this failure to recruit the hTREX complex abolishes ORF57-mediated export of viral intronless mRNAs. We propose a model for herpesvirus mRNA export, whereby ORF57 mimics splicing in order to recruit the cellular mRNA export machinery to intronless viral mRNAs.

Innate immune control of hepatitis B virus by interferon γ and tumor necrosis factor α

R.J. Schneider

New York University School of Medicine, USA

Abstract not received

XBP-1 transactivates the KSHV ORF50 promoter linking plasma cell differentiation to KSHV reactivation from latency

Sam Wilson, Edward Tsao, Lucy Dalton-Griffin, Hongtao Ye, Adrian Whitehouse, Ming Du & **Paul Kellam**

Dept of Infection, University College London, 46 Cleveland Street, London W1T 4JF

Reactivation of lytic replication from viral latency is a defining property of all herpesviruses but the authentic cellular cues that result in the lytic switch are poorly understood. Kaposi's sarcoma-associated herpesvirus (KSHV) can establish latency in human B-cells and this latent infection is associated with primary effusion lymphoma (PEL). Reactivation from latency should ensure virus production in tissues that allow transmission of KSHV, such as the oral mucosa. We hypothesize that terminal differentiation of B-cells into plasma cells is the cue for lytic reactivation of KSHV and that B cell transcription factors mediated this process. Here we show that the B-cell terminal differentiation and Unfolded Protein Response transcription factor XBP-1 induces the KSHV lytic cycle through binding to and transactivating the virus immediate early promoter ORF50/RTA. These data suggest KSHV remains latent until B-cell terminal differentiation into plasma cells, where the virus responds to the presence of new host transcription factors that induce lytic reactivation.

Investigation of the role(s) of the essential tegument protein VP1-2 of HSV

Fernando Abaitua[1], Raquel N. Souto[2] & Peter O'Hare[1]

[1]Marie Curie Research Institute, Oxted, Surrey; [2]Dept of Pathology, University of Cambridge

VP1-2, encoded by the UL36 gene of HSV, is a large structural protein assembled into the tegument compartment of the virion, conserved across the herpesviridae, and essential for virus replication. It has been implicated in early entry, in late assembly, in virion transport and contains an N-terminal ubiquitin hydrolase domain. However a mechanistic understanding of its assembly and function remain incomplete. We have characterized VP1-2 localization by immunofluorescence and electron microscopy and examined a reversible ts VP1-2 mutant. The protein is normally present in cytoplasmic and nuclear compartments, frequently in clusters in both locations. In the ts mutant virus electron microscopy revealed VP1-2 coalescence into aberrant foci with VP16 in the cytosol, while in the nucleus, defective capsid assembly (or release) was observed. Within the nucleus distinct subpopulations of VP1-2 were also observed dependent upon differential association with capsid proteins, or with conjugated ubiquitin. This differential localization may reflect involvement in multiple steps including recruitment to deubiquitinating intermediates, and assembly of capsids competent for exit.

The role of Rev in translation of human immunodeficiency virus type-1 (HIV-1) RNA

Harriet C.T. Groom, Emma C. Anderson, John A. Dangerfield & Andrew M.L. Lever

Dept of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ

Full length HIV-1 viral RNA acts as both messenger RNA, encoding Gag and Gag-Pol polyproteins, and genomic RNA. Translation of this RNA must be tightly controlled to allow sufficient protein synthesis prior to a switch to particle production. We have previously shown that Gag plays a role in this. The aim of this project is to investigate other factors that may contribute to the control of translation of HIV-1 RNA. Rev stimulates nuclear export of unspliced HIV-1 RNAs containing the Rev Response Element (RRE) but may also stimulate translation of these RNAs. Our laboratory has identified an additional Rev binding site in the 5? UTR of HIV-1 RNA. We tested the effect of Rev on translation of HIV-1 RNA to dissect the roles of these Rev binding sites. *In vitro* Rev inhibits translation non-specifically at high concentrations and stimulates at intermediate concentrations. Stimulation appears to be dependent on the presence of the Rev 5? UTR binding site rather than the RRE. Preliminary results in cells supports these data.

RNA viruses

Diversity, co-operation and viral mutual aid

Raul Andino, Marco Vignuzzi, Cecily Burrill & Emily Wendt

Dept of Microbiology and Immunology, University of California, San Francisco, CA 94143-2280, USA

An RNA virus population does not consist of a single genotype; rather, it is an ensemble of related sequences, termed quasispecies. High mutation rates of RNA viral replication create a 'cloud' of potentially beneficial mutations at the population level, which afford the viral quasispecies a greater probability to evolve and adapt to new environments and challenges during infection. Using poliovirus as our model we developed strategies to increase or reduce the mutation rate of the viral polymerase thus changing the levels of genomic diversity in the viral population. In infected animals, reducing or increasing viral diversity leads to loss of neurotropism, and an attenuated pathogenic phenotype. These findings suggest that quasispecies diversity is finely tuned to ensure evolutionary survival of the virus and is a biological determinant for the outcome of poliovirus infection. Our study uncovered a surprising property of the virus population, in which different variants within the quasispecies experiment a co-operative interaction so that some variants allow others to enter the brain. Furthermore, while the viral population with restricted genomic diversity replicate robustly in small intestine, we were unable to isolate viruses from feces of infected mice. This observation suggests that quasispecies diversity plays an important role in virus spread from individual to individual. Interestingly, Sabin vaccine strains are restricted quasispecies, suggesting that population diversity is, at least in part, the basis of attenuation in poliovirus vaccine strains. Thus, altering the structure of the quasispecies result in attenuation of the virus and may provide a novel, rational and general approach for the development of safe live-attenuated virus vaccines.

Association of the influenza virus RNA polymerase with host factors

Ervin Fodor

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE

The RNA-dependent RNA polymerase complex of influenza A virus that consists of three subunits, PB1, PB2, and PA, transcribes and replicates the eight segments of the negative-sense single-stranded RNA genome in the nucleus of infected cells. The aim of our research is to understand how the transcriptional and replicative activities of the viral RNA polymerase are regulated during the viral life cycle and what the role of interacting host factors is in these regulatory processes. We have identified host chaperons and import factors, e.g. Ran Binding Protein 5 (RanBP5), that play a role in the nuclear import and assembly of the viral RNA polymerase complex in infected cells. In the nucleus, the viral RNA polymerase associates with the host transcriptional machinery by interacting with the C-terminal domain (CTD) of the large subunit of cellular RNA polymerase II (Pol II). We hypothesized that this interaction is required for viral mRNA synthesis since viral transcription is dependent on cellular functions, i.e. capping and splicing – activities associated with Pol II transcription. In addition, the viral RNA polymerase, by binding to the CTD of the large subunit of Pol II and cleaving the nascent host transcript, might interfere with Pol II function that could contribute to the virus-induced host shut-off phenomenon.

Functional analysis of calicivirus gene expression

Ian Goodfellow

Dept of Virology, Faculty of Medicine, Imperial College London (Email I.Goodfellow@Imperial.ac.uk)

The *Caliciviridae* family of positive-stranded RNA viruses cause a number of diseases in both man and animals. Members of the norovirus and sapovirus genera are a significant cause of gastroenteritis and have been responsible for the recent outbreaks across the UK. Despite the impact of noroviruses on society, progress on understanding the mechanisms of virus replication and progress towards methods of either preventing or controlling outbreaks has been somewhat limited.

We have previously demonstrated that members of the *Caliciviridae* use a novel mechanism for the initiation of protein synthesis that involves the interaction of initiation factors with the VPg protein covalently linked to the viral RNA. Using both feline calicivirus (FCV) and murine norovirus (MNV) as model systems we have examined the possibility of targeting protein synthesis as an antiviral strategy. During our more recent work we have identified a number of proteins which interact with the calicivirus genome and using a reverse genetics system for MNV, recently developed in our lab, we have begun to characterize the role of RNA structures on norovirus replication. This is the first report of a functional role for RNA structures in norovirus replication.

The role of small non-coding RNAs in flavivirus replication and pathogenicity

Gorben P. Pijlman[1,3], Anneke Funk[1], Natasha Kondratieva[1], Jason Leung[1], Lieke van der Aa[1], Wen Jun Liu[1], Ann C. Palmenberg[2], Roy A. Hall[1] & **Alexander A. Khromykh**[1]

[1]School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, Australia; [2]Institute for Molecular Virology, University of Wisconsin, 1525 Linden Drive, Madison, WI 53706; [3]Laboratory for Virology, Wageningen University, Binnenhaven 11, 6709PD, Wageningen, The Netherlands

West Nile Virus (WNV) belongs to a group of highly pathogenic Flaviviruses which pose a risk to public health. In addition to the full length genomic RNA of ~11 kb, a subgenomic fragment of about 0.5 kb can be detected in infected cells. Here we show that the accumulation of this small RNA (small flavivirus RNA, sfRNA) is a unique feature of the genus Flavivirus and that it is derived from the 3′ untranslated region (UTR) of genomic RNA. Surprisingly, its production was independent of viral RNA replication and did not require viral proteins or the 5′ UTR. We have identified the cellular exoribonuclease XRN1 as the enzyme responsible for generation of sfRNA via 5′–3′ degradation of genomic RNA. RNA structure prediction and extensive mutagenesis analyses revealed that rigid RNA structures within 3′ UTR are likely to be responsible for the stalling of XRN1 and generation of sfRNAs of different sizes. Mutant WNV viruses producing sfRNA of less than 0.5 kb or not producing sfRNA at all showed a decrease in virus replication efficiency and loss of virus-induced cytopathicity in cells, as well as loss of virulence in mice. Thus, the generation of 0.5 kb sfRNA by cellular exoribonuclease XRN1 is essential for flavivirus replication and pathogenicity.

The picornavirus avian encephalomyelitis virus possesses a hepatitis C virus-like internal ribosome entry site (IRES) element

Mehran Bakhshesh, Elisabetta Groppelli, Margaret M. Willcocks, Elizabeth Royall, Graham J. Belsham & **Lisa O. Roberts**

Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH

Translation initiation on picornavirus RNA is directed by an internal ribosome entry site (IRES) element within the 5′ untranslated region (UTR) of the mRNA. Avian encephalomyelitis virus (AEV) is a picornavirus that causes disease in poultry worldwide; it is currently classified within the hepatovirus genus since its proteins are most closely related to those of hepatitis A virus (HAV). We have demonstrated that the 494 nucleotide long 5′ UTR of the AEV genome contains an IRES element but, unlike the HAV IRES, the AEV IRES is relatively short and functions in the presence of cleaved eIF4G, it is also resistant to an inhibitor of eIF4A. These properties are reminiscent of the recently discovered class of IRES elements within certain other picornaviruses, those with significant similarity to the hepatitis C virus (HCV) IRES in sequence, function and predicted secondary structure. Mutational analysis of the predicted pseudoknot structure at the 3′ end of the AEV IRES lends support to the secondary structure we present. This suggests that picornaviruses, from different genera, contain an IRES element that is closely related to the HCV IRES; classification of AEV within the hepatovirus genus may need to be reassessed in the light of these findings.

HCV NS5A inhibits cell proliferation via a block to EGF-stimulated Ras-Erk signalling

Jamel Mankouri & Mark Harris

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

HCV NS5A inhibits EGF-stimulated activation of the Ras-Erk pathway. We have previously demonstrated that NS5A mediates this effect via a novel mechanism whereby internalized EGFR is diverted away from the late endosomal signalling platform and protected from lysosomal degradation. The cellular consequences of this inhibition are however, not understood.

Activation of the Erk pathway by EGF is required for progression through a restriction point in the late G_1 phase. We show that in cells harbouring the HCV subgenomic replicon, cellular proliferation is inhibited in a similar fashion to treatment of naïve cells with a Ras-Erk inhibitor. Consistent with this inhibition the progression of cells from the G_1 to S phase is decreased in both replicon and inhibitor treated cells.

Overall, we conclude that NS5A mediates an anti-proliferative action through inhibition of EGFR Ras-Erk activation. This may explain the high proportion of G_1 arrested cells observed in chronically infected HCV liver.

Prize Lectures

Marjory Stephenson Prize Lecture

Studies with an oncogenic virus: how to survive a lifetime with EBV

A.B. Rickinson

University of Birmingham

Alan Rickinson did his PhD in Radiotherapeutics in Cambridge and, after spending three years as a post-doctoral fellow in Sydney, moved to Bristol in 1972 to join Tony Epstein's group working on Epstein–Barr virus. There he began to study EBV's interaction with the B lymphoid system, the role of the virus in B cell lymphomagenesis, and later (in collaboration with Denis Moss in Brisbane) the host's cellular immune response to virus infection. Since moving to become Head of the CRUK Institute for Cancer Studies in Birmingham in 1983, he has continued these themes, working in the area where virology, immunology and oncology meet, and is currently developing and testing therapeutic vaccines against EBV-positive malignancies. He was elected a Fellow of the Royal Society in 1997.

Fleming Prize Lecture

Challenges in emerging infectious diseases – dengue in Vietnam

Cameron Simmons

Oxford University Clinical Research Unit, Hospital for Tropical Diseases, 190 Ben Ham Tu, District 5, HCMC, Vietnam

Cameron Simmons is a Reader in Tropical Medicine at Oxford University and based at the Hospital for Tropical Diseases in Ho Chi Minh City, Viet Nam. He completed his PhD at the University of Melbourne (Australia) and post-doctoral studies at Imperial College (UK). Since arriving in Vietnam in 2001, Cameron's research focus has been in understanding the pathogenesis of important diseases in the region, beginning with tuberculous meningitis and more recently, H5N1 influenza and dengue. These studies have been deliberately holistic and encompass investigations of the pathogen, immune response and host genetics. It is hoped these investigations will provide the foundation for improved patient management and new clinical interventions in these diseases. Cameron's contributions to dengue research have seen him appointed to various WHO advisory committees.

Dengue has emerged as an enormous public health problem in many parts of the tropical developing world. The clinical phenotype of dengue likely reflects a complex interaction between the host and virus. In the host, sub-neutralizing levels of dengue virus reactive IgG (acquired passively or by previous infection) are postulated to be a critical risk factor for severe dengue. Accordingly, we found a strong temporal association between the Fc-dependent, dengue virus infection-enhancing activity of neat plasma from healthy Vietnamese infants and the age-related epidemiology of severe dengue in this population. This is the first evidence linking the antibody-dependent enhancement phenomenon to disease epidemiology. Identifying viral prognostic markers of severe disease is clinically important. We found early plasma NS1 concentrations to be associated with progression to dengue shock syndrome, opening a pathway to developing rapid prognostic tests for the most common severe manifestation of dengue in children. Few determinants of dengue virus virulence have been identified and selective pressures driving evolution are not well understood. By whole-genome sequencing, we have monitored Dengue virus evolution in southern Vietnam for the last 6 years to reveal frequent serotypic and clade replacement events that co-incide with changes in disease incidence. The implications of these data for models of dengue pathogenesis, vaccines and diagnostic tests will be discussed.

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

Posters

Cells & Cell Surfaces Group

CCS 01 Characterizing the autotransporter proteins of Pseudomonas aeruginosa

O. Darch, S. Heeb, S. Pommier, M. Cámara & K.R. Hardie

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

Pseudomonas aeruginosa is an opportunistic human pathogen, and possesses a large battery of virulence determinants which have contributed to its ability to become recognized as a 'superbug' causing havoc in our hospitals. Autotransporters are the largest family of secreted proteins in Gram-negative bacteria, and those characterized are virulence factors. The *P. aeruginosa* genome encodes at least three proteins exhibiting the characteristic three domain structure of autotransporters which allows secretion of the functional passenger domain to the extracellular environment via the inner membrane Sec machinery and an outer membrane β-barrel. Little is known about the functions of these three proteins or their role in pathogenicity. Here, we describe the consequences of mutagenesis of these three autotransporters upon *P. aeruginosa* alongside studies to determine their function and impact upon virulence.

CCS 02 Localization of fluorescently labelled EspC in *Escherichia coli*

Stéphanie Pommier, Mahmoud Ashawesh & Kim R. Hardie

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

Autotransporter proteins are major virulence determinants in many Gram-negative pathogens. They possess a cleavable N-terminal signal peptide conferring translocation across the inner membrane and a C-terminal β-barrel domain required for transit through the outer membrane. These two features surround the passenger domain which constitutes the extracellular mature protein, which is often released from the cell by proteolytic cleavage.

Different indirect techniques have shown that several autotransporters such as IcsA and SepA of *Shigella flexneri*, AIDA-I of diffusely adherent *Escherichia coli* and BrkA of *Bordetella pertussis*, are localized in the cytoplasm to the bacterial pole. This study aims to determine the localization of EspC, an autotransporter originating from Enteropathogenic *E. coli*, at the cell surface by the insertion of a red fluorescent protein in the passenger domain.

Fluorescent microscopy has revealed that this hybrid protein is successfully transported to the cell surface, and distributed in foci over the entire cell including the poles, potentially indicating a different secretion route.

CCS 03 Studying the roles of cyclic-di-GMP and cytoskeletal and sepatation proteins in the predatory life cycle of *Bdellovibrio bacteriovorus*

Andrew Fenton, Laura Hobley, Rashidah Ahmad, Rowena Fung, Carey Lambert & Liz Sockett

Institute of Genetics, School of Biology, University of Nottingham, Medical School, QMC Nottingham NG7 2UH

Bdellovibrio are predatory bacteria which invade and digest Gram-negative bacteria, penetrating and squeezing through the prey outer membrane into the periplasm of their prey where they grow forming a long filamentous cell structure. Resolution of this structure with multiple cell division septa gives rise to multiple progeny. It is hypothesized that this novel form of cell division allows the growing *Bdellovibrio* cell to maximize its use of available nutrients within a prey cell and to co-ordinate its hydrolytic digestion and uptake of prey cytoplasmic contents. Accurate positioning of the septum machinery will be critical to the cell in order to divide one filamentous cell into multiple progeny. The *Bdellovibrio* genome contains an interesting array of cell division genes to achieve this and we present transcriptional data on their expression during the predatory cycle. In order to maintain this filamentous cell structure and also to gain entry to the prey *Bdellovibrio* need a highly adaptive cell cytoskeleton and may also need to rearrange it to squeeze through the smallest possible pore in the preys membrane. *Bdellovibrio* contain multiple *mreB* homologues, one of these homologues is expressed only in the growth phase and there is evidence to suggest that this could be linked to the establishment or accurate resolution of the filamentous cell structure.

The *Bdellovibrio* genome contains multiple genes encoding proteins with the *pilZ* motif sensitive to c-di-GMP, a bacterial metabolite known to be involved in sensory and developmental responses to bacterial cell starvation. Could di-c-GMP, formed in the *Bdellovibrio* when the cell senses resources in the prey are depleted, be one of the molecular triggers for cell division? Could prey c-di-GMP levels also be sensed by *Bdellovibrio*? We have begun to investigate these questions by directed mutagenesis and we present evidence that the predatory efficiency is changed in mutant prey with altered c-diGMP levels and describe the phenotype of mutant *Bdellovibrio* with elevated c-diGMP levels.

CCS 04 Fold and structural properties of the essential Omp85-family POTRA domains

Saeeda Bobat[1], Timothy J. Knowles[2], Felician Dancea[2], Mark Jeeves[2], Tracy Palmer[3], Michael Overduin[2] & Ian R. Henderson[1]

[1]Division of Immunity and Infection; [2]CRUK Institute for Cancer Studies, School of Medicine, University of Birmingham, Edgbaston, Birmingham B15 2TT; [3]School of Life Sciences, University of Dundee, Dow Street, Dunde DD1 5EH

The outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts contain integral β-barrel outer-membrane proteins (OMPs). Folding and insertion of OMPs into membranes utilizes a multiprotein complex employing Omp85 family proteins, represented by YaeT in *Escherichia coli*. Members of the Omp85 family contain a characteristic set of N-terminal repeats, known as POTRA domains. The precise function of the POTRA domains is unclear but is important in recruiting nascent OMPs from the periplasm and directing them towards the Omp85 complex. The NMR structure of tandem POTRA domains of YaeT has been determined, revealing the POTRA domain fold. Each POTRA domain comprises a three-stranded β-sheet overlaid with a pair of antiparrallel helices in a β-α-α-β-β order. Titrations were performed with PhoE, and weak binding was observed on the β-sheets within the tandem POTRA domains, which could provide an ideal folding platform.

CCS 05 The unusual extended signal peptide region of a type V-secreted protein is not essential for secretion or function

M.G. Luna, Anthony Scott-Tucker, Mickaël Desvaux, Sue M. Turner & Ian R. Henderson

Division of Infection and Immunity, The Institute for Biomedical Research, University of Birmingham, Edgbaston, Birmingham B15 2TT

The Pet autotransporter is synthesized as a single polypeptide composed of three main domains: an N-terminal signal peptide; a central passenger domain, the functional portion; and a C-terminal translocation unit (TU), which forms a β-barrel pore within the OM, and permits translocation of the passenger domain to the cell surface. Pet contains an extended signal peptide region (ESPR), which is conserved, an indication of an important biological function. However, removal of the ESPR does not abolish Pet secretion. In order to assess Pet translocation across the inner membrane in the absence of the ESPR, a series of inducible-expression constructs were engineered in which the signal sequence of Pet was replaced for alternative sequences possessing different targeting mechanisms. Each of the heterologous constructs was secreted and isolated from the supernatant fraction, when applied to the HEp-2 cell monolayers, presented cytotoxic effect, which is indicative of the presence of functional Pet toxin.

CCS 06 Heterologous assembly of outer membrane cytochromes on the surface of *Escherichia coli*

James Donald[1,3], Matthew Hicks[2], David Richardson[2] & Tracy Palmer[3]

[1]Dept of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH; [2]School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ; [3]College of Life Sciences, University of Dundee, Dundee DD1 5EH

The Gram-negative marine organism *Shewanella oneidensis* assembles a number of lipid-anchored multihaem cytochromes on its surface, allowing it to grow using a range of different insoluble compounds as terminal electron acceptors. This process requires the removal of electrons from insoluble compounds at the cell surface, their passage across the outer membrane and the periplasm to reach the electron acceptor in the inner membrane. Study of the proteins involved in the electron transport chain required for this process, is hampered by the large number of *c*-type cytochromes produced by the organism. In order to dissect the proteins involved in one of these pathways and to understand how they are assembled, I am expressing components of one such electron transport system heterologously in *E. coli*.

I have heterologously expressed the *S. oneidensis omcA* gene, which encodes an outer membrane decahaem cytochrome, able to reduce insoluble iron compounds, in different *E. coli* host strains. All *E. coli* strains expressing *omcA* showed a characteristic u.v/visible spectra consistent with the production of a *c*-type cytochrome. Moreover, incubation with soluble Fe (III) compounds (that are outer membrane permeable) resulted in oxidation of OmcA, indicating that it is able to transfer electrons to Fe (III). These results demonstrate that OmcA is actively assembled in the heterologous host.

In order to determine whether OmcA is found on the cell surface in the heterologous host, I carried out cell fractionation and topological studies. Analysis of OmcA produced in the *E. coli* B strain, BL21, showed that at least some of the OmcA is found on the outer face of the outer membrane, indicating that this strain of *E. coli* is able to correctly localize the protein to the cell surface. By contrast however, when expressed in the *E. coli* K12 strain MC4100, the protein is not exposed at the cell surface but is associated with the inner surface of the outer membrane. I propose that in BL21, OmcA is translocated across the *E. coli* outer membrane by the Type II secretion machinery. Previous reports have shown that the Type II system is not expressed in MC4100 (Francetic & Pugsley. (1996) *J. Bacteriol.* 178:3544–3549), which would account for the mislocalization of OmcA in this strain.

Production of OmcA in both *E. coli* strains, leads to the increased release of outer membrane vesicles (OMVs) by the cells. This is may occur as a result of cell envelope stress caused by the increased amount of lipoprotein being inserted into the cell membranes, or as a result of additional protein being transported into the periplasm.

CCS 07 Serine protease autotransporters of *Citrobacter rodentium*

Robert A. Kingsley, Song Choon Lee, Stephania Bambini & Gordon Dougan

Sanger Institute, Cambridge CB10 1SA

Serine protease autotransporters of the enterobacteriaceae (SPATE) have been described in several pathovars of *Escherichia coli*, although their role in pathogenesis remains largely unknown. *Citrobacter rodentium* is a close relative of *E. coli* and a member of a group of pathogens, including the human restricted pathogens EPEC and EHEC, that cause colitis following colonization of the intestine associated with the formation of attaching and effacing (A/E) lesions. *C. rodentium* infections of mice are an important surrogate model of these human infections. We have identified the full repertoire of SPATE's in *C. rodentium* by in silico analysis of the complete genome nucleotide sequence. Three SPATE's designated *creA*, *creB* and *creD* exhibited significant identity to the *espC* nucleotide sequence, a SPATE encoded by EPEC. Each of these predicted proteins had features typical of autotransport proteins and contained a serine protease motif in the N-terminal passenger domain. A specific immune response to each of the putative SPATE's was detected in mouse serum four weeks following oral inoculation suggesting that these proteins are expressed during infection and immunogenic. Mutations in *creA* and *creB* had no detectable effect on colonization or persistence but exhibited marked depression of hyperplasia and crypt depth.

CCS 08 SepL binds Tir to co-ordinate type III secretion from *Escherichia coli* O157:H7

D. Wang, A.J. Roe, J.R. Emmerson & D.L. Gally

Centre for Infectious Diseases, University of Edinburgh, Edinburgh EH16 4SB

Type III secretion (T3S) from enteric bacteria is a co-ordinated process with a hierarchy of secreted proteins. In enteropathogenic and enterohaemorrhagic *Escherichia coli*, SepL and SepD are essential for translocator but not effector export but how they function to control this differential secretion is not known. This study has focused on the different activities of SepL. Analyses with C-terminus SepL truncates demonstrated that the different functions associated with SepL can be separated. In particular, SepL with a deletion of 11 amino acids from the C-terminus was able to localize to the bacterial membrane, export translocon proteins but not regulate Tir secretion. Tir was shown to bind directly to full length SepL but not to this truncated protein. By synchronizing induction of T3S, it was demonstrated that full length SepL delays release of Tir whereas this secretion hierarchy was lost when the 11 amino acid C-terminal truncate of SepL was used. Finally, it is proposed that a competitive interaction between EscD/Tir/SepL may release sequestered Tir for export following translocon assembly.

CCS 09 Galacto-oligosaccharide, an effective pre-biotic against *Salmonella* Typhimurium infection

Laura Searle[1,2], Angus Best[1], Martin J. Woodward[1], Bill A. Cooley[1], George Tzortzis[2], Bob Rastall[2], Glenn Gibson[2], Roberto M. La Ragione[1] & James Collins[1]

[1]Dept of Food and Environmental Safety, Veterinary Laboratories Agency, Weybridge, Addlestone, Surrey KT15 3NB; [2]Dept of Food Biosciences, University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP

Prebiotics have been advocated as genuine alternative therapies to antibiotics. One such prebiotic is Galacto-oligosaccharide (GOS). Oligosaccharides are selectively fermented by a sub population of the normal microbiota in the intestine and can confer health benefits to the host.

Here we utilized *in vitro* and *in vivo* techniques to determine the therapeutic potential of a novel GOS, produced using galactosidase from *Bifidobacterium bifidum*, against *Salmonella* Typhimurium DT104. *In*

vitro tissue culture studies using HT29 and 3D IPEC-J2 cells, revealed that GOS may mitigate against *S.* Typhimurium adherence and invasion, the latter by significantly impeding membrane ruffling formation.

A BalbC mouse model was employed to evaluate the efficacy of GOS against *Salmonella* Typhimurium colonization. In these studies the administration of GOS prior to the challenge with *S.* Typhimurium reduced the severity of clinical Salmonellosis and the number of bacteria colonizing the liver, spleen, and intestinal tissues.

CCS 10 Crystal structure of a bacterial signal peptide peptidase

Apollos C. Kim, **David C. Oliver** & Mark Paetzel

Dept of Molecular Biology and Biochemistry, Simon Fraser University, South Science Building, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6

Escherichia coli signal peptide peptidase ($SppA_{EC}$), also known as protease IV, is a 618 amino acid periplasmic protein that is anchored to the cytoplasmic membrane by an N-terminal transmembrane domain. As its name implies, SppA has been shown to degrade signal peptides left behind after Sec-dependent protein export across the inner membrane. Here we report the first structure of the periplasmic region of $SppA_{EC}$ ($SppA_{EC}\Delta 2\text{-}46$). $SppA_{EC}\Delta 2\text{-}46$ forms a tetrameric assembly with a novel bowl shaped architecture which has a dramatically hydrophobic interior and contains four separate active sites that utilize a Ser/Lys catalytic dyad mechanism. The structure reveals a similarity in the protein fold between the periplasmic Ser/Lys protease SppA and the cytoplasmic Ser/His/Asp protease ClpP. We propose that SppA may, in addition to its role in signal peptide hydrolysis, have a role in the quality assurance of periplasmic and membrane bound proteins, similar to the role that ClpP plays for cytoplasmic proteins.

CCS 11 Auto-catalytic cleavage of the AIDA-I autotransporter of *Escherichia coli* depends on an aspartate residue of the junction region

M.-E. Charbonneau, J. Janvore & M. Mourez

Canada Research Chair on Bacterial Animal Diseases, Université de Montréal, Faculté de Médecine Vétérinaire, St-Hyacinthe, 3200 Sicotte, St-Hyacinthe, Québec, J2S 7C6, Canada

The cleavage of the autotransporter adhesin involved in diffuse adherence (AIDA-I) of *Escherichia coli* yields a membrane-embedded fragment, AIDAc, and an extracellular fragment, the mature AIDA-I adhesin. The latter remains noncovalently associated with AIDAc, and can be released by heat treatment. The mechanism of cleavage of AIDA-I is thought tobe autocatalytic but it is not understood. In this study, we observed that refolding of purified uncleaved proprotein from inclusion bodies result in a partial folding and an extensive degradation, suggesting that the proprotein indeed possesses an active proteolytic catalytic site. Furthermore, deletions and site-directed point mutations we engineered in mature AIDA-I and AIDAc show that a region between Ala_{667} and Thr_{953} is sufficient for cleavage and that Asp_{878} is essential for cleavage. The acidic side chain of Asp_{878} appears involved in the proteolytic reaction, since modification into a Glu permitted processing whereas the mutations into Ala or Asn completely abolished cleavage. The uncleaved Asp mutants are still as functional as the wild-type protein in adhesion, auto-aggregation and biofilm formation assays, suggesting that their overall structure is not perturbed and, therefore, that auto-catalytical cleavage of AIDA-I could be directly mediated by Asp_{878}.

CCS 12 The type I signal peptidase as a tool for screening of potential anti-staphylococcal drug candidates

Smitha Rao C.V., Katrijn Bockstael, Jozef Anné & **Nick Geukens**

Laboratory of Bacteriology, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

Staphylococcus aureus causes a wide array of life threatening infections. It is also the leading overall cause of nosocomial infections. *S. aureus* has two signal peptidases (which functions to remove signal peptides from secretory proteins after their translocation across the membrane) SpsA and SpsB, of which SpsA is inactive. SpsB has been shown to be essential for the viability of the cell and is an attractive target for development of anti-staphylococcal drugs due to its unique properties. With the objective of screening compounds that can inhibit the activity of SpsB (and therefore might have a potential to be developed into drugs), we have cloned, expressed and purified this enzyme in *E. coli* and used it to set up an *in vitro* assay based on an internally quenched fluorescent peptide (FRET). The assay has been validated and scaled up for screening of peptide and chemical libraries. This approach resulted in the identification of some interesting compounds, some of which showed strong antibacterial activity.

CCS 13 Characterization of the *Streptomyces lividans* PspA response

Kristof Vrancken, Lieve Van Mellaert & Jozef Anné

Laboratory of Bacteriology, Rega Institute KULeuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

The phage shock protein (Psp) response is induced by extracytoplasmic stress that may reduce the energy status of the cell. In *E. coli,* the Psp system consists of the *pspABCDE* operon and of *pspF* and *pspG*. The entire system is highly conserved among a large number of Gram-negative bacteria. However, many bacterial genomes, including that of *Streptomyces lividans*, only contain a *pspA* homologue but no other *psp* homologues. This conservation indicates that PspA alone might play an important role in these bacteria.

In this report we show that *S. lividans pspA* encodes for a 28 kDa protein that is present in both the cytoplasmic and the membrane fraction. We demonstrated that the *pspA* gene is strongly induced under stress conditions that attack the membrane integrity, and that it is essential for growth and survival under most of these conditions.

The data reported here clearly show the importance of PspA in *S. lividans* under stress conditions, despite the absence of homologues of the other *psp* genes. These results therefore suggest that in many bacteria PspA on its own is more important than previously thought.

CCS 14 Members of the *Bartonella quintana* (BQ) vomp family of four, trimeric autotransporter adhesins (TAA) are differentially expressed, required for virulence *in vivo*, and each confers distinct binding specificities

Peng Zhang, Joanna MacKichan & **Jane E. Koehler**

University of California-San Francisco, San Francisco, CA 94143-0654, USA

Background BQ is a Gram-negative human pathogen causing relapsing/prolonged bloodstream infection. BQ occupies two disparate niches, requiring different binding specificities for: the gastrointestinal epithelium of the body louse (28°C); and erythrocytes and endothelial cells in the mammalian bloodstream (37°C). BQ has four highly conserved, tandemly arranged TAA: the variably expressed outer membrane proteins (Vomp). We hypothesized that the Vomp are critical virulence determinants necessary for infection *in vivo*, with expression regulated by environmental cues.

Results By quantitative RT-PCR, 2-D-SDS-PAGE, and immunoblotting, we found that expression of each *vomp* is differentially regulated at the replication level by *vomp* deletion, and at the transcriptional level by temperature. We also developed the first strategy for in-frame deletion in BQ, finding the *vomp* null mutant is unable to infect an animal that is always infected by wildtype BQ.

Conclusion The Vomp are unusual TAA that permit adaptation to different niches by differential expression of individual *vomp*; deletion of all four *vomp* renders BQ avirulent *in vivo*.

CCS 15 The role of toxin-antitoxin systems in *Mycobacterium tuberculosis* persistence

Holly R. Ramage & Jeffery S. Cox

Dept of Microbiology and Immunology, University of California, San Francisco, USA

Mycobacterium tuberculosis is a bacterial pathogen that persists in host tissues by establishing a latent, asymptomatic infection. In other bacteria, expression of toxin-antitoxin (TA) systems is involved in establishing similar persistent states. Thus, we hypothesize that TA systems of *M. tuberculosis* allow the pathogen to respond to environmental stress and enter into a nonreplicating state following infection. To begin to test this idea, we have used bioinformatic tools to identify 87 putative TA operons in *M. tuberculosis*. We have expressed 80 individual toxin genes in *M. smegmatis*, a fast-growing, nonpathogenic relative of *M. tuberculosis*. Expression of 35 of these genes resulted in toxicity, 28 of which were rescued upon co-expression of the putative antitoxin. Several of these toxins cleave RNA *in vitro* and inhibit translation *in vivo*. We are continuing studies to examine the role of TA systems in establishing the persistent state of *M. tuberculosis* both *in vivo* and *in vitro* dormancy models.

CCS 16 Structure/function analyses of the outer membrane protein FhaC from *Bordetella pertussis*

A.S. Delattre[1], B. Clantin[2], N. Saint[3], C. Locht[1], V. Villeret[2] & F. Jacob-Dubuisson[1]

[1]INSERM U629, Institut Pasteur de Lille, IFR142, Lille, France; [2]UMR8161 CNRS, Universités de Lille 1 et 2, Institut Pasteur de Lille, IFR142, Lille, France; [3]INSERM U554, UMR5048 CNRS, Universités de Montpellier 1 et 2, Montpellier, France

The Gram-negative bacterium *Bordetella pertussis* is the causative agent of whooping cough. The filamentous hemagglutinin (FHA) of *B. pertussis* is secreted by the two-partner secretion pathway. The specific transporter of FHA across the outer membrane is FhaC of the TpsB/YaeT superfamily. To decipher the structure/function relationship of FhaC, we have solved its structure by X-ray crystallography. FhaC is composed of a C-terminal 16-stranded β barrel and two N-terminal periplasmic POTRA domains involved in FHA recognition. To characterize the interactions between the two proteins, a number of surface-exposed residues of the POTRA helices were replaced by site-directed mutagenesis. While some of these substitutions perturb FHA-FhaC interactions, they do not significantly affect FHA secretion, indicating that the POTRA helices are minor interaction determinants. These interactions are being mapped by using various recombinant portions of the two proteins in co-precipitation assays.

CCS 17 Cyanobacterial extract enhances cell division rate in excised *Cucumis sativus* L. cotyledons

Anwar Hussain & Shahida Hasnain

Dept of Microbiology and Molecular Genetics University of the Punjab, Lahore, Pakistan (Email anwarhpu@yahoo.com)

The present work was intended to isolate and screen cyanobacterial strains for cytokinin production. Ten cyanobacterial strains from Rice fields in different locations were screened for cytokinin production by developing a simple and rapid screening technique modified from Fletcher and McCuliagh (1971). Chlorophyll content was enhanced up to 65.41% by extract from *Synecosystis sp*. and upto 85.71% by water biomass extract from *Oscillatoria sp*. Thin Layer Chromatography (TLC) and HPLC analysis of the extract from cyanobacterial culture confirmed the presence of at least two cytokinin species. Maximum free trans-zeatin (48ng/ml) was detected in the two months cultural filtrate of *Synecosystis sp*. Enhanced cell division rate was seen in dark grown excised cotyledons in the presence of KCl solution alone, supplemented with cyanobacterial extract or trans-zeatin. Significant inhibition of root elongation and enhanced root hair formation was observed in *Cucumis sativus* L. grown in the presence of the selected strain as compared to control. Data from present work confirmed that Cyanobacteria improved physiological responses in plants.

Posters

Clinical Microbiology Group

CM 01 Interaction between *Campylobacter jejuni* and commensal micro-organisms at the mucin interface

Sarah Cabry, Caroline Dalton & Karen Stanley

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

Once *Campylobacter jejuni* has been ingested and passed to the small intestine, a key stage of the infection process occurs at the interface between the lumen of the gastrointestinal (GI) tract and mucin layer. The aim of our work is to investigate the interactions between *C. jejuni*, commensal micro-organisms and GI tract mucin. We are using the mucin overproducing cell line HT29-MTX to investigate the effect of mucin on growth of *Campylobacter jejuni*. Results indicate that *C. jejuni* NCTC 11168 increases mucin production by HT29-MTX. HT29-MTX fed with 10% spent culture supernatant from *C. jejuni* NCTC11168 significantly increased mucin production compared to controls. *C. jejuni* NCTC 11168 grew significantly faster in the presence of a mature mucin layer from HT29-MTX than in culture medium alone. We are also investigating the effect of commensal GI tract organisms *Lactobacillus plantarum* and *Enterococcus* sp. on HT29-MTX and, using western blotting to examine changes in MUC proteins, whether commensal microflora modify mucin production in such a way to affect *C. jejuni* growth or infection.

CM 02 Molecular methods to study infection control in the intensive care unit environment

G.L. Kay, T.J. Smith & K.N. Stanley

Biomedical Research Centre (BMRC), Sheffield Hallam University, Sheffield S1 1WB

Hospital-acquired infections (HAIs) are an increasing clinical problem. The majority of organisms that cause such infections are both opportunistic and antibiotic-resistant. Whilst antibiotic resistance of clinical isolates has been extensively studied, relatively little work has been done on the role of the hospital environment as a reservoir of micro-organisms and resistance determinants. This research aims to establish a robust sampling regime and down-stream sample processing for molecular and cultural analysis of environmental microbiology in an intensive care unit (ICU). The laboratory was used as a model environment and sampled (benches, computers, floors, sinks, taps and chairs) by swabbing and contact plates. Various DNA extraction methods have been investigated and a method that uses lysozyme for cell-wall lysis has been found to work well for PCR detection of a range of genes: 16S rRNA genes and the *tet*(w), bla_{TEM}, bla_{SHV}, bla_{CTX-M} and *mec*A (MRSA) resistance determinants. Establishment of these methods will facilitate molecular analysis of micro-organisms and resistance determinants in the ICU environment.

CM 03 Use of antibiotics to eradicate bacterial pathogens colonizing the colonic mucosa in ulcerative colitis patients

Tatsuya Nakayama, Katie Blackett, Helen Steed, Sandra Macfarlane, John H. Cummings & George T. Macfarlane

University of Dundee, Division of Pathology and Neuroscience, Ninewells Hospital Medical School, Dundee DD1 9SY

Ulcerative colitis is (UC) is an idiopathic inflammatory bowel disease of unknown aetiology. Commensal bacteria colonizing the colonic mucosa are known to play a key role in its initiation and/or maintenance. The failure of antibiotics in the treatment of UC may be attributed to the protection of organisms in mucosal biofilms. This study aimed to identify putative pathogens colonizing mucosal surfaces in UC patients, and to determine their antibiotic sensitivities, with a view towards developing antibiotic therapeutic strategies for their eradication. Combinations of antibiotic pairs were given to 20 UC patients enrolled in a controlled trial for a period of one month. Clinical status was scored and rectal biopsies were taken for analysis of biofilm structure, antibiotic sensitivity, and the spread of antibiotic resistance genes. Results showed Bacteroides, Staphylococcus and Streptococcus were major strains present in original biopsies. After one month antibiotic treatment 73% (11) of patients had a reduced clinical score (SCCAI), with removal of putative pathogenic bacteria and low antibiotic resistance. In 13% of patients (2) SCCAI increased, with *E. coli*, Fusobacterium, Peptostreptococci, and Clostridium species showing high antibiotic resistance against specifically prescribed antibiotics. In conclusion antibiotic induced manipulation of UC biofilms may improve patient quality of life.

CM 04 Microbial colonization of the oesophageal mucosa

K.L. Blackett, G.T. Macfarlane, J.F. Dillon & S. Macfarlane

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

The human gut is colonized extensively by dense communities of micro-organisms. The stomach and small bowel are known to contain relatively few organisms in health, and most studies have focused on the mouth and large intestine. As a consequence, little is known about the oesophagus. The aim of this study was to characterize microbial communities colonizing mucosal surfaces in the upper gastrointestinal tract, and to compare these microbiotas to planktonic communities in oesophageal aspirates by culturing and real-time PCR, together with FISH and confocal laser scanning microscopy. Biopsies and aspirates were taken from healthy subjects at endoscopy. Bacterial isolates were identified by 16S rRNA gene sequencing and fatty acid methyl ester analyses. Results showed that mucosal surfaces were more heavily colonized than oesophageal aspirates and that there were marked differences in species composition. Bifidobacteria, actinomycetes, streptococci, staphylococci, rothia and veillonellas predominated in mucosal biofilms (ca $\log_{10}$ 4.5 cm^2), whereas lactobacilli, streptococci (ca $\log_{10}$ 2.8 ml^{-1}) and yeasts such as *Candida* and *Saccharomyces* (ca $\log_{10}$ 4.3 ml^{-1}) were the principal species identified in aspirates. Microscopy showed that bacteria in mucosal biofilms often occurred in microcolonies. These data show that in health, the oesophageal mucosa is colonized by unique biofilm populations.

CM 05 Investigation into the role of bacteria–platelet interactions in the pathogenesis of *Staphylococcus aureus* infective endocarditis

S. Daga[1,2], J.G.S. Callaghan[1,2], J.G. Shepherd[1,2], D.E. Newby[2] & J.R. Fitzgerald[1]

[1]Centre for Infectious Diseases, Chancellor's Building, University of Edinburgh, Edinburgh EH16 4SB; [2]Centre for Cardiovascular Sciences, Chancellor's Building, University of Edinburgh, Edinburgh EH16 4SB

Bacteria-induced platelet activation leading to aggregation may play an important role in the pathogenesis of infective endocarditis (IE). Although the *Staphylococcus aureus* surface proteins (MSCRAMMs) and their interaction with platelet receptors leading to activation has been well characterized *in vitro*, the relevance to pathogenesis is not clear.

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

We determined the frequency of platelet FcγRIIa H131R, GPIIb/IIIa PlA and the GPIb Kozak, VNTR and HPA-2 polymorphisms among 21 IE patients and 168 healthy volunteers and investigated the contribution of these polymorphisms to *S. aureus*-induced platelet aggregation. There was no significant difference in the prevalence of polymorphisms in patients with IE versus healthy volunteers. However, platelets with the GPIb Kozak T/C genotype had an increased lag time to *S. aureus*-induced platelet aggregation (p=0.038) suggesting an influence on bacteria-platelet interactions.

CM 06 Antibiotic resistance and persister cells in *Staphylococcus epidermidis* infections

Mark Prince, Olivia L. Champion & Rick W. Titball

University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD

S. epidermidis is a common cause of nosocomial infections. It is an opportunistic pathogen which has a tendency to form biofilms that exhibit resistance to standard antibiotic therapy. It has been proposed that this resistance may be due to the presence of persister cells which have the ability to switch to a dormant state when under stress and thereby tolerate antibiotic treatment.

Growth curves of *S. epidermidis* were measured to determine early stationary phase at which persister cells are most prevalent in bacterial populations. The minimum inhibitory concentration (MIC) of vancomycin for planktonic *S. epidermidis* was determined using a gradient plate method (2 µg/ml). Persister cells were selected for by treating broth cultures of *S. epidermidis* with concentrations of vancomycin >5× than the MIC. In addition, the genome of *S. epidermidis* was investigated for putative persister cell genes *in silico*.

Research into persister cells in microbial populations will lead to a greater understanding of the mechanism of antibiotic resistance and re-infection following antibiotic treatment.

CM 07 Identification of plant natural products with antimicrobial activity

Andrew Mitchell[1], Olivia L. Champion[1], Michael Stavri[2], Winnie K.P. Shiu[2], Simon Gibbons[2] & Rick Titball[1]

[1]Exeter University, School of Biosciences, Geoffrey Pope Building, Exeter EX4 4QD; [2]Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, London WC1N 1AX

Due to the prevalence of antibiotic-resistant bacterial strains, the discovery of new antimicrobial compounds with novel modes of action is essential. Previous studies show that compounds isolated from plants synergistically act as antibiotics, working together to provide the plant a degree of protection against pathogens. We have tested three plant natural products, PNP1, PNP2 and PNP3, for antimicrobial activity against Gram-negative and Gram-positive bacteria. Using a gradient plate method, macrodilutions and microdilutions, the minimum inhibitory concentration (MIC) of gentamicin against *Y. pseudotuberculosis* was determined to be 2 µg/ml. An MIC of 3 µg/ml and a minimum bactericidal concentration (MBC) of >6 µg/ml for PNP1 against *Y. pseudotuberculosis* was observed using the same methods. In addition, an MIC of 2 µg/ml of PNP1 for *S. epidermidis* was also observed. We aim to establish MIC and MBC values for PNP2 and PNP3 and to carry out assays to establish toxicity to eukaryotic cells of all compounds.

CM 08 Real-time PCR for the detection of *qnrA* gene in *Enterobacteriaceae*: the incidence of plasmid mediated quinolone resistance in a London teaching hospital

Sakina Naqvi, **Emma Cunningham**, Claire Jenkins, Timothy D. McHugh & Indran Balakrishnan

Royal Free Hospital, London NW3 2QG

Mechanisms of resistance to quinolones are classically chromosomally mediated. However, the plasmid encoded gene, *qnrA*, encodes a protein that binds to the subunit of topoisomerase IV, preventing quinolone binding and conferring resistance. The plasmid encoded nature of the gene may facilitate spread in a hospital environment. A previously described PCR for the detection of *qnrA* was modified to a real-time PCR format and used retrospectively to screen 45 extended-spectrum-β-lactamase (ESBL) enterobacterial isolates collected at the Royal Free Hospital between January 2005 and December 2007. In addition, 59 isolates of quinolone-resistant *Enterobacteriaceae* were screened prospectively between October and December 2007. Of the isolates from the retrospective and prospective study, 4.4% and 1.7% carried the *qnrA* gene, respectively. Enterobacteriaceae exhibiting quinolone resistance encoded by the plasmid-medicated *qnrA* gene are present in our hospital but the incidence is low.

CM 09 Comparison of two different approaches to 16S rRNA gene sequencing for clinical samples

Julianne Lockwood, Holly Ciesielczuk, Claire Jenkins, Clare Ling, Timothy D. McHugh & Christopher Kibbler

Royal Free Hospital & University College Medical School, London NW3 2QG

Amplification and sequencing of the 16S rRNA gene is used to detect and identify bacteria in clinical samples. Analysis of a large fragment of DNA may enable greater differentiation between species, although amplification of shorter fragments may be more sensitive and result in better quality sequence data. To test this, we analysed 48 clinical samples (two not cultured, 20 culture-positive and 26 culture-negative) using two 16S PCR protocols to amplify one long fragment (LF; 1343bp) or two short fragments (SF; 762 and 598bp). The LF-PCR was positive for 13 of the 48 samples tested and sequencing successfully identified clinically relevant bacteria in 12 of them (92.3%). The SF-PCRs were positive for 40 of the 48 samples tested and sequencing successfully identified clinically relevant bacteria in 19 of them (47.5%). This study has shown that the SF-PCR is more sensitive than the LF-PCR, but as a consequence is more prone to contamination.

CM 10 Fluoroquinolone resistance in *Burkholderia cepacia* complex

C.F. Pope, S. Khwaja, S.H. Gillespie & T.D. McHugh

Centre for Medical Microbiology, Dept of Infection, University College London, NW3 2PF

Members of the *B. cepacia* complex (BCC) cause chronic infection in the lungs of cystic fibrosis patients who are often subject to repeated treatments with antibiotics. The impact of such treatment on the development of resistant *B. cepacia* is not clear. It is often assumed that antibiotic-resistant mutants are disadvantaged in the absence of the antibiotic and our group have previously found the presence of 'no cost' fluoroquinolone (FQ)-resistant mutations in BCC. Here we report the mechanisms of resistance in laboratory derived mutants and clinical isolates.

In vitro mutants were selected using 2xMIC clinafloxacin. For 25 mutants the phenotype was checked and the *gyrA* quinolone resistance

determining region (QRDR) genotype confirmed. No QRDR mutants were found, but using a resperine assay, the MIC to clinafloxacin was reduced, suggesting the presence of an efflux pump. For the 8 clinical isolates investigated QRDR mutations were recorded and the location of the codon change affected the FQ MIC. Also, the MIC was reduced in some clinical isolates in the presence of reserpine.

We conclude that low level resistance is primarily a result of efflux mechanisms and in clinical isolates resistance is through both mutation and efflux pumps.

CM 11 Novel antimycobacterial agents targeting mycolate synthesis: are they pyrazinamide-like?

Amani Alnimr[1], Geoff Coxon[2], Tim D. McHugh[1] & Stephen H. Gillespie[1]

[1]Royal Free and University College Medical School, London; [2]Strathclyde Institute of Pharmacy and Biomedical Sciences

Background Tuberculosis is a major public health concern especially with the growing problem of multi-resistance. The development of potent new anti-tubercular compounds with no cross-resistance to existing drugs is urgently needed. We investigated a library of chemical compounds formulated specifically to interfere with the FAS II system in *M. tuberculosis*.

Methods 85 compounds were screened for antimycobacterial activity using tube macrodilution method and *M. tuberculosis* H37Rv. Activity was confirmed by resazurin microdilution assay, agar dilution propotion method and MB/BacT ALERT 3D System. Compounds which showed significant activity were tested against a panel of mono and multi-drug-resistant clinical isolates.

Results 3/85 compounds showed good activity against *M. tuberculosis* H37Rv (MIC = 0.1–0.2 µg/ml) and clinical isolates but were less active against pyrazinamide-resistant strains (MIC = 16–32 µg/ml).

Conclusions Theses data suggest these compounds have activity analagous to pyrazinamide. This hypothesis is currently being tested by sequence analysis of *pncA* in the resistant mutants. These compounds with a known target may serve as promising leads for future drug development.

CM 12 Epidemiology and molecular characterization of renal *Staphylococcus aureus* bacteraemia isolates: future rapid typing applications

M. Stone[1], A. Ivens[2], J. Wain[3] & K. Bamford[1]

[1]Dept of Infectious Diseases and Immunity, Imperial College, London; [2]Pathogen Microarray Team, Wellcome Trust Sanger Institute, Cambridge; [3]Molecular Microbiology, Wellcome Trust Sanger Institute, Cambridge

S. aureus is an important pathogen within the haemodialysis setting, responsible for high levels of morbidity and mortality. The aim was to work towards the development of a rapid typing system for use within the routine diagnostic setting. Thirty-nine consecutive renal *S. aureus* bacteraemia isolates collected between July 2002 and March 2003 were characterized by antibiogram, phage typing, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). A subset of isolates were also characterized using a staphylococcal microarray. Meticillin resistance was detected in 46% of isolates. PFGE revealed eight patterns, clustering into 2 major types, EMRSA-15 and EMRSA-16. MLST showed that the MRSA isolates belong to ST22 and ST36. Isolates that were identical by MLST had either identical or similar PFGE fragment patterns. Microarray analysis allowed the definition of 'core' and 'accessory' genomes and the identification of potential amplification targets for use in a relevant and rapid typing scheme.

CM 13 Initial reduction of *Salmonella* burden in chickens following oral bacteriophage administration is proportional to titre delivered

B.W. Gannon, V.N. Krylov, J. Azeredo & J.M. Roe

Clinical Veterinary Science, University of Bristol, BS40 5DU (Email ben.gannon@bristol.ac.uk)

This study was conducted as part of '*Phagevet-P*', a European Union-funded project evaluating bacteriophage therapy to combat salmonella and campylobacter infections in commercial poultry. One-day-old broiler chicks were orally dosed with *Salmonella* Enteritidis PT4. One week later they were divided into 4 groups and given 0, 10^4, 10^6, or 10^8 PFU of bacteriophage PVP38. Throughout the following 7 days the numbers of salmonella in the caecal contents of birds that had received bacteriophage were significantly lower ($P<0.05$) than those in the control group. The greatest difference was one day post-phage inoculation (dpi), when the reductions in salmonella numbers were 3.07, 2.37 and 1.34 $\log_{10}$c.f.u./g in the high, medium and low dose groups respectively. This study shows that the initial reduction in salmonella numbers was directly proportional to the titre of bacteriophage administered, but after 2 dpi there was no correlation between dose titre and reduction in salmonella. Therefore, to achieve the greatest reduction of salmonella numbers using bacteriophage therapy the chickens should be treated one day prior to slaughter.

CM 14 Investigation into the changing colonization of skin bacteria during isotretinoin treatment for acne vulgaris

A.E. Thomas[1], D.R. Williams[1], T. Watts[2], N.C. Hepburn[2] & R.A. Dixon[1]

[1]Dept of Forensic and Biomedical Sciences, University of Lincoln, Brayford Pool, Lincoln LN6 7TS; [2]Dept of Dermatology, Lincoln County Hospital, Greetwell Road, Lincoln LN2 5QY

Isotretinoin, a retinoid – can be used to treat patients with moderate to severe acne despite the adverse effects of mucosal surface drying and the contraindication of use during pregnancy. Commonly, patients prescribed systemic isotretinoin have previously not responded to treatment with oral or topical antibiotics. Broad-spectrum antibacterial therapy attempts have, however, promoted changes in the diversity and antibiotic resistance status of the patients' skin microbiota. The present study involved the recovery and analysis of skin organisms from 53 patients (mean age 23 y range 15–37 y) before, during and after treatment with a 16-week course of isotretinoin. Isotretinoin has been shown to lead to profound reductions in the microflora of patients. Many bacteria had high levels of antibiotic resistance from the outset. The work has progressed with the analysis of the diversity and resistance of *Propionibacterium acnes* strains present originally and during recolonization.

CM 15 Virulence of environmental isolates of *Pseudomonas aeruginosa* in the invertebrate model *Galleria mellonella*

Eleanor Pinnock[1]; David Honeybourne[2] & Christopher Dowson[1]

[1]Warwick University, Biological Sciences Dept, Gibbet Hill Road, Coventry; [2]West Midlands Adult CF Unit, Heart of England Foundation Trust, Bordesley Green East, Birmingham

Pseudomonas aeruginosa is the most common source of bacterial infection in patients with cystic fibrosis (CF). The decline in lung function associated with infection is one of the best predictors of mortality in this group.

P. aeruginosa is commonly isolated from a wide range of environmental niches and these organisms may pose a significant infection risk for patients. The potential virulence of these strains is currently underreported.

Using *Galleria mellonella*, an established invertebrate model for investigating virulence in *P. aeruginosa*, we screened a strain panel

sourced from: a commercial mushroom growing unit, chronically colonized CF patients and epidemic outbreaks.

Environmental strains were significantly more virulent in the model than strains isolated from clinical infection. Some strains isolated from chronically colonized patients were avirulent.

This indicates that environmental isolates of *P. aeruginosa* may be capable of initiating infection. Their presence in commercial mushroom production may have implications in food preparation for CF patients in both nosocomial and home settings.

CM 16 The effect of garlic on the sensitivity of meticillin-resistant *Staphylococcus aureus* to oxacillin

Jonathan Wood[1,2], Iveta Garaiova[2], Sue Plummer[2], Carsten Müller[1], Martin Day[1] & David Lloyd[1]

[1]Microbiology, Cardiff University, Park Place, Cardiff; [2]ORL, Unit 2, Christchurch Road, Port Talbot

Meticillin-resistant *Staphylococcus aureus* (MRSA) is known to be sensitive to the antimicrobial effects of garlic. The effects of combining garlic and oxacillin on the growth of four strains of MRSA (NCTC 6571, N315 and two clinical strains, 4500 and JW4) have been assessed here. The MICs of garlic and oxacillin alone ranged from 0.5–1 mg/ml and 0.125–256 µg/ml respectively. A 50% decrease in the MIC of oxacillin occurred in the presence of 50–60% of the garlic MIC with all strains. The MIC of oxacillin could be reduced by 75% when combined with 60% of the garlic MIC for N315 and 4500. For JW4 (highly oxacillin-resistant) and NCTC 6571 (meticillin-sensitive) a 75% reduction in the MIC of oxacillin was only seen in combination with >90% of the garlic MIC.

These results illustrate different strain dependent responses of a pathogenic organism when exposed to a combination of antimicrobials.

CM 17 The management of candidaemia in intensive care

Hannah Welbourn

St James's University Hospital, Leeds LS9 7TF

Background Candida is a leading cause of infection in critical care and is associated with considerable mortality. The incidence of Candidaemia is increasing, as is the prevalence of non-albicans *Candida* (NAC) species, which are less susceptible to azoles. In 2003 the British Society for Medical Mycology (BSMM) published guidelines for Candidaemia management.

Objectives Analyse the local Candida epidemiology and ascertain if BSMM standards are being met.

Methods Retrospective casenote review of intensive care patients with Candidaemia 2004–2007.

Results 35 episodes of Candidaemia were identified in 35 patients. NAC species accounted for 49% of isolates; 50% of these had only limited susceptibility to fluconazole. The audit standard for removal of central venous catheters (CVCs) was only achieved in 50% of cases. Only half received treatment for the recommended duration. Screening echocardiography and fundoscopy were performed in 38% and 23% respectively.

Conclusions The emergence of NAC strains means that first-line treatment with fluconazole may be ineffective in 25% of Candidaemias. This study highlights the need for increased diligence in the removal of CVCs, more consideration of appropriate treatment duration, and increased screening for complications in Candidaemia.

CM 18 Quinolone resistance in non-Typhi salmonellae isolated from patients in Liverpool, UK

Manar Al-Mashhadani, D. Ryan & C.M. Parry

Division of Medical Microbiology & GUM, University of Liverpool, L3 5BA

Background Non-Typhi *Salmonella* (NTS) gastrointestinal infections are usually treated with fluoroquinolones. However, low and high-level quinolone resistance has emerged. We studied the incidence and mechanism of quinolone resistance in NTS and the associations between serovar, phage type and resistance.

Methods Low-level FQ resistance was identified by nalidixic acid resistance (NAR) in NTS isolated from patient samples submitted to the Royal Liverpool University Hospital between 2003 and 2007. Point mutations in *gyrA* were detected by PCR and sequencing of the QRDR and the *qnr* genes by multiplex PCR.

Results 286 NTS isolates were studied; 186 (65%) serovar Enteritidis. NAR was detected in 67 (23%) (ciprofloxacin MICs 0.125–0.500 µg/ml). NAR was associated with serovars Enteritidis (PT1 and PT21), Newport and Virchow. The most common *gyrA* point mutation was Asp87Tyr. A *qnrS* gene was detected in two of the 67 NAR isolates.

Conclusions NAR was detected in one quarter of the NTS isolates, was associated with particular serovars and phage types, and although mostly associated with point mutations of gyrA, *qnr* genes were also detected.

CM 19 A methacrylate polymer is bactericidal to Gram-positive and Gram-negative bacteria

Lee-Anne B. Rawlinson & David J. Brayden

UCD School of Agriculture, Food Science and Veterinary Medicine, UCD, Belfield, Dublin 4, Ireland

Poly(2-(dimethylamino ethyl)methacrylate)) (pDMAEMA) is a mucoadhesive cationic polymer. pDMAEMA has been shown to have antimicrobial effects against *E. coli* and *B. subtilis* and has been found to inhibit adhesion and invasion of *S. typhimurium* to the intestinal cell line, E12. The aim of this study was to further elucidate the antibacterial potential of pDMAEMA and to investigate its mode of action. The antimicrobial effect of pDMAEMA was investigated by determining the MIC and the MBC against bacteria. Effect of temperature and pH were determined by assessing variations in MIC values. Interaction of the polymer with *S. typhimurium* and *S. epidermidis* was determined at various time points using fluorescent microscopy. pDMAEMA was found to be bactericidal to both Gram-negative and Gram-positive bacteria at concentrations between 0.1–10 mg/ml. Its antimicrobial effect is both temperature- and pH-dependent with optimal activity at 37–43°C and pH 7.5–8. Fluorescently tagged pDMAEMA was found to bind to the surface of bacteria and may internalize. Due to its bactericidal effect against a range of bacteria and its ability to inhibit adhesion, formulated p(DMAEMA) may be useful as a topical treatment for bacterial infections or as a coating for prosthetics and catheters to inhibit biofilm formation.

CM 20 Epidemiology and mechanisms of multi-drug resistance in Gram-negative clinical isolates

Micheál Mac Aogáin[1], Jim Clair[2], Claire Adams[1] & Fergal O'Gara[1]

[1]Biomerit Research Centre, Dept of Microbiology, Biosciences Institute, National University of Ireland, Cork, Ireland; [2]Consultant Microbiologist, Mercy University Hospital, Cork, Ireland

The emergence of multi-drug resistance (MDR) in bacterial pathogens threatens the efficacy of antibiotics used commonly in the clinical setting. In this project we survey resistance mechanisms in a collection of 82 MDR Gram-negative bacteria isolated from patients attending the Mercy University Hospital, Cork. The population consists largely of *Enterobacteriaceae* (>90%). Extended-spectrum β-lactamase (ESBL)

production was detected phenotypically in 54% of isolates. Strains were typed at the sub-species level using randomly amplified polymorphic DNA (RAPD) analysis. PCR screening revealed a diversity of β-lactamase genes among ESBL producers. Seventeen *E. coli* isolates were found to harbor the CTX-M-15 ESBL gene, 14 of which gave identical RAPD profiles. Transfer of resistance was demonstrated by conjugation experiments in which increased resistance to β-lactams, aminoglycosides and ciprofloxacin was detected in *E. coli* J53 transconjugants. The level of resistance transferred was investigated by disk diffusion and broth micro-dilution. This study highlights the diversity of transferable resistance mechanisms harbored by MDR Gram-negative pathogens at this institution.

CM 21 Detection of anaerobic bacteria in sputum from patients with bronchiectasis

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

[1]School of Pharmacy; [2]School of Medicine & Dentistry, Queen's University of Belfast

We have previously shown by anaerobic culture that the lungs of Cystic Fibrosis (CF) patients with bronchiectasis are not only colonized by chronic infecting opportunist pathogens such as *Pseudomonas aeruginosa*, but also by an array of other bacterial species, many of which are anaerobes, which would not be routinely considered as pathogens in CF pulmonary infection. The aim of this study was to determine whether anaerobes are also present in the sputum of non-CF patients with bronchiectasis.

Sputum samples were collected and processed, using strict anaerobic bacteriological techniques, from adult patients attending the respiratory outpatient clinic. Bacteria within the samples were detected by plating on selective agars, quantified by total viable count and identified by PCR and sequencing of 16S ribosomal RNA genes.

Sputum samples were collected from 18 patients with bronchiectasis. *P. aeruginosa* was identified as the predominant aerobic bacteria in all 18 samples and was present in numbers ranging from 6.4×10^3 to 2.7×10^6 c.f.u./g of sputum. Anaerobes from a range of species including *Prevotella, Actinomyces* and *Streptococci* were detected in similar numbers, ranging from 1.5×10^4 to 5.6×10^6 c.f.u./g of sputum, from 16/18 (89%) patients. These results indicate that anaerobes are present in large numbers within the lungs of non-CF patients with bronchiectasis. Their presence could be of important clinical relevance to these patients as they may contribute significantly to infection and inflammation.

CM 22 Influence of green tea on the antimicrobial activity of some antibiotics against multiresistant clinical isolates

Nourhan H. Fanaki, **Mervat A. Kassem**, Mohamed A. Fawzi & Fatma S.E. Dabbous

Dept of Microbiology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

Antibiotics belonging to different groups were tested separately and in combinations with green tea against *Staphylococcus* spp, Gram-negative isolates and 3 standard strains using the disc agar diffusion technique. Subinhibitory concentrations of green tea showed marked increase in the sensitivity of tested isolates to most of the antibiotics tested. Green tea enhanced the bactericidal activity of all tested antibiotics especially when green tea-chloramphenicol combination was tested against isolate $P_{2,}$ using the surface viable count. Moreover, green tea had the ability to cure the resistance, of one out of the tested 5 isolates, to cefuroxime. In addition, the effect of green tea on direct inhibition of β-lactamases production was conducted using the nitrocefin method. The effect of omeprazole, proton pump inhibitor, and subinhibitory concentration of green tea on the antimicrobial activity of tetracycline HCl was studied. In conclusion, beneficial outcomes of concomitant administration of green tea with tetracycline, chloramphenicol as well as β-lactam antibiotics were observed.

CM 23 Effect of prebiotics on the growth of potential probiotic human faecal lactobacilli

Tejpal Dhewa[1], Shailja Pant[1], Nishant Goyal[1] & R.K. Saxena[2]

[1]Dept of Microbiology, Bundelkhand University Jhansi, (UP) India; [2]Dept of Microbiology, Dolphin PG Institute of Biomedical & Natural Sciences, Dehradun, (Uttarakhand) India

Intake of probiotics (living micro-organisms), prebiotics (non-digestible oligosaccharides) and synbiotics (mixture of probiotics and prebiotics) has been demonstrated to modify the composition of the microflora, restore the microbial balance and therefore have the potential to provide health benefits.

In the present study the effect of prebiotics on the growth of potential probiotics human faecal lactobacilli was observed. For this, total of 26 isolates of *Lactobacillus* sp. were recovered from human faecal samples. The recovered isolates were enriched in MRS broth followed by their maintenance in chalk litmus milk. All the isolates were screened for utilization of non digestible oligofructose (prebiotics) by agar plate assay. The 8 screened isolates of different species were further grown in presence of prebiotics (Inulin, honey and gum acacia) at different concentration (0.5, 1.0, 3.0 and 5.0%) during 0–24 h at intervals of 0–6 h, 6–18 h and 18–24 h. Maximum specific growth rate with glucose as sole energy (Control) source was $\mu = 0.26\ h^{-1}$ during 0–6 h (Isolate HF20; *Lactobacillus plantarum*). Maximum specific growth rate utilizing prebiotics was recorded with honey (μ, 0.41 h^{-1}) at 0.5% during 0–6 h (Isolate HF20; *Lactobacillus plantarum*). The best effect was shown by Inulin on probiotics at 5% within the time interval of 0–6 h. Honey came up with best utilization by the probiotic Lactobacilli at 0.5% during 0–6 h. Gum acacia gave remarkable results with probiotics at 3% during 0–6 h and 6–18h.

Considering all the observations and results we can conclude that among the three prebiotics, honey (0.5% during 0–6 h interval) was utilized efficiently giving highest specific growth rate (μ, 0.41 h^{-1}). Among all the different species of probiotic lactobacilli isolates, *Lactobacillus plantarum* showed appreciable results and could be designated as a potential probiotic.

Posters

Clinical Virology Group

CV 01 An outbreak of parainfluenza 3 in patients with haematological malignancies

A. Lee, D. Bibby, M. Cummings, H. Oakervee, D. Clark & F. Mattes

Barts and The London NHS Trust, Virology, 3/F Pathology & Pharmacy, 80 Newark St, London E1 2ES

Parainfluenza (PF) virus causes mild upper respiratory tract infections in immunocompetent persons. However, in haematopoetic stem cell transplant patients, infection is associated with high morbidity and mortality. We report an investigation into a PF3 outbreak involving 18 patients with haematological malignancies. Laboratory diagnosis was made using a multiplex real-time PCR screen for 10 respiratory viruses including PF1, 2 and 3. Sequence analysis of an 800bp region of the HN gene was performed on PF3 positive samples from these patients and non-outbreak associated patients. Phylogenetic analysis showed that 17/18 outbreak samples had identical sequences across the HN region studied, whereas unrelated community strains diverged by between 1.9 and 3.3%. The one divergent outbreak sequence differed by a single nucleotide from the outbreak sequences. Despite prompt isolation of symptomatic patients, transmission occurred over a 6 week period. Epidemiological analysis suggested transmission of PF3 within the hospital occurred via staff members. This incidence highlights the importance of sufficient infection control measures, especially within high risk wards.

CV 02 HIV-2 infection and HAART in a northern Ghanaian cohort

D. Bibby[1], G. Wall[1], M. Dittmar[2], D. Clark[1] & D. Chadwick[3]

[1]Virology Dept, Barts & the London NHS Trust, E1 2ES; [2]Centre for Infectious Disease, ICMS, Barts and The London, London E1 2AT; [3]The James Cook University Hospital, Middlesbrough

Studies of highly active anti-retroviral therapy (HAART) in HIV-2-infected individuals are rare, and confined either to small cohorts or to case studies. Here, we describe a large cohort (100 patients) of HIV-2-infected individuals attending a teaching hospital in Kumasi, northern Ghana, at six-monthly intervals. Fifty-nine patients are receiving HAART. However, many initial diagnoses failed to distinguish HIV-1 from HIV-2 infection, and 69 are dually infected with HIV-1 and HIV-2.

Using newly developed in-house plasma viral load assays, no significant difference in HIV-2 RNA detectability was observed between treatment-naïve and experienced patients (41.3% and 43.9% respectively, p=0.79), whereas HIV-1 RNA was detected more frequently in treatment-naïve dually infected individuals (92% versus 46.8%, p<0.0002). We conclude that the inclusion of NNRTIs in HAART regimens has led to suboptimal HIV-2 treatment in this cohort, emphasizing the need for discrminatory diagnosis prior to initiation of therapy.

CV 03 Comparative evaluation of the Roche Taqman quantitative HIV-1 assay and the Roche Amplicor version 1.5 PCR

X. Couto-Parada, I. Ushiro-Lumb, A. Oliver, A. Lee, S. Pereira, D. Perinpanathan & D. Clark

Dept of Virology, Barts & the London NHS Trust, London E1 2ES

We conducted an evaluation of the automated Roche Taqman HIV-1 Viral Load assay with the Roche Amplicor version 1.5 PCR (Oliver et al, J Clin Microbiol, 2007) prior to the introduction of the former into our diagnostic service in July 2005. Our evaluation showed that there was a good correlation between the two assays for the majority of samples tested. However, another laboratory has reported that the Roche Taqman assay was under-quantifying in a significant number of samples across a wide range of HIV subtypes compared to the Roche Amplicor test (Damond et al. J Clin Microbiol, 2007), In light of this study, we decided to conduct a further evaluation of the two systems.

We are presently conducting paired testing of 200 plasma samples with both Roche assays and determining HIV subtype for each sample. In addition samples are being tested on an alternative platform (Abbott real time HIV-1 assay). The results will be presented and discussed.

CV 04 Serological responses in health care workers (HCWs) to the live attenuated varicella vOka vaccine

Suzanna McDonald, Fiona Scott, Mary Leedham-Green, Chris Maple & Judy Breuer

Center of Infectious Diseases, Institute of Cell and Molecular Science, Bart's & the London School of Medicine and Dentistry, Queen Mary's University of London, 4 Newark St, Whitechapel, London E1 2AT

Aim To investigate humoral immune responses to the varicella vaccine, in relation to immune status at enrolment and ethnicity. To determine if the time resolved immunoflouresence assay (TRIFA) is a more accurate methodology for determining immune status, than the currently used assay.

Methodology Non-immune HCWs were enrolled in the study. Participants (n = 100) were given 2 doses of vaccine (Merck) 6 weeks apart. Serology testing was carried out using TRIFA and Avidity assays. A subset of TRIFA readings where correlated with fluorescent antigen to membrane antigen (FAMA) results.

Results Study participants were from a diverse ethnic background. Only 70% were deemed to be non-immune at enrolment by TRIFIA. 93% of the non-immune HCWs seroconverted, (51% with one dose, 41% with 2 doses). 7% failed to seroconvert and were all of West African/Caribbean ethnicity. High avidity results correlated strongly with immune status at enrolment. Immunity by TRIFA (using a cut off determined in this study) correlated strongly with the FAMA.

Conclusions Humoral responses to the vaccine varied. An association between ethnic origin and failure to seroconvert was evident. Avidity maturation is a good correlate of existing immunity. TRIFA is a preferable assay to determine immune status to ELISA and FAMA.

CV 05 Quantitative detection of VZV in serial plasma samples of a 76-year-old male with VZV pneumonitis

G. Wall, M. Quinlivan, D. Clark & J. Breuer

Virology Dept, Barts & the London NHS Trust, 80 Newark Street, London E1 2ES

Varicella Zoster Virus (VZV) typically infects children and results in a mild, febrile illness and a vesicular rash. Pneumonitis is a less common complication, with older individuals acquiring a primary infection at a higher risk. Here we present a 76 year old male who was admitted to ITU with pneumonitis and acute respiratory distress syndrome (ARDS), in addition to a vesicular rash as a result of infection with VZV. VZV DNA was quantified by our in-house quantitative real time PCR which amplifies a 77bp region of the ORF 29 gene. We received serial plasma samples during the course of infection to monitor the levels of VZV DNA in peripheral blood, and to help guide antiviral treatment. The VZV blood viral load declined from 6.29 Log_{10} copies/ml to 4.04 Log_{10}

copies/ml over a period of 11 days on IV aciclovir therapy. Aciclovir therapy was continued with further decrease in viral load and eventual resolution at day 11 of vesicles, but the patient continued to decline clinically due to the underlying ARDS. Despite IV steroids, the patient died. VZV viral load can be a useful guide to the management patients with chickenpox or zoster who become seriously ill.

CV 06 Calicivirus antigen engineering for possible vaccine development

A.K. Khaled Shawki & Mike J. Carter

University of Surrey, Guildford

The *Caliciviridae* is a family of related viruses infecting a wide variety of animals. Human pathogens belong to the genera Norovirus and Sapovirus and induce enteric infections. Of these, the noroviruses are by far the most important and account for the bulk of infectious gastroenteritis in adults. Noroviruses are antigenically diverse viruses and cross-reactive immunity is poor, serial infections occur even with the homologous virus in susceptible individuals. These features present a considerable challenge for vaccine development but approaches have been based around the use of empty virus-like particles produced by self-assembly of the virus capsid protein. Such structures are acid-stable and withstand passage through the gut and thus raising the possibility of edible vaccines for enteric immunization. Here we attempt to induce particles that would be suitable for such a route but which should induce more broadly cross-reactive immunity by deleting those immunodominant regions that evoke type-specific responses and replacing them with polyglycine amino acid chain to maintain particle stability.

CV 07 Protection against heterologous SHIV challenge following vaccination with HIV-1_{W61D} recombinant envelope in the macaque model

M. Page[1], N. Berry[1], R. Stebbings[1], D. Ferguson[1], R. Hull[1], D. North[1], S. Harron[1], W. Elsley[1], C. Ham[1], G. Voss[2], N. Mathy[2] & N. Almond[1]

[1]National Institute for Biological Standards & Control, Division of Retrovirology, Potters Bar; [2]GSK, Rixensart, Belgium

Background Passive transfer of potent neutralizing antibodies has been demonstrated to protect macaques against challenge with SHIV. However, the goal is to elicit these serological responses by vaccination. We have developed and applied a SHIV based model to investigate the role of serological responses in protection elicited by HIV-1 rgp120 based vaccines.

Objectives To investigate the breadth of protection conferred by HIV-1_{W61D} rgp120 vaccination and to identify the correlates of this protection.

Methods *M. fascicularis* received 8 immunizations with rgp120 HIV-1_{W61D} expressed in mammalian cells and formulated in the adjuvant AS02A followed by challenge 4 weeks after the last immunization with SHIVsbg intravenously, along with groups of 4 naïve challenge controls. A second group of 4 naïve *M. fascicularis* received 50mls of a pool of immune serum collected from the rgp120 immunized macaques between their third and 8th immunizations. The recipients were challenged 24 hrs later with SHIVsbg along with a group of naïve challenge controls.

Results High titres of anti-envelope antibodies were detected in all vaccinates that bound to both the homologous HIV-1_{W61D} and heterologous HIV-1_{IIIB} envelope rgp120 by ELISA. Significant titres of virus neutralization were detected against $SHIV_{W61D}$ expressing an envelope homologous with the vaccine, but only limited cross neutralization of $SHIV_{sbg}$ that expressed an envelope homologous with HIV-1_{IIIB} envelope. Protection against $SHIV_{sbg}$ was observed in all 4 vaccinated animals. Rechallenge of protected macaques 32 weeks later with the same virus resulted in infection of all vaccinates. Serum transfer did not result in protection from $SHIV_{sbg}$ challenge in spite of the protection observed against this same virus challenge stock following vaccination.

Conclusions HIV envelope vaccines may protect against heterologous virus challenge. The mechanism of heterologous protection is short lived and does not correlate with neutralizing antibody titres. Serum transfer does not confer protection. It is unlikely that protection is antibody mediated. Other mechanisms of protection by rgp120 HIV-1 vaccination need to be investigated.

CV 08 Multiplex real-time PCR and low density oligonucleotide microarray for the early differential diagnosis of acute viral respiratory tract infections

Michael J. Carr[1], **Gemma Cannon**[1], Paul O'Reilly[1], Edwin O'Kelly[1], Jeff Connell[1] & William W. Hall[1,2]

[1]National Virus Reference Laboratory, University College Dublin, Dublin 4, Ireland; [2]Centre for Research in Infectious Diseases, University College Dublin, Dublin 4, Ireland

Acute respiratory tract infections (ARTIs) currently account for approximately 3% of deaths annually among children < 5 years of age in the industrialized countries and up to a quarter of deaths among children < 5 years of age in the non-industrialized countries (1). The development of new diagnostic methodologies is thus urgently required to address the challenges of ARTIs. An accurate, sensitive and rapid differential diagnosis can alter individual patient clinical treatment, as well as facilitate the recognition of outbreaks in hospital settings and in the wider community that have serious public health implications.

Furthermore, the early recognition of a viral pathogenic agent, or co-infections, may allow appropriate therapeutic intervention that serves to reduce attendant morbidity and mortality; the resulting economic consequences associated with losses in productivity; and the emergence of bacterial drug resistance, drug side effects and the cost associated with inappropriate empirical antibiotic therapy.

We have established and validated a multiplex real-time PCR and a corresponding low density oligonucleotide microarray system using PCR and immunofluorescence positive clinical specimens and external QC proficiency panels. This methodology allows the detection, differentiation and sub-typing of respiratory viruses including: Influenza A and B, Parainfluenza viruses 1–3, Respiratory syncytial virus A/B, the genus Rhinovirus, Human Metapneumovirus A/B, Coronaviruses types 229E, OC43 and NL63, Adenoviruses and the newly identified Bocavirus. End-point multiplex PCR and hybridization to the low density microarray exhibited comparable sensitivities and specificities to the multiplex real-time PCR. This data can be used to be better assess the viral etiopathogenic agent responsible for patient respiratory distress in the case of viral (and bacterial) co-infections.

Reference Williams *et al.* (2002) Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis* 2, 25–32.

CV 09 CMV genotyping in an Irish immunocompromised patient group: correlation with disease and immunity

Allison Waters, J. Hassan, S. Coughlan, S. Dooley, J. Connell & W.W. Hall

NVRL, University College Dublin, Dublin 4, Ireland

Human cytomegalovirus infects a diverse range of cell types and virulence may be linked to genetic variations within the viral genome. The UL144 gene is retained only by clinical isolates and lost in laboratory-attenuated strains, implicating it in viral pathogenesis. We examined UL144 genotypes in CMV-infected transplant recipients (n=19), neonates (n=38) and HIV positive individuals (n=8). All UL144 genotypes (A, B, C, A/C, A/C) were detected in the patient groups. Genotype B was the most common strain detected. However, the 'less common' genotypes were present in significant numbers also (C=17, A=25, recombinants=9) and were detected in a much higher proportion

than previously shown for other patient groups. There was no correlation between genotype and viral load. Th1- and Th2-type cytokines were examined by multiplex bead arrays and IL-10 was significantly upregulated in all CMV positive sera tested ($p<0.001$), suggesting that CMV may direct the host immune response towards a Th2-type response. This study highlights the possibility that both viral genes and host immune response factors may impact on the pathogenesis of CMV in the different patient groups.

CV 10 Prevalence and genetic diversity of norovirus infection in Irish children

Allison Waters[1], L. Dunford[1], G. Tuite[1], J. Connell[1], S. Dooley[1], B. Foley[2], P. McKeown[2], W.W. Hall[1] & S. Coughlan[1]

[1]NVRL, University College Dublin, Dublin 4, Ireland; [2]HPSC, 25–27 Middle Gardiner St, Dublin 1, Ireland

Paediatric gastroenteritis places a considerable burden on children worldwide, however the aetiology of the disease is not well defined in Ireland. The national surveillance of gastroenteritis in Ireland is a combined virological and epidemiological surveillance program. The objectives of this study were to characterize the norovirus genotypes associated with viral gastroenteritis in children ≤5 years, and compare these strains with those detected in adults. A total of 5 different Norovirus genotypes were associated with infection in children (GII/2,GII/4,GII/6,GII/b,GII/unknown) whereas the GII/4 genotype was the only strain detected in adults. This genotypic difference between paediatric and adult infection was found in both community and hospital-based infection. Norovirus was the most common cause of gastroenteritis in hospitalized children of 4 months–5 years and these patients group reported diarrhoea as their most common symptom. This raises the question whether Kaplan criteria are the most effective method for clinically diagnosing outbreaks of enteric infection in children.

CV 11 A laboratory developed quadruplex assay for the dual detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine

M.J. Hopkins[1], L. Ashton[1], H.M. Palmer[2] & I.J. Hart[1]

[1]Liverpool SVC, Royal Liverpool University Hospital; [2]SBSTIRL, Royal Infirmary of Edinburgh

Objectives To develop and evaluate a real-time PCR assay capable of detecting *Chlamydia trachomatis (CT)* and *Neisseria gonorrhoeae (GC)* in urine, for use in a diagnostic laboratory.

Methods Diagnostic performance of a laboratory developed quadruplex assay (LDQA) for *CT* and *GC* was assessed using 1028 urine specimens. The LDQA was compared to the Roche COBAS Taqman CT test and the Roche COBAS Amplicor GC assay. Positive urines (*CT* or *GC)* were identified on the basis of at least two positive PCRs.

Results Of the 88 urines identified as Chlamydia positive, 82 were positive and 6 equivocal (total 88) by the LDQA and 83 positive and 3 equivocal (total 86) by the Roche Taqman CT assay. Two urines were inhibitory to the commercial assays but none were inhibitory to the LDQA. Weighted kappa analysis showed 96% concordance between these two *CT* testing systems. The LDQA also detected *GC* in 17 specimens; fourteen were confirmed using another laboratory developed GC PCR and the other 3 were positive by the Roche Amplicor GC test. Of the 19 positives by the Roche GC test only 14 were confirmed positive by another GC PCR.

Conclusions The LDQA was found to be an effective method for detection of *C. trachomatis* and *N. gonorrhoeae* in urines in comparison to commercial methodology.

Posters

Environmental Microbiology Group

EM 01 Analyses of spatial and temporal diversity of methanotrophs in a landfill cover soil using a functional gene array

Deepak Kumaresan[1], Guy C.J. Abell[2], Nancy Stralis-Pavese[2], Levente Bodrossy[2] & J. Colin Murrell[1]

[1]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL; [2]Austrian Research Centre, Siebersdorf, Austria

Understanding of microbial functional diversity in an ecosystem requires taking into consideration the spatial heterogeneity, both horizontal and vertical, of the system. Moreover, temporal variations such as temperature and precipitation can also have a profound impact on the functional diversity and stability of micro-organisms. Landfills are the largest anthropogenic source of methane (CH_4) and contribute about 13% of total CH_4 emissions. Landfill cover soils are a rich environment sustaining active methanotroph populations above landfills that could potentially oxidize up to 100% of the CH_4 produced in the landfill. Henceforth, it is important to understand the impact of spatial and temporal heterogeneity of soil on functional diversity methanotrophs in landfill cover soils thereby harnessing the potential of methanotrophs in reducing CH_4 emissions from landfills. In this study, we determined the spatial and temporal diversity of methanotrophs using a functional gene array (*pmoA* microarray) and the diversity was correlated to the physico-chemical parameters and soil CH_4 oxidation potential. Moreover, previous studies have suggested that size of soil samples used to determine the molecular microbial diversity exerts a great influence on the results. In this study, we compared the effect of soil sample size on reproducibility of the results (0.5/5/50g homogenized samples) in determining the methanotroph community structure.

EM 02 Purification, characterization and cloning of dimethylsulfide mono-oxygenase from *Hyphomicrobium sulfonivorans*

Rich Boden[1], Elena Borodina[2], Ann P. Wood[2], Donovan P. Kelly[1], J. Colin Murrell[1] & Hendrik Schäfer[1]

[1]Biological Sciences, University of Warwick, Coventry CV4 7AL; [2]King's College London Dental Institute, Guy's Campus, London SE1 9RT

Dimethylsulfide (DMS) is an organosulfur trace gas accounting for the majority of biogenic atmospheric sulfur. Its atmospheric oxidation products backscatter heat radiation and promote cloud formation, giving DMS a role in climate cooling. To date, two pathways of DMS metabolism by bacteria have been described, however, no biochemical or genetic studies have been carried out. DMS mono-oxygenase activity has been described in *Hyphomicrobium* spp., *Thiobacillus* spp. and *Arthrobacter* spp. many times since its discovery in 1981, however, it has never previously been purified to homogeneity or cloned and the genes have remained unknown. Here DMS mono-oxygenase has been purified from *H. sulfonivorans* and is shown to consist of two subunits comprising a reduced flavin mononucleotide-dependent mono-oxygenase (54kDa, DmoA) and an NADH-dependent flavin oxidoreductase (20kDa, DmoB) showing similarity to alkanesulfonate mono-oxygenase. Activity with a range of substrates and inhibitors has been assessed and the genes encoding the enzyme subunits have been cloned and sequenced.

EM 03 A novel pathway of bacterial dimethylsulfide metabolism in *Methylophaga thiooxidans* sp. nov.

Rich Boden, J. Colin Murrell & Hendrik Schäfer

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

Dimethylsulfide (DMS) is an organosulfur trace gas accounting for the majority of biogenic atmospheric sulfur. Its atmospheric oxidation products backscatter heat radiation and promote cloud formation, giving DMS a role in climate cooling. To date, two pathways of DMS metabolism by bacteria have been described in isolates. The pathway of DMS catabolism by the facultative marine methylotroph *Methylophaga thiooxidans* has been shown to produce tetrathionate from DMS and is associated with an energy-gaining step from the oxidation of thiosulfate intermediates. The kinetics of growth of *M. thiooxidans* on a range of carbon and energy source combinations has been assessed and chemolithoheterotrophic growth on DMS has been demonstrated for the first time. The production of polythionates by a marine methylotroph is also novel and distinct from other DMS-oxidizing *Methylophaga* spp. which have been found to produce thiosulfate as an end product of DMS metabolism.

EM 04 Life without light: bacterial, archaeal and eukaryotic communities in a sulfur- and methane-driven ecosystem (Movile Cave, Romania)

Y. Chen[1], R. Boden[1], A. Hillebrand[2], D. Kumaresan[1], H. Moussard[1] & J.C. Murrell[1]

[1]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL; [2]Institute of Speleology 'Emil Racovita', Str. Frumoasa, nr. 31, sect. 1, 010987 Bucharest, Romania

The discovery of Movile Cave in Romania attracted great attention due to the fact that the cave is completed sealed and life in this ecosystem is entirely independent of light. Scientific evidence demonstrated that this cave is driven by chemosynthesis, gaining energy mainly by sulfur oxidation and methane oxidation. In this study, Bacterial, Archaeal and Eukaryotic diversity has been analysed by cloning and sequencing of the small subunits of ribosome RNA (16S rRNA and 18S rRNA). Further analyses of related functional genes, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and sulfur oxidation gene (soxB) involved in sulfur oxidation; particulate and soluble methane monooxygenase (pMMO and sMMO) and methanol dehydrogenase (MDH) involved in methane oxidation, revealed that specialized microbial guilds are dominant in the cave system. DNA-Stable Isotope Probing experiment with $^{13}CH_4$ and ^{13}C bicarbonate demonstrated the 13-carbon flow from sulfur and methane oxidizing bacteria to other prokaryotes and eukaryotes.

EM 05 Diversity of active marine methanotrophs and methylotrophs in Colne estuary sediments

Hélène Moussard[1], Nancy Stralis-Pavese[2], Josh D. Neufeld[3], Levente Bodrossy[2] & J. Colin Murrell[1]

[1]Biological Sciences, University of Warwick, Coventry CV4 7AL; [2]Dept of Bioresources, Austrian Research Centre, A-2444 Seibersdorf, Austria; [3]Dept of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

Aerobic surface sediment from the Colne Estuary was examined for the presence of C_1-utilizing communities using DNA-stable isotope probing (SIP) with ^{13}C-labelled methane, methanol and monomethylamine, with and without the addition of supplemental nutrients. Active methylotrophic bacteria were profiled using DGGE and clone libraries of 16S rRNA gene amplicons, revealing methylotrophic bacteria affiliated with *Methylophaga* spp. in methanol and monomethylamine incubations. The addition of nutrients increased the rate of substrate

metabolism, but affected the fingerprints associated with ^{13}C-labelled DNA. The $^{13}CH_4$ SIP incubations suggested the activity of both Type I and Type II methanotrophs. Microarray hybridization of amplified particulate methane monooxygenase (*pmoA*) genes revealed the role of Type Ia methanotrophs in SIP incubations, with and without added nutrients. Type II methanotrophs were only detected without added nutrients, suggesting that Type II methanotrophs may be more active under conditions similar to those occuring *in situ*.

EM 06 Meticillin-resistant staphylococci in the farm environment

C.E. O'Neill[1], W. Gaze[1,3], L. Tisi[2], K. Hardy[3], P.M. Hawkey[3] & E.M.H.Wellington[1]

[1]Dept of Biological Sciences, University of Warwick, West Midlands CV4 7AL; [2]Lumora Ltd, Cambridge; [3]West Midlands Public Health Laboratory, NHS Trust and University of Birmingham

Staphylococcus aureus is part of the normal human flora, but it can also be pathogenic in immunocompromised individuals. *S. aureus* contains a range of potentially mobile resistance and pathogenicity islands on its chromosome; such as SCC*mec*, carrying meticillin resistance. Meticillin-resistant *S. aureus* (MRSA) poses significant risk due to decreased susceptibility to β-lactam antibiotics. The aim of this research is to determine the distribution of MRSA and meticillin-resistant coagulase negative staphylococci in the agricultural environment. A selective medium was used to screen 100 bulk milk samples from dairy farms in geographically dispersed locations around the UK. MRSA strains were typed using VNTR-based SIRU-typing. Transfer of virulence toxins increases virulence of community MRSA, therefore key toxin genes were screened for by PCR. Environmental samples were screened for MRSA using the IDI – MRSA real-time PCR method. Loop-mediated isothermal amplification (LAMP) allows fast amplification using a single incubation temperature, negating the need for specialized thermocyclers. Specific LAMP primers were designed to MRSA and validated using a selection of MRSA and MSSA strains. Sensitivity and specificity of real-time PCR and LAMP were compared in a range of environmental samples.

EM 07 Validation of a reliable tool for genetic population studies in *Paracoccus denitrificans*

A.P. Natale & K.J. Purdy

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

Paracoccus denitrificans is a soil alphaproteobacterium characterized by its versatile growth under various environmental conditions. Its aerobic respiratory pathway and its ability to reduce nitrate to dinitrogen gas under anaerobic and microaerophilic conditions have made this bacterium a popular model for molecular and ecological studies.

The aim of this study is to test whether 'an organism's metabolic flexibility is maintained *in situ* by environmental heterogeneity' by investigating if the selective pressure of long-term steady states leads to the loss of functions through the accumulation of mutations in unused genes. In this study, we validate the use of Capillary Electrophoresis Single Strand Conformation Polymorphism (CE-SSCP) as a suitable tool to detect the occurrence of point mutations in a growing population. This was achieved by using a mutagenesis system and by comparing profiles of flat techniques such as DGGE and SSCP with CE-SSCP. The most reliable, less labour intensive and most sensitive tool is CE-SSCP. This technique is therefore an ideal tool for genetic evolutionary studies of model microbial populations.

EM 08 Metagenomic analysis of human tongue using phage display

Samantha Easton & John Ward

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

Microbial infections are dependent on a series of changes in both host and bacterium, in response to bacterial attachment. Commensal bacteria also develop and maintain an intimate relationship with mammalian cells, without triggering invasion mechanisms. Phage display is a simple molecular technique for the identification of protein-ligand interactions, such as microbial attachment to mammalian cells, and is currently used successfully in epitope mapping, antibody tailoring and receptor agonist and antagonist screening. It is well established that the adhesion of enteric, oral and respiratory bacteria is required for colonization and, once bound, the bacteria are less likely to succumb to host defences. Indeed, bacteria-host interactions are very often synergistic, the microbial community displaying stability in ever changing environments. This study aims to identify bacterial adhesins which are vital for bacterial colonization of the human tongue dorsum, using Phage Display. Screening of the Phage Display library has been carried out using a technique called Bio-panning, against human IgA, and is expected to yield one or more bacterial adhesins. The analysis of many IgA binding proteins highlighted by the study is underway, and the process of characterizing 'real' binders is ongoing.

EM 09 Identification of a link between *Pseudomonas phylogeny* and phosphate solubilization activity

Patrick Browne[1], Olivia Rice[1], Simon Miller[1], Jimmy Burke[2], John Morrissey[1] & Fergal O'Gara[1]

[1]BIOMERIT Research Centre, University College Cork, Ireland; [2]Teagasc, Oak Park Research Centre, Carlow, Ireland

Phosphate contamination from agricultural sources is a significant water pollution problem. Soluble phosphate is readily immobilized in soil and consequently phosphate fertilizers are often over-applied. Microbially mediated phosphate solubilization in the soil by species such as *Pseudomonas* could improve the availability of phosphate to crops thus reducing the requirement for phosphate fertilizer application. Knowledge of how plant species and varieties influence the phosphate solubilizing microbial populations could facilitate strategies to reduce phosphate pollution. *Pseudomonas* isolate collections were established from the rhizospheres of wheat and barley grown in conventional or low input cropping systems. Isolates from these collections were classified as strong, weak or non-phosphate solubilizing on the basis of screening for phosphate solubilization on buffered NBRI agar. ARDRA and sequencing of the 16S rDNA and *gyrB* genes was used to assess the phylogeny of isolates representative of strong, weak and non-phosphate solubilizing classes. A link between *Pseudomonas* phylogeny and phosphate solubilization ability was identified.

EM 10 Identification and functional analysis of *Pseudomonas fluorescens* f113 genes involved in phosphate mobilization

Simon Miller, Olivia Rice, Pat Browne, John Morrisey & Fergal O'Gara

[1]Biomerit Research Centre, University College Cork, Cork, Ireland

Soil bacteria and fungi, including *Pseudomonas* are able to mobilize sources of insoluble phosphate into bioavailable forms for uptake by plants via the production of organic acids, phosphatases and phytases in a process termed biofertilization. The genetic mechanisms behind biofertilization in *Pseudomonas* sp. are poorly understood and how expression of these genes is regulated in the rhizosphere is of great interest. We have undertaken two approaches to identify the mechanisms involved in phosphate biofertilization in the model biocontrol strain *P. fluorescens* F113. 1) Genes predicted to be involved in gluconate production/utilization and organic phosphate mineralization were targeted for mutagenesis. 2) A transposon mutant library of F113 was screened for phosphate solubilization/mineralization deficient mutants. The impact of these genes on the biofertilization ability of F113 was also examined. Through this screen we have identified genes which have an impact on phosphate mobilization that

were not previously anticipated. This study suggests that there are additional factors to those presently described involved in determining phosphate mobilization abilities of rhizosphere-associated bacteria.

EM 11 Phenotypic and molecular characterization of potential plant growth promoting Pseudomonas from rice and maize rhizospheres

Phrueksa Lawongsa[1], Neung Teaumroong[1], Simon Miller[2] & Fergal O'Gara[2]

[1]School of Biotechnology, Suranaree University of Technology, Nakhon Ratchasima, Thailand; [2]Biomerit Research Centre, University College Cork, Cork, Ireland

Plant growth promoting *Pseudomonas* have received particular attention because of their potential as biocontrol agents, biofertilizers and phytostimulators. In this study, *Pseudomonas* species were isolated from rhizospheres of two plant hosts, rice and maize. The biocontrol and plant growth promotion abilities of these strains were confirmed by bioassays on fungal and bacterial plant pathogens, as well as IAA production and carbon source utilization. Comparative analysis of ARDRA profiles grouped all rice isolates together in a cluster while the maize isolates assembled in their own individual group. This work identified a number of isolates with potential for use as plant growth promoting and biocontrol agents on rice and maize. Furthermore, *phlD* specific primers were used to detect 2,4-diacetylphloroglucinol (DAPG)-producing pseudomonads. The result showed only 2 of 138 isolates were *phlD*$^+$. However, neither of these two strains produced a peak for DAPG detected by HPLC. The status of the DAPG genes in these two isolates was therefore investigated further.

EM 12 Effect of previous exposure on the rate and extent of polycyclic aromatic hydrocarbons degradation in soil

P. Sawulski, N. Clipson & E. Doyle

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

Polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous group of compounds that can be introduced to the environment both by anthropogenic and natural activity. Due to their deleterious effect on biological systems, PAHs have been rated by the US Environmental Protection Agency as priority toxic substances. Natural degradation of PAHs occurs within the soil, with the rate of disappearance depending upon contaminant concentration, composition and previous soil exposure to the PAH in question. The aim of this study was to examine the effect of previous exposure to PAHs on the rate of degradation of various PAHs in soil. Two soils were selected; the first was an agricultural soil with no history of PAH contamination and the second soil was obtained from a former creosote manufacturing facility. A series of microcosm experiments were set up containing both soils amended with a 3 ring (phenanthrene), a 4 ring (fluoranthene) and a 5 ring (benzo[a]pyrene) PAH. The rate of disappearance of these compounds was measured by gas chromatography. The influence of the PAHs on microbial communities was assessed using both culture-dependent and -independent methods (ARISA, TRLFP). Dehydrogenase activity was used as an indication of general microbial activity in the soil. Results indicated that the degradation of PAHs occurs in both types of soil although is considerably faster in pre-exposed soil. Phenanthrene was readily degraded in both soils, whereas fluoranthene degradation proceeded at a much faster rate in the soil with a known history of PAH contamination. No significant removal of the five ring benzo[a]pyrene occurred in either soil during the course of the experiment.

EM 13 Improved detection of environmental *Vibrio parahaemolyticus* through the use of chromogenic media

B.A. Daramola, D.R. Williams & R.A. Dixon

Dept of Forensic & Biomedical Sciences, University of Lincoln, LN6 7TS

Background Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar has been used selectively for many years to differentiate *Vibrio parahaemolyticus* from other Gram-negative bacteria such as *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Vibrio vulnificus* and *Vibrio mimicus*. The use of chromogenic Vibrio (CV) agar (CHROMagar, Paris, France), however, improves the selection of *V. parahaemolyticus* strains within a mixed population of other Gram-negative bacteria.

Methods The procedure has involved an enrichment of a portion of marine sample in Alkaline Peptone Water (APW) containing 3% NaCl, incubation at 37^{0}C overnight, and inoculation of the enriched sample onto CV agar. *V. parahaemolyticus* colonies develop a purple colour on CV agar which distinguishes them from other related bacterial strains.

Results In this study, CV agar has been used to enhance the recovery of *V. parahaemolyticus* in marine samples.

Conclusion The use of CV agar is more sensitive and accurate in detecting *V. parahaemolyticus* than previously used media and although the medium is more expensive, it increases the efficiency of laboratory testing.

EM 14 Biodegradation of resistant oil fractions

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

[1]School of Biosciences, University of Exeter, EX4 4PS; [2]Petroleum & Environmental Geochemistry Group, SEOES, University of Plymouth

Fractions of oil which are resistant to weathering represent large volumes of environmentally toxic waste comprise complex mixtures of chemicals. Such fractions are unresolved by conventional analytical methods. A bacterial consortium enriched from Whitley Bay sediment, UK, has been previously reported to degrade alkylcyclohexyltetralins proposed as model compounds for such oil fractions. In the present study, a single bacterial strain capable of oxidizing 1-(3′-methylbutyl)-7-cyclohexyltetralin, was isolated from the Whitley Bay consortium. Hydrocarbon extraction and GC-MS analysis revealed that after 21 days incubation 63% of the compound had been degraded when compared to abiotic controls. Sequencing of the 16R rRNA gene revealed the organism to have greatest homology with *Rhodococcus* sp. Isolation and fraction of total soluble cell proteins yielded a fraction that exhibited increased oxygen consumption in the presence of tetralin. Observations indicated partial degradation of the compound had occurred; this holds promise for future remediation of sites contaminated with such chemicals.

EM 15 Seasonal effects on microbiological methane cycling in UK forest soils

T.M. Hudson[1], E.I. Vanguelova[2], J.M. Lynch[2], S.K. Burton[1] & H.M. Lappin-Scott[1]

[1]School of Biosciences, University of Exeter, EX4 4QD; [2]Forest Research, Alice Holt GU10 4LH

Oak and Corsican pine stands are being studied over 1 year, to see how seasonality affects methanotroph and methanogen communities in soils. 30 sample points from 90m^2 grid plots were sampled every 3 months. DGGE analysis was carried out on the 16s rRNA genes, and functional genes *mxaF* for methane oxidation and *mcrA* for methanogenesis.

All samples showed a strong presence of the 16s rRNA genes. There is greater presence of the *mxaF* gene in the organic layer compared with the mineral layer for both sites, with wide diversity of methanotrophs present in all organic layer samples. Methanotrophs were present in only half of the mineral layer samples. Methanogens were present in half of organic layer samples and a quarter of mineral layer samples, with lower functional gene replicates overall. Much greater diversity and abundance of bacterial species were seen in oak soils, and seasonal variation was seen at both sites. The data will help refine the quantification and understanding of methane cycling in UK forest soils.

EM 16 Fate of human pharmaceuticals in the aquatic environment: biodegradation and toxicity on aquatic microbes

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

[1]School of Biosciences, University of Exeter; [2]School of Life Sciences, University of Sussex, Falmer

Pharmaceuticals are continuously released into the environment via sewage treatment plants (STP). Although detected in low concentrations in the aquatic environment, many of these substances remain bioactive. Therefore, chronic exposure may be detrimental to naturally occurring microbial communities. In this study, activated sludge collected from a local STP was spiked with different non-steroidal compounds to assess their biodegradation potential. Pharmaceutical degradation was quantified using SPE and LC/ESI-TOF-MS. Changes in the microbial community were monitored by PCR-DGGE. The bacterial consortium was unable to successfully degrade the compound diclofenac suggesting that it may be micro-toxic. Microtoxicity studies of the pharmaceuticals were performed using *Vibrio fischeri* Microtox® test. The assessment of diclofenac toxicity on *Pseudomonas putida*, *Micrococcus luteus*, *Zoogloea ramigera and Comamonas denitrificans* indicated different responses to acute exposure both as mono-cultures and mixed cultures. This evaluation of the laboratory stability of microbial communities exposed to diclofenac is in contrast to its observed degradation in the aquatic environment.

EM 17 Microbial communities in long-term heavy-metal-contaminated ombrotrophic peats

Patricia E. Linton[1], Andrew D.Thomas[1] & Laura Shotbolt[2]

[1]Dept of Environmental & Geographical Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD; [2]Dept of Geography, Queen Mary, University of London, Mile End, London

The southern Pennines of the UK are characterized by expanses of ombrotrophic peat soils that have experienced deposition of high levels of heavy metals since the mid to late 1800s. Concentrations of metals in the peat remain high but the effect of the contamination on the *in-situ* microbial communities is unknown. Geochemical and molecular polymerase chain reaction-denaturing gradient gel electrophoresis and sequencing techniques were used to derive new information on the metal chemistry and microbial populations in peat soils from six locations in the southern Pennines. All sites were highly acidic (pH 3.00–3.14) with high concentrations of potentially toxic heavy metals. The results reveal a split in site characteristics between the most polluted sites with the highest levels of bioavailable metals and those with much lower bioavailable metals. There was no difference in the number of dominant bacterial species between the sites but there were significant differences in the species composition. At the three sites with the highest levels of bioavailable metals, bacterial species with a high similarity to acidophilic sulfur- and iron-oxidizing bacteria and those from high metal environments were detected. Bacteria with similarity to those typically found in forest and grassland soils were documented at the three sites with the lowest levels of bioavailable metals. The data highlight the need for further studies to elucidate the species diversity and functionality of bacteria in heavy metal contaminated peats in order to assess implications for moorland restoration.

EM 18 Effects of phosphate limitation on *Pseudomonas* biofilms – biofilm formation and gene expression

Herve Sanguin[1], Agnieszka Zyśko[1], Eric Paterson[2], Brajesh K. Singh[2] & Michael A. Kertesz[1]

[1]Faculty of Life Sciences, University of Manchester, Oxford Rd, Manchester M13 9PT; [2]The Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH

The bacterial biofilm lifestyle is highly regulated by environmental cues. In the rhizosphere, fluctuations of growth-limiting nutrients such as phosphate (Pi) lead to important changes in bacterial gene expression, colonization and growth. The effect of Pi limitation on biofilm formation was assessed for a range of *Pseudomonas* species isolated from various environments. The results showed a differential effect of Pi concentrations (30 µM–1 mM) on adhesion and biofilm formation by different *Pseudomonas* species. These contrasts may reflect the different host interactions of the various species tested.

The influence of Pi-supply on *Pseudomonas* gene expression in biofilms was then studied in sand microcosms inoculated with the rhizobacterium *P. aeruginosa* 7NR, using Affymetrix microarrays. The results confirmed the Pi limitation effect on well known, Pi-regulated genes implied in phosphorus metabolism during planktonic growth, but also revealed novel Pi-regulation of genes implied in biofilm architecture or the iron starvation response.

EM 19 Molecular responses of *Pseudomonas aeruginosa* 7NSK2 to phosphate deficiency in the rhizosphere

Agnieszka Zyśko[1], Hervé Sanguin[1], Brajesh K. Singh[2], Eric Paterson[2] & Michael A. Kertesz[1]

[1]University of Manchester, Oxford Road, Manchester M13 9PT; [2]Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH

Phosphate availability is often limited in the soil, and plants and microbes have therefore evolved mechanisms to sense phosphate limitation and optimize phosphate scavenging mechanisms. Plant root exudates are important in mobilizing bound P for plants, but they also modulate bacterial gene expression in the rhizosphere. Little is known about how these exudates mediate molecular signals between rhizobacteria and plants in phosphate-deficient environments.

In this study, the interactions between P-starved ryegrass (*Lolium perenne*) and gene expression in the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 were investigated under phosphate limiting conditions. Bacterial gene expression in the rhizosphere was measured by microarray analysis, and a rhizosphere P-regulon was identified. RT-qPCR was then used to compare the expression of 23 bacterial genes in cells grown in the rhizosphere, in liquid culture and on artificial roots under two Pi regimes (1mM/0.33mM and 0.03mM). Analysis of differentially expressed genes allowed us to identify new genes responsible for plant-microbe interactions and survival in phosphate-deficient environments.

EM 20 Diversity of sulfonate utilizing bacteria in grasslands

Achim Schmalenberger[1,2] & Michael Kertesz[1]

[1]University of Manchester, Faculty of Life Sciences, Manchester; [2]University of Sheffield, Kroto Research Institute, Sheffield

The rhizosphere is known as a hot spot for bacterial growth since plant roots secrete compounds that can be utilized by the bacteria. However, the rhizosphere is also depleted of sulfate due to efficient sulfate-uptake by the plant root. Growing bacteria have to utilize alternative sulfur sources such as sulfonates, a major sulfur source in soil.

This study identified high concentrations of cultivable desulfonating bacteria in rhizospheres of *Agrostis* in semi-improved grasslands. A significantly higher concentration of cultivable desulfonating bacteria was found in the rhizosphere compared to the bulk soil. Isolates harbouring the desulfonating key gene *asfA* were affiliated to the genera *Variovorax*, *Polaromonas*, *Flavobacterium* and *Rhodococcus*. Microcosm studies with *Agrostis* identified a shift in the bacterial community when the microcosms where starved of inorganic sulfate and compared to ones with increased sulfate levels using DGGE on the 16S rRNA gene and T-RFLP on the *asfA* level. The results suggest that desulfonating bacteria play an important role in sulfate limited, non-agricultural environments.

EM 21 Biologically functionalized sol-gel coatings for combating corrosion

Jeanette Gittens[1,2], Heming Wang[1], **Thomas J. Smith**[2] & Robert Akid[1]

[1]Materials and Engineering Research Institute; [2]Biomedical Research Centre, Sheffield Hallam University, Sheffield S1 1WB

Corrosion of metals, alloys and other materials is a major cost to many industries and a significant threat to safety. Microbial-induced corrosion (MIC), which includes attack of materials by corrosive species produced by sulfate-reducing bacteria (SRB), is a major form of corrosion that is traditionally minimized by the use of biocides, which are both toxic and prone to leach into the environment. An alternative approach is to use bacteria that actually inhibit corrosion, possibly via depleting oxygen at the material surface and/or by secreting antibiotics that inhibit growth of SRB. Recently we have developed sol-gel coatings inoculated with bacterial endospores that protect against corrosion in field trials for at least six months and perform substantially better than similar coatings without added bacteria. Here we describe optimization of the procedure for immobilizing the bacterial spores toward effective industrial application of this technology.

EM 22 Ectomycorrhizal-mediated apatite and biotite weathering

M.M. Smits[1], S.J. Haward[2], S. Bonneville[3], J.R. Leake[1], T.J. McMaster[2], K.V. Ragnarsdottir[2], L.G. Benning[3] & S.A. Banwart[1]

[1]Dept of Animal & Plant Sciences, University of Sheffield, S10 2TN; [2]University of Bristol, BS8 1TH; 3University of Leeds, LS2 9JT

Ectomycorrhizal fungi extend into the soil from tree roots, obtaining plant carbon in return for supplying soil nutrients. These fungi are known to accelerate mineral weathering, but the exact rates and mechanisms involved are unclear. We have developed novel microcosm reactors enabling plant-ectomycorrhizal fungal interactions with minerals to be quantified with nano-scale physical and chemical imaging technologies. Pine (*Pinus sylvestris*) trees mycorrhizal with *Paxillus involutus* were grown with apatite grains or biotite flakes in specific weathering arenas that only the fungus can colonize. Plant-to-fungus carbon flux, measured with ^{14}C tracer, was preferentially allocated to arenas with apatite grains and these experienced threefold increased weathering. Atomic-force microscopy of hyphae on biotite flakes confirmed their strong hydrophobicity and revealed rod-like putative hydrophobin domains, whereas FIB sections and TEM showed biotite alteration starting to occur underneath hyphae 20 µm from the growing tip. These studies are starting to reveal the nano-scale interactions between mycorrhizal hyphae and weathering minerals.

EM 23 The application of quantitative polymerase chain reaction to determine the relative contributions of difference sources of faecal pollution impacting a shell fishery

A.W. Davies, G.C. Thomas, A. Murray, **S.M. Parker** & R. Sleat

EnviroGene Ltd, Tredomen Innovation & Technology Centre, Ystrad Mynach, Hengoed CF82 7FQ

Molecular microbial source tracking seeks to identify the source of faecal bacterial contamination in the environment through the identification of gene sequences within micro-organisms that are specific to their host animal (i.e. bovine or human). Once the gene sequences have been identified they can be detected in environmental samples through the use of polymerase chain reaction (PCR). However, conventional PCR is a qualitative technology that simply allows for the determination for the presence or absence of faecal bacteria of a specific host origin. It is important to understand the relative contributions of individual sources under circumstances where multiple sources are impacting. This can be achieved through the application of quantitative polymerase chain reaction (qPCR).

This quantitative approach was applied to study the relative contributions of difference sources of faecal pollution impacting on a shell fishery farm in the Fal estuary, Cornwall. Target faecal bacteria were *Enterococcus* sp., *Bacteroides* sp. and *E. coli*. Multiple sources of faecal pollution were identified with bovine sources predominating following rainfall events. Lower background levels of human faecal pollution occurred irrespective of rainfall events.

EM 24 A dimethylsulfonioproprionate lyase enzyme that liberates the climate-changing gas dimethyl sulfide in marine bacteria

Andrew R.J. Curson, Rachel Rogers, Jonathan D. Todd, Charles A. Brearley & Andrew W.B. Johnston

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

The bacterial breakdown of dimethylsulfonioprionate (DMSP) to dimethyl sulfide (DMS), a gas which affects cloud formation over the oceans, was postulated to occur via a bacterial 'DMSP lyase', yielding DMS and acrylate. Until recently, very little was known about the molecular aspects of this process, but Todd *et al.* (2007) showed that some bacteria use an acyl-CoA transferase, DddD, as the first step in DMSP catabolism, not the previously predicted 'lyase'.

Some DMS-producing bacteria lack *dddD* in their genomes, so must make DMS by other methods. We identified a single gene, *dddL*, from one such strain, the marine alphaproteobacterium *Sulfitobacter* EE-36, which when transferred to *E. coli* conferred the ability to form DMS plus acrylate from DMSP. This first DMSP lyase to be identified at a molecular level belongs to a wholly novel family of enzymes whose mode of action is unknown, but which has functional homologues in other marine bacteria, including the much-studied *Rhodobacter sphaeroides*.

EM 25 Ammonifying bacteria and nitrous oxide

Daniela Hensen & David Richardson

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

As the gas nitrous oxide (N_2O) plays a vital role in global warming processes, it is important to clearly identify different emission sources and the amount of nitrous oxide produced, respectively. Agricultural soil management is responsible for the largest amount of nitrous oxide emitted into the atmosphere. The gas may be a result of different processes in soil: nitrification, nitrifier denitrification, anaerobic and aerobic denitrification and ammonification. The contribution of nitrate ammonifiers to the N_2O emission on the whole is still unknown.

We used pure culture approaches with different ammonifying proteobacteria (genera *Escherichia*, *Salmonella*, *Klebsiella* and *Wolinella*) to shed some light on this issue.

Despite ammonification being the main pathway for nitrite reduction, we found significant amounts of nitrous oxide released by the organisms under the chosen experimental conditions. Several proteins involved in dissimilatory nitrogen metabolism were shown not to be essential for N_2O production. However, a reduction of approximately 50% could be achieved in a *Salmonella* mutant deficient in *nrfA*, *norV* and *hmpA*. Additional proteins involved in this process remain to be identified.

EM 26 From chicken feather to feather meal – a protein-rich feed

Priya Ramnani[1] & Rani Gupta[2]

[1]University of Reading, Reading RG6 6AP; [2]University of Delhi, New Delhi 110021, India

Feather wastes are generated in burgeoning amounts every year leading to troublesome environmental pollution. Chicken feathers comprise almost 90% structural protein and can thus serve as a rich protein source. Recycling of these wastes is tough owing to the highly rigid, strongly cross-linked structure of keratin. Until recent years, this waste was baked at high temperatures and pressure which is an energy intensive process and resulted in destruction of essential amino acids.

Keratinolytic micro-organisms serve as attractive biotechnological alternatives to convert feather into nutritionally rich feather meal. In the present study a potentially keratinolytic strain of *Bacillus licheniformis* RG1 was used for degrading chicken feather. Complete degradation (97%) by the bacterium was statistically optimized using Response Surface methodology – Face Centered Central Composite design (RSM-FCCCD). The feather meal generated was scaled up in a 50L bioreactor and subjected to freeze drying. The amino acid profile was comparable to soybean meal – the most popular chick feed. It was rich in essential amino acids particularly tryptophan, phenylalanine and tyrosine and can thus replace soybean meal as the dietary supplement.

EM 27 Development of an *in situ* method to visualize bacterial cells at high hydrostatic pressures

L. Black[1], G. Ferguson[2], H. Vass[1], F.B. Ward[1] & R. Allen[1]

[1]University of Edinburgh; [2]University of Aberdeen

The physiological effect of hydrostatic pressure on bacteria is being investigated for two main reasons. Firstly, in food processing, high hydrostatic pressure is a potential method to inactivate bacteria and to reduce spoilage. A second important motivation is to understand the physiological adaptations of deep sea bacteria which make them resistant to hydrostatic pressure. The majority of commercial pressure cells are large in size; they are designed to withstand pressure greater than 1 kBar in order to study the bactericidal effects of high pressure. These instruments also lack instrumentation to measure cell physiology. Deep sea bacteria, such as *Photobacterium profundum*, have a much lower optimum pressure for growth (280 bar). Here we review the development of small high pressure cells to study bacteria *in situ* and report a method of imaging single cell bacteria at pressures up to 1 kBar. The method allowed visualization of single fluorescently labelled cells and will facilitate future studies of cell behaviour.

EM 28 Molecular ecology of green sulfur bacteria in a meromictic Swiss lake (Lago Cadagno)

Raymond P. Cox[1], Lea H. Gregersen[3], Donald E. Canfield[2], Niels-Ulrik Frigaard[3], Kirsten Habicht[2] & Mette Miller[1]

[1]Dept of Biochemistry & Molecular Biology and [2]Nordic Center for Earth Evolution, University of Southern Denmark, 5230 Odense M, Denmark; [3]Dept of Molecular Biology, University of Copenhagen, 2200 København N, Denmark

Lago Cadagno is a meromictic lake in the Swiss Alps. The anoxic layers of the water column contain sulfide and large populations of phototrophic green and purple sulfur bacteria. High concentrations of bacteriochlorophyll e (more than 100 µg/L) were found in samples from all depths in the anoxic water column. Clone libraries were made from water samples taken at different depths in August 2006 using universal primers for the 16S rRNA gene in Bacteria. More than 60% of the recovered sequences both from the top of the anoxic zone (11.5 m) and from 15 m were identical to each other and closely related to the brown-coloured green sulfur bacterium *Chlorobium pheoclathratiforme* 4DE. Light absorbtion from the pigments the water column makes it highly unlikely that the bacteria in the lower sample are actively photosynthetic.

EM 29 Auxin producing bacteria: 16S rRNA identification and their auxin quantification

Ambreen Ahmed & Shahida Hasnain

Dept of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

Plant associated bacteria are involved in symbiotic microbial activities and play a major role in the growth and development of plants. Beneficial effects of these plant growth promoting rhizobacteria have been attributed to mechanisms such as biological nitrogen fixation and secretion of phytohormones such as IAA, cytokinins, gibberellins etc. The aim of the present work is the isolation and identification of the auxin producing bacterial strains and quantification of auxins produced by the bacteria. Auxin producing bacteria were isolated from rhizosphere of different plants. These bacteria were then characterized morphologically, physiologically and biochemically. Auxin production by the bacteria was investigated using colorimetric method and then auxin production was confirmed and quantified through high performance liquid chromatography (HPLC). Bacterial DNA was isolated and purified and then amplified by polymerase chain reaction (PCR). The amplified DNA is then used for the identification of bacteria through 16S rRNA ribotyping and the isolated auxin producing bacteria were found to belong to *Bacillus* and *Enterobacter* species.

Keywords *Enterobacter*, *Bacillus*, PGPR, auxin, HPLC, ribotyping

EM 30 Restoration of bioavailability of BTEoX in soil slurries by a non-ionic surfactant, overall effect on BTEoX and MTBE biodegradation

Juan Francisco Villarreal-Chiu, Miriam Veronica Gracia-Lozano, Maria Teresa Garza-Gonzalez & Karim Acuna-Askar

Laboratorio de Biorremediacion Ambiental, Facultad de Medicina, UANL, Madero Pte y Dr. Aguirre, 64460 Monterrey, N.L., Mexico

Hydrocarbons spills are considered to be environmental emergencies due to the fact that they can put in danger the integrity of the ecosystems, as well as the preservation of the natural resources. In this study the biodegradation rates of BTEoX (Benzene, Toluene, Ethylbenzene and ortho-Xylene) and MTBE (Methyl tert-Buthyl Ether) by a BTEX-acclimatized biomass and the effect of soil and a non-ionic surfactant on the biodegradation efficiency were evaluated. Controls and three sets of samples were measured (Control1: 50mg/L of every compound, Control2: Control1+1g sterile soil, Control3: Control2+25mg/L Tergitol NP10; Sample1: Control1+800 mg/L VCC, Sample2: Sample1+1g of sterile soil, Sample3: Sample2+25 mg/L Tergitol NP10). BTEoX and MTBE were analysed by a Varian 3400 GC/FID chromatograph. The biomass was able to degraded the compounds as follow: T(99.8%) >E(99.2%) >B(99.0%) >>oX(50.1%) >>MTBE(9.9%) after 36h of experiment. The addition of soil produces several changes in the biodegradation rates: E(98.2%) >T(97.9%) >B(77.0%) > oX(54.8%) > MTBE(16.1%), while the addition of Tergitol NP10 produced an increase on the biodegradation rates: E(99.7%) >T(99.6%) >B(96.3%) >oX(65.3%) >MTBE(43.0%).In conclusion, soil produces a negative effect on BTE biodegradation, probably attributable to the physicochemical properties of soil´s surface which may adsorb these compounds, while MTBE and oX consumption were benefited by the diminution of the bioavailability of BTE. Tergitol NP10 restores the bioavailability of BTE by increasing the solubility of these compounds and making oX and MTBE more suitable for assimilation.

EM 31 The comparative characteristic of morphological and functional parameters of two nostoc species, growing in water and terrestrial environments

E.N. Patova & **M.D. Sivkov**

Institute of Biology Komi Sci Center Ural Div., Russian Academy of Science (Email pat_el@mail.ru)

Study of morphological and functional features of two cyanobacteria species which grow in different ecological conditions was carried out. The materials were collected in natural habitats in East-European tundras. Populations of two species that are capable to form macroscopic colonies were investigated. *N. pruniforme* Ag. inhabits water environment and *N. commune* Vach. is typical for the terrestrial ecosystems.

		Photosynthesis, mg CO_2		Respiration, mg CO_2		Nitrogenase activity, mkg C_2H_4	
		on g_{dw}^{-1} h^{-1}	on dm^{-2} h^{-1}	on g_{dw}^{-1} h^{-1}	on dm^{-2} h^{-1}	on g_{dw}^{-1} h^{-1}	on dm^{-2} h^{-1}
N. commune	Mean	**4.05**	**0.34**	**2.24**	**0.21**	**167.0**	**15.5**
	SD	1.28	0.11	0.52	0.05	79.8	7.4
	Max.	5.90	0.55	2.83	0.26	296.7	27.5
N. pruniforme	Mean	**1.47**	**0.52**	**0.54**	**0.19**	**72.2**	**28.1**
	SD	0.31	0.11	0.13	0.05	5.8	3.5
	Max.	1.69	0.60	0.63	0.22	76.3	30.6

The investigation of morphological characteristics, ecological conditions of natural habitat, functional characteristics (carbon dioxide gas exchange and nitrogen-fixing activity) of both species was carried out.

N. pruniforme typically forms large spherical colonies up to 4 cm in diameter, that weight up to 12 g. The thickness of periderm reaches up to 1 mm. The trichomes are 4.5–8 μm wide, cells – 3.2–7.5 μm long, heterocytes – 6–9 μm diameter.

For *N. commune* plane growth is common. The size of colonies is up to 5 cm^2; thickness is up to 3 mm. Cells sizes are 2.5–4.5 μm wide, 3.5–5 μm long, heterocytes 5–6 μm.

Functional parameters of *Nostoc* species are shown in the table above.

In the table: dw – dry weight; average, standard deviations of average and the maximal size (from 5 replicated observations in each variant). Measurements are carried out at 20°C.

The comparison of findings for dry weight showed essential differences of functional parameters between two species. The reason of divergence between parameters of water and terrestrial *Nosto*cs is due to different distribution of heterocytes in the macrocolony volume. In *N. pruniforme* colonies the majority of heterocytes are located along a periderm surface, in contrast to *N. commune* there they are situated in regular intervals. This fact is confirmed by calculations on area unit of macrocolonies that revealed practically identical speeds of the investigated functional parameters. Higher speed nitrogenase activity of *N. pruniforme* (table) is explained by rather large size of its heterocytes.

Thus, comparative measurements had shown, that *N. pruniforme* as well as *N. commune* is capable to fix nitrogen of atmosphere with high speeds that specifies high status of this cyanobacteria in the general nitrogen exchange in tundra environment.

EM 32 Detection of microcystin synthetase genes in cyanobacteria from Lake Baikal and water reservoirs of East Siberia

A.S. Gladkikh, I.V. Tikhonova & O.I. Belykh

Limnological Institute of Siberian Branch of Russian Academy of Sciences, 3, Ulan-Batorskaya, P.O. Box 278, Irkutsk 664033, Russia

Cyanobacteria is a worldwide group of organisms, some of them are known to produce toxins implicated in animal and human poisoning incidents. Therefore, early revealing of toxin producing cyanobacteria is very important for studies of ecological state of a water body.

The aim of our work was to analyse populations of cyanobacteria from Lake Baikal, as well as from Irkutsk, Bratsk, Ust-Ilimsk, Boguchansk, and Bereshskoye reservoirs. The species composition was studied by light microscopy. We studied toxin producing species with polymerase chain reaction (PCR) and determined nucleotide gene sequence of toxin synthesis. Gene *mcy*A was used as a marker, a coding fragment of microcystine peptide synthetase.

Gene *mcy*A was not revealed in the total DNA of Lake Baikal cyanobacteria and in phytoplankton of Irkutsk and Boguchansk reservoirs. PCR-products of gene mcyA were obtained in positive control reactions and as a result of testing of phytoplankton DNA samples from Ust-Ilimsk, Bratsk and Bereshskoye reservoirs. Nucleotide sequences of these fragments and the subsequent BLAST-analysis showed that they belong to *Microcystis aeruginosa* and cyanobacteria of the genus *Anabaena*. Thus, potentially toxic cyanobacteria was detected in these reservoirs.

EM 33 Effect of sugar factory wastes on physicochemical and microbial soil properties

M. Nagaraju, G. Narasimha & V. Rrangaswamy

Dept of Microbiology, National P.G. College, Nandyal – 518502, India

The influence of pollution by sugar industry effluents on physicochemical and microbial properties of black soils of the southern Indian was investigated. To achieve these objectives, soil samples with (test) and without (control) effluent discharges were collected from selected surrounding areas of Sri Rayalaseema Sugar and Energy Limited, Nandyal, Andrapradesh, India.

In this work we found that soils exposed to pollution by effluents have undergone changes in all measured parameters. Physicochemical and microbial properties of soils samples were determined by standard procedures. The soil texture in terms of percentage of sand, silt and clay were 51, 29, 20 of the test and 64, 22 and 14 of the control respectively. These results indicated that the effluents discharged soil had relatively lower sand and higher silt and clay contents than the control soil. High water holding capacity and electrical conductivity were observed in contaminated soil than in the control. The values were 0.34 ml/g, 1.71 μS/cm and 0.28 ml/g, 0.24 μS/cm of the test and the control respectively. The pH of the polluted soil was reduced from 8.30 to 7.62 upon the release of effluents from sugar factory. Organic matter, total nitrogen and phosphorous contents of the test soil were nearly two fold higher than the control soil and the values of the polluted soils were 6.432 g/kg, 0.22 g/kg and 8.21 mg/g against 3.602 g/kg, 0.14 g/kg and 4.25 mg/g of the control respectively. The microbial flora of both soil samples were enumerated. Three fold higher bacterial and two fold higher fungal populations were observed in the polluted soils over the control soils.

It is concluded that discharge of sugar industry effluents into soils increased all the measured physicochemical and microbial parameters except pH and sand percentage over undischarged soils.

EM 34 The influence of sulfur compounds on phosphorus removal from wastewater

M. Lai, A. Kulakova, J.P. Quinn & J.W. McGrath

QUESTOR Centre and School of Biological Sciences, Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Rd, Belfast BT9 7BL

Phosphorus (P) is one of the most important factors contributing to the eutrophication of freshwater. Therefore its removal from industrial and municipal wastewaters is required by European legislation with a biological process regarded as preferable by the water industry.

In this project six sulfur-containing compounds were tested regarding their influence on the P removal performance of activated sludge and Polytox (a surrogate activated sludge inoculum). It was shown that three compounds – sodium sulfite, hydrosulfite and potassium tetrathionate – significantly increased P removal, by up to 40%. Our analysis has shown an increase in yeast cell numbers in the activated sludge. DAPI and Neisser staining demonstrated the presence of polyphosphate granules in bacterial as well as yeast cells.

Posters

Fermentation & Bioprocessing Group

FB 01 Analysis of recombinant protein production in *Escherichia coli* and its effects on the organism, during batch and fed-batch fermentation

Andrew J. Want[1], Owen R.T. Thomas[1], Christopher J. Hewitt[2], Bo Kara[3] & John Liddell[3]

1University of Birmingham, Dept of Chemical Engineering, Edgbaston Park Road, Edgbaston, Birmingham B15 2TT; [2]Interdisciplinary Centre for Biological Engineering, Chemical Engineering, Loughborough University, Leicestershire; [3]Avecia Biotechnology, P.O Box 2, Belasis Avenue, Billingham TS23 1YN

Due to the overwhelming body of knowledge surrounding *E. coli*, it is a natural choice for use as a vector for the manufacture of recombinant biological products. The aim of this work was to attempt to improve the fate of the micro-organisms and gain some measure of control over the breakdown of said organisms by adjusting fermentation parameters or other extraneous factors. We have utilized flow cytometry alongside more traditional techniques, to better understand the effect of protein overexpression on the *E. coli* cell using differing modes of operation and inducer concentration. We have seen that the effort expended in directing the product in question to the periplasm has been misspent, with widespread cell fragmentation and product aggregation resulting from induction of protein expression.

FB 02 The effect of cryopreservation on α-amylase production by 5 l batch *Bacillus licheniformis* fermentations

Nichola H. Hancocks[1], Colin Thomas[1], Stuart M. Stocks[2] & Christopher J. Hewitt[3]

[1]Chemical Engineering, University of Birmingham, Edgbaston; [2]Novozymes Fermentation Pilot Plant, Smørmosevej 25, 2880 Bagsvaerd, Denmark; [3]Interdisciplinary Centre for Biological Engineering, Chemical Engineering, Loughborough University, Leicestershire

Excellent product quality coupled with a high yield is the ultimate goal for any research and development programme leading to the large-scale production of microbial based products at the commercial scale. This paper discusses the optimization of the cryopreservation of *Bacillus licheniformis* cell banks used as inoculum for α-amylase producing 5L batch fermentations, as the quality of the inoculum is often overlooked which is surprising since it can have a significant effect on overall process performance. The effect of the presence of various cryopreservants including glycerol, Tween 80, dimethyl sulfoxide and trehalose on final biomass and α-amylase concentration was investigated using optical density, dry cell weight, colony forming units, and multi-parameter flow cytometry. It was found that the concentration and type of cryopreservent used had a profound effect on resuscitation recovery immediately after thawing but counter intuitively little effect on overall process performance.

FB 03 The characterization and optimization of biological hydrogen production by *Escherichia coli* HD701: a complex problem

Michael Sulu[1], C.R. Thomas[1] & C.J. Hewitt[2]

[1]Chemical Engineering, University of Birmingham, Birmingham; [2]Interdisciplinary Centre for Biological Engineering, Chemical Engineering, Loughborough University, Leicestershire

E. coli HD701, a hydrogenase up-regulated strain has the potential for industrial–scale H_2 production on an energetically self-sufficient basis. Current experimental work is investigating transferring growth from shake flask culture to 5 l lab-scale fermenters. The accompanying increase in cell density is not the only factor that needs to be addressed when making such a process change; *E. coli*, converts formate to H_2 using the membrane bound formate hydrogen lyase (FHL) complex, The transcription of this complex is dependent on the intracellular [formate], which is driven by the [formate] in the medium. As a result of this complication a process has been derived that allows the bacteria to grow with approximately the same amount of oxygen limitation in a 5L lab scale fermenter as is present in a shake flask culture, giving mixed acid fermentation products that include a high [formate] whilst simultaneously increasing cell density.

FB 04 Mutagenesis of soluble methane monooxygenase toward novel applications in bioremediation synthetic chemistry

Tim Nichol[1], Elena Borodina[2], Hélène Moussard[2], Thomas J. Smith[1] & J. Colin Murrell[2]

[1]Biomedical Research Centre, Sheffield Hallam University, Sheffield S1 1WB; [2]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

As well as its role in biological methane oxidation, soluble methane monooxygenase (sMMO) co-oxidizes diverse adventitious substrates and consequently has many suggested applications in bioremediation and synthetic chemistry. The catalytic versatility of sMMO, however, has significant limitations. For example, triaromatic hydrocarbons (which are serious pollutants) are not oxidized, probably because steric factors prevent their access to the active site. Previous site directed mutagenesis of the proposed gating residue Leu110 showed that this is a critical residue in determining regioselectivity with smaller substrates, but none of the mutants could oxidize triaromatics. Here we report development of a new vector system and screening methods for directed evolution of sMMO toward oxidation of triaromatic compounds and identification of the residues that limit the size of substrate that can enter the active site. Our expression system for sMMO, which uses a methane-oxidizing bacterium as the expression host, also permits production of biocatalysts using methane as the starting material.

Posters

Food & Beverages Group

FdBev 01 Investigating the effect of homogenization methods and detergents on recovery of micro-organisms from fatty minced beef

Priya Ramnani & Bernard Mackey

Dept of Food Biosciences, University of Reading, Reading RG6 6AP

There is concern that standard sample preparation methods may not be adequate for recovering micro-organisms from fatty foods including certain meat preparations and cheeses. The effects of detergents (Tween-80, SDS and Triton X-100) were examined on recovery of bacteria from minced beef containing 25% fat. Two methods of homogenization – stomacher where paddling action was used for bacterial recovery and pulsifier in which a metallic ring produced shock waves to dislodge bacteria were compared. Samples were homogenized in diluent containing 0, 0.2, 0.5, 1, 2, 2.5% Tween-80, SDS or Triton X-100. Tween-80 at a concentration of 2.5% improved recovery of aerobic plate count and Enterobacteriaceae count by approximately 2-fold. Triton X-100 and SDS on the other hand, decreased recovery. There were no significant differences in recovery using stomacher or pulsifier. Recovery of known numbers of *Escherichia coli*, *Staphylococcus aureus* and *Campylobacter jejuni* added to irradiated minced beef. showed that both Gram-positive and -negative bacteria were recovered at 94–100% indicating that variable surface properties of bacteria may not affect interaction with fat particles. At the concentrations used, Tween was not inhibitory to freeze- or heat-injured cells.

FdBev 02 Isolation and characterization of anti-*Listeria* bacteriocin producing lactic acid bacteria and their bacteriocins from raw milk

Panagiotis Chanos & D. Ross Williams

University of Lincoln, Brayford Pool, Lincoln LN6 7TS

This work aimed to investigate bacteriocins active against *Listeria monocytogenes*. The bacteriocins were obtained from lactic acid bacteria isolated from raw sheep milk originating from small producers in northern Greece. Following isolation on differential media and at different temperatures, the bacteriocin producing strains were physiologically characterized and the activity of the bacteriocins produced was tested against other common food-borne pathogens. A molecular characterization using rep-PCR and $(GTG)_5$ primer combined with sub-species specific PCR was also done. The presence of genes encoding the production of known bacteriocins in the bacterial genome was also investigated with specific PCR. Results were accumulated regarding Gram reaction; growth at different temperatures and NaCl concentrations; catalase and oxidase reaction and heterofermentation of the strains. A phylogenetic tree was derived from the rep-PCR and correlated with the results from the specific PCR of the strains.

Posters

Microbial Infection Group

MI 01 Antimicrobial activity of nanoparticulate metals and metal oxides

Miguel Vargas-Reus[1], Guogang Ren[2] & Rob Allaker[1]

[1]Institute of Dentistry; [2]Dept of Materials, Queen Mary, University of London, Blizard Building, 4 Newark Street, London E1 2AT

The field of nanotechnology is experiencing rapid growth, with many and diverse potential applications being explored in the biomedical field, including the control of infectious diseases. Nanoparticulate silver and copper, and their compounds have been widely studied. In particular, nano silver particles have been reported to inactivate most micro-organisms, including HIV-1. Thirteen different latest generation nanoparticle preparations, including those based upon metals and metal oxides, were examined for their bacteriostatic and bacteriocidal effects. Significant activity with 5 of these preparations against bacterial pathogens, including meticillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, was demonstrated. Minimum bacteriocidal concentrations were found to be in the 100 to 5000 µg/ml range. In order to minimize potential toxic and resistance problems, mixtures of different nanoparticles were also tested. A 100% reduction of microbial populations (10^7 c.f.u./ml) within 60 minutes was achieved using this approach. Exploitation of the toxic properties of nanoparticulate metals and metal oxides are now being explored in antimicrobial formulations within and outside the hospital environment.

MI 02 Targeted lethal photosensitization of meticillin-resistant *Staphylococcus aureus*

Samantha Packer, Sean Nair & Michael Wilson

Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD

Light-activated antimicrobial agents are promising alternatives for the treatment of topical infections, particularly meticillin-resistant strains of *Staphylococcus aureus* (MRSA) which are one of the major causes of hospital-acquired infections worldwide. Lethal photosensitization involves the use of a photosensitizer which, when exposed to light of a suitable wavelength, produces singlet oxygen which destroys cell walls and membranes resulting in cell death. We have investigated the use of white light irradiation in combination with a novel targeted photosensitizer, comprising a tin (IV) chlorin e6 (SnCe6)-bacteriophage conjugate, for the photodynamic inactivation of *Staphylococcus aureus*. Substantial kills of *S. aureus* 8325-4 and MRSA-16 were achieved using low concentrations of the conjugate (containing 1.75 µg/ml SnCe6). On a concentration equivalent basis, the conjugate was a more effective bactericide than the unconjugated SnCe6 when irradiated with white light. The results of this study have shown that a bacteriophage can be used to deliver a photosensitizer to *S. aureus*, including MRSA, resulting in enhanced killing over that obtained using a photosensitizer alone upon irradiation with white light.

MI 03 Lethal photosensitization of *Staphylococcus aureus* using a tin chlorin e6-gold nanoparticle

Linda Dekker[1], Jesús Gil-Tomas[2], Naima Narband[2], Sean Nair[1], Ivan Parkin[2], Cale Street[3] & Michael Wilson[1]

[1]Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD; [2]Dept of Chemistry, University College London, 20 Gordon Street, London WC1H OAJ; [3]Ondine Research Laboratories, 19017-120th Ave NE, Bothell, WA 98011, Canada

Light-activated antimicrobial agents (LAAAs) such as tin chlorin e6 (SnCe6) are promising alternatives to conventional antibiotics for the treatment of topical infections. Upon exposure to light of a suitable wavelength these reagents produce free radicals and reactive oxygen species that cause non-specific damage to the bacterial cell which leads to death. Gold nanoparticles have unique physical and chemical properties that make them attractive as molecular scaffolds and which we have hypothesized may potentiate the effectiveness of LAAAs. In this study we set out to synthesize a light-activated antimicrobial nanoparticle by covalently attaching SnCe6 to gold nanoparticles through a glutathione linker. This novel nanoparticle had potent antimicrobial activity towards *Staphylococcus aureus* when activated by white light or 632 nm laser light.

MI 04 Inhibition of staphylococcal virulence factors using light-activated antimicrobial agents

S. Tubby[1], M. Wilson[1], C. Street[2] & S. Nair[1]

[1]Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD; [2]Ondine Research Laboratories, 19017-120th Ave NE, Bothell, WA 98011, Canada

A limitation of antibiotic therapy is that even after successful elimination of the infecting organism, virulence factors may still be present and cause significant damage to the host. Light-activated antimicrobials show potential for the treatment of topical infections; therefore if these agents can inactivate virulence factors, this would represent an advantage over antibiotic treatment. *Staphylococcus aureus* produces a number of virulence factors that contribute to its success as a pathogen by facilitating colonization and destruction of host tissues. In this study, the effect of the light-activated agent methylene blue in combination with laser light of 665nm on the activity of staphylococcal virulence factors was investigated. Virulence factors were exposed to laser light in the presence of methylene blue and their activities determined. The activities of V8 protease, α-haemolysin and sphingomyelinase were shown to be inhibited in a dose-dependent manner, suggesting that photodynamic therapy may be able to reduce the virulence potential of *S. aureus*.

MI 05 The effect of fluence rate on the lethal photosensitization of wound-associated organisms using indocyanine green and near-infrared laser light

G. Omar, M. Wilson & S.P. Nair

Division of Microbial Diseases, UCL Eastman Dental Institute, University College London

The management of wound infections is a serious problem due to the emergence of antibiotic-resistant bacteria; consequently novel antimicrobial approaches are needed. The effect on the viability of *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* of exposure to indocyanine green using high and low fluence rates of 808 nm laser light was investigated. Bacterial suspensions were irradiated using high or low fluences in the presence or absence of indocyanine green and the surviving bacteria enumerated by viable counting. Both a high fluence of 1.37 W/cm^2 and a low fluence of 0.05 W/cm^2 were able to kill up to 99.999% of *S. aureus* and *Strep. pyogenes*. A high fluence irradiation results in kills of 99.998% of *P. aeruginosa*; while a kill of 80% was achieved using a low fluence of 0.07 W/cm^2. These findings imply that indocyanine green in combination with high or low fluence 808 nm laser light may be an effective means of eradicating bacteria from wounds.

MI 06 The conjugative transposon Tn*5397* specifically disrupts the fic genes in the hypervirluent *Clostridium difficile* 027 strain R20291

Haitham A. Hussain, Adam P. Roberts & Peter Mullany

Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD

Tn*5397* is a tetracycline resistance encoding conjugative transposon that was originally isolated from *Clostridium difficile* 630. Work from our lab has shown that this element can be transferred between *C. difficile* strains and to and from *Bacillus subtilis*. Previous work has also shown that the element has a preferred insertion site into which it will always insert. However if the site is absent then it will insert into other sites in the genome, the only obvious sequence requirements being a GA dinucleotide at the cross over region. In this work a second preferred insertion site was identified in the hyper virulent 027 strain R20291. Tn*5397* uses these sites with equal frequency. The construction of a chloramphenicol-resistant derivative of Tn*5397* Tn*5397*ΔC, showed that both sites can also be occupied simultaneously. The target site for Tn*5397* is within an open reading frame that has the potential to encode a protein homologous to Fic from *E. coli*. Although these genes have not been well investigated in bacteria they have a role in maintaining cell shape and in cell division. The strain that contains a Tn*5397* insertion in 2 *fic* homologues no longer forms filamentous cells.

MI 07 Molecular detection of leptospirosis in UK wildlife

Sally J. Cutler[1], Dikber Hussain[1], Lidia Lasecka[1], Anna Meredith[2], Darren J. Shaw[2] & Sarah Cleaveland[2]

[1]University of East London; [2]R(D)SVS, University of Edinburgh

Background Leptospirosis is a neglected, yet significant global zoonosis affecting numerous mammalian species. We looked at molecular detection of leptospirosis in wild rodents and foxes, as a preliminary part of an ongoing study evaluating the use of carnivores as sentinels for infectious disease.

Methods Rodents and foxes were euthanased, kidneys collected and stored frozen at –70°C until analysis. Isolated DNA was initially screened using real-time PCR targeting the rRNA gene, with reactive samples confirmed by conventional PCR using the *gyr*B gene. Identification of the causative *Leptospira* spp. was achieved using a 'species-specific' PCRs and DNA sequencing of a sub-set of reactive samples.

Results A total of 17.5% 268 of rodent kidneys showed evidence of infection. Of these, 68% could be confirmed using conventional PCR, however, this proved to be less sensitive than the real-time screen used. Identification of leptospires present revealed that most 28/47} belonged to *L. borgpetersenii*, while only 2/47 were *L. icterohaemorrhagiae*. 17/47 failed to react with species specific primers. None of 18 fox kidneys were positive for *Leptospira*.

Conclusion The techniques described successfully detected infection with a number of different leptospires among UK wild rodents. *Leptospira* species were not detected in any of the foxes samples examined.

MI 08 Genomic islands (O-islands) and their potential role in the regulation of type III secretion in enterohaemorrhagic *Escherichia coli* O157:H7

Allen Flockhart[1], John Leong[2], David Gally[1] & Christopher Low[3]

[1]Centre for Infectious Diseases, University of Edinburgh; [2]Dept of Molecular Genetics and Microbiology, University of Massachusetts, USA; [3]SAC, Animal Health Group, Edinburgh

Complete genomic comparison of pathogenic *E. coli* O157:H7 with avirulent *E. coli* K-12 has revealed the presence of some 177 genomic islands, termed O-islands (OI) that are only present in the genome of the pathogen. Many of these OI consist of phage-like elements that are also present in other enteric pathogens. These OI can therefore be viewed as discrete elements, like the locus of enterocyte effacement (LEE). However, unlike the LEE, the contribution these islands make to the virulence and physiology of the bacterial cell is unknown. The main objective of this study was to investigate the potential role of these OI in the regulation of type III secretion in *E. coli* O157:H7. Initial investigations focused on the phenotypic characterization of a large selection of OI mutants that were constructed using the lambda red system. SDS-PAGE and Western Blot analysis of TCA precipitated culture supernatants identified a subset of OI deletions that either up-regulate or down-regulate secretion compared to the WT strain, indicating some control by genes on the deleted island. To determine if the effects on secretion can be complemented and therefore indicate which genes might be responsible, specific OI clones were constructed and are currently being analysed.

MI 09 Genome scale analysis of the role of superantigens in the pathogenesis of staphylococcal mastitis

Gillian J.C. Wilson[1], Caitriona M. Guinane[1], Timothy K. Connelly[2], W. Ivan Morrison[2] & J. Ross Fitzgerald[1]

[1]Laboratory of Bacterial Evolution and Pathogenesis, Centre for Infectious Disease, Chancellor's Building, New Royal Infirmary, University of Edinburgh, Edinburgh EH16 4SB; [2]Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Roslin EH25 9RG

Staphylococcus aureus is a major cause of bovine mastitis. Analysis of the genome sequence of the bovine isolate RF122 revealed genes encoding 10 predicted superantigens which may contribute to enhanced persistence in the host. Expression of all superantigen genes was detected *in vitro* but those located in the staphylococcal pathogenicity island (SaPIbov) were expressed at higher levels than those present in the enterotoxin gene cluster (*egc*). Transcriptional analysis of the *egc* indicated that the *seln* and *selu* genes were co-transcribed in both mid-exponential and stationary phases of growth *in vitro*, whereas *seg, seln, selu,* and *sei* were co-transcribed in mid exponential phase only.

In order to investigate the role of superantigens in disease pathogenesis, a superantigen-deficient strain of *S. aureus* has been constructed by sequential allele replacement, which will be used in experimental infections of dairy cows and to examine lymphocyte expansion of particular T-cell receptor β variable (TRBV) genes in response to superantigens *in vitro*.

MI 10 Pathogenomic analysis of the common bovine *Staphylococcus aureus* clone (ET3): emergence of a virulent subtype with potential risk to public health

Caitriona M. Guinane[1], Daniel E. Sturdevant[2], Michael Otto[2], Davida S. Smyth[4], Amer E. Villaruz[2], Patrick J. Hartigan[3], Cyril J. Smyth[4] & J. Ross Fitzgerald[1]

[1]Centre for Infectious Diseases, The Chancellor's Building, New Royal Infirmary, University of Edinburgh, Edinburgh EH16 4SB; [2]Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 South 4th St., Hamilton, MT 5984, USA; [3]Dept of Physiology and [4]Dept of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland

Genomic variation within natural populations of *Staphylococcus aureus* is large. However, our understanding of how this contributes to differences in pathogenic potential is limited. Molecular population analyses have shown that a single clone (ET3) of *S. aureus* is responsible for nearly 30% of all cases of mastitis worldwide. In this study, we identified 3 subtypes of ET3 by multilocus sequence typing

which differed in intramammary virulence in a mouse model of mastitis. One group in particular, ST151 resulted in very elevated levels of mammary tissue damage and mouse mortality. Comparative genomic studies revealed that the ST151 clone has undergone extensive genome diversification in virulence and regulatory gene content including the acquisition of genetic elements encoding toxins not made by the other ET3 subtypes. Further, levels of cytolytic toxin gene expression by the subtypes *in vitro* correlated with levels of tissue damage and mortality during intra-mammary infection. Previously, the ST151 clone has been shown to be hyper-susceptible to the acquisition of vancomycin-resistance genes from Enterococcus spp. Taken together, these data indicate the emergence of a virulent subtype of the ET3 clone that has undergone considerable genomic diversification and could present an enhanced risk to public health.

MI 11 Impact of horizontal gene transfer on the evolution of the canine pathogen *Staphylococcus pseudintermedius*

Nouri L. Ben Zakour[1], Jeanette Bannoehr[1], Caitriona M. Guinane[1], Adri Van Den Broek[2], Keith L. Thoday[2] & J. Ross Fitzgerald[1]

[1]Centre for Infectious Diseases, Royal (Dick) School of Veterinary Studies, The Chancellor's Building, New Royal Infirmary, University of Edinburgh, Edinburgh EH16 4SB; [2]Dermatology Unit, Division of Veterinary Clinical Sciences, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, University of Edinburgh, Roslin, Midlothian EH25 9RG

Staphylococcus pseudintermedius is a major skin pathogen of dogs and occasionally causes severe zoonotic infections of humans. Using a comparative genomic approach, we investigated the role of horizontal gene transfer (HGT) in the evolution of *S. pseudintermedius*. We identified putative mobile genetic elements (MGE) related to MGEs previously identified in other staphylococci species such as insertion sequences, transposons, and pathogenicity islands. Several *S. pseudintermedius* MGEs contained genes associated with pathogenesis, colonization and antibiotic resistance. Importantly, partial metabolic pathways have been acquired by lateral gene transfer which may have contributed to host adaptation. Our findings provide evidence for the important role of HGT in the emergence of *S. pseudintermedius*, as a major animal pathogen.

MI 12 Novel toxin secretion systems identified in *Photorhabdus* species

G. Yang, I. Vlisidou, M. Sanchez-Contreras & **N.R. Waterfield**

Dept of Biology and Biochemistry, University of Bath, BA2 7AY

Photorhabdus is an enterobacterial pathogen of insects (and man) which is a symbiont of entomopathogenic *Heterorabditid* nematodes. *P. luminescens* species are known to secrete high molecular weight Toxin complexes (Tc) with oral activity against a range of insects. Tc toxins are released into the supernatant of strain W14 but remain cell associated in the closely related strain TT01. Heterologous expression in *E. coli* and a comparison of the Tcd pathogenicity islands of W14 and TT01, identified genes involved in the release of the membrane-associated Tc toxins. Central to this is a class III lipase domain protein, Pdl. Interestingly, *pdl* homologues are also associated with *vgrG*-containing pathogenicity islands in the dual human/insect species *P. asymbiotica* and in other bacteria including *Vibrio cholera*. VgrG encodes a toxic component of the *V. cholera* type VI secretion system and database analysis has revealed the presence of VgrG-lipase islands in many other bacteria. The implications for the role of lipases in toxin secretion are discussed.

MI 13 Dissecting the function of *Photorhabdus* virulence cassettes

Isabella Vlisidou, Guowei Yang & Nicholas R. Waterfield

Dept of Biology & Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY

The study of specialized bacterial toxin secretion systems has revolutionized our understanding of the evolution of bacterial virulence. We investigate a novel toxin secretion and delivery system identified in the human and insect pathogenic enterobacterial genus *Photorhabdus*. The system, designated the '*Photorhabdus* virulence cassettes' (PVCs), resembles the antibacterial R-type pyocins containing conserved phage-like structure. A variable number of putative toxic effectors are encoded at one end. Despite its similarity to R-type pyocins it lacks antibacterial activity. Surprisingly, it appears to be toxic for eukaryotic host cells. In this study, we functionally characterized the chromosomal region of *P. asymbiotica* PVC encoding a cytotoxic necrotizing factor homologue designated Pnf. We demonstrated that Pnf was functional and able to modify Rho GTPases. We also used RT-PCR and GFP translational fusions to characterize the *in vitro* and *in vivo* conditions required for activation of PVCs structural and toxic components.

MI 14 The KdpDE sensor-regulator of *Photorhabdus asymbiotica* is sufficient to allow survival of non-pathogenic *Escherichia coli* in insect phagocytes *in vivo*

Isabella Vlisidou[1], Ioannis Eleftherianos[2], Nicholas R. Waterfield[1] & Stuart E. Reynolds[1]

[1]Dept of Biology & Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY; [2]CNRS-UPR9022, Institut de Biologie Moléculaire et Cellulaire, 15, rue René Descartes, F-67084 Strasbourg Cedex, France

Phagocytosis is used throughout the animal kingdom to kill bacterial pathogens. The method by which bacteria are destroyed nevertheless remains incompletely understood. Many bacterial pathogens are able to persist within phagocytes, deploying complex sets of tightly regulated virulence factors and secretion systems to do so. So far, however, no single factor has been identified that is in itself sufficient to allow intracellular persistence of otherwise harmless bacteria. Here we report that a single pair of co-expressed genes, the two component sensor-regulator *kdpDE* from the model emerging human pathogen *Photorhabdus asymbiotica*, is sufficient to allow a standard laboratory strain of *E. coli* to persist in insect phagocytes, and eventually to kill the host. We show that this requires *Photorhabdus-KdpDE*-regulated expression of the native host *E. coli kdp* structural genes, known to encode a high affinity potassium transporter. These findings reveal the central role of potassium sensing in intracellular survival of bacteria even in the absence of more complex and specialized virulence factors, and indicates the importance of potassium ions during cell killing by insect phagocytes.

MI 15 Identification of genes differentially expressed in *Escherichia coli* lysogens of the Shiga-toxin-encoding bacteriophage $\Phi 24_B$

Marta Veses Garcia, Alan McCarthy & Heather Allison

Microbiology Research Group, Biosciences Building, University of Liverpool, Crown Street, Liverpool L69 7ZB

The enterohaemorrhagic *Escherichia coli* (EHEC), including O157:H7, are food borne pathogens that have became a worldwide public health concern during the last two decades. Symptoms range from mild diarrhoea to haemorrhagic colitis with haemolytic uraemic syndrome or thrombotic thrombocytopenia purpura as potentially fatal complications. The major virulence determinant of EHEC is the production of the Shiga toxin, either Stx1 or Stx2, and both are encoded on temperate bacteriophages (Stx-phages). Stx-phages possess similar genomic organization to λ phage and the regulatory functions are also conserved. However, Stx-phage genomes can be as much as 50% larger, and many phage genes encode unknown functions. There are examples of bacteriophage λ-encoded proteins that enhance the survival of the lysogen, and we are identifying hypothetical Stx phage gene products that might function similarly, especially as many are

conserved across a range of otherwise heterogeneous bacteriophages. Thus far,11 differentially expressed proteins have been found, and are currently being identified by 2D-PAGE and MALDI-TOF mass spectometry analyses.

MI 16 Identification of Stx-phage genes expressed by their lysogens

Laura Riley, Alan McCarthy & Heather Allison

Microbiology Research Group, Biosciences Building, University of Liverpool, Crown Street, Liverpool L69 7ZB

Shigatoxigenic *Escherichia coli* (STEC) are zoonotic pathogens that cause human disease subsequent to colonization of the intestinal tract. Infection can lead to downstream sequalae such as haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura, which can be fatal. Though multiple traits contribute to the pathogenic profile of STEC strains, their main virulence determinant is the ability to produce Shiga-toxin (Stx). The Stx genes are encoded on lambdoid Stx-phages, the genomes of which can be up to 50% larger than that of λ. Many genes that are conserved amongst Lambda and the heterogeneous Stx-phages have no assigned function and at least some are very likely to have roles in phage replication or lysogen survival.

Genome annotation of a model detoxified short-tailed Stx-phage, $\Phi 24_B$ (57.7 kb), has revealed a number of ORFs that encode hypothetical proteins. Using a novel technique, Change Mediated Antigen Technology (CMAT, iviGene Corporation), we have identified several genes encoded by $\Phi 24_B$ and expressed only in the *E. coli* lysogen. Expression studies to validate these results are underway, and bioinformatic analyses combined with biological assays are being used in an effort to assign functions to these genes.

MI 17 Genetic analysis of invasive African non-typhoidal salmonellae

Christina Bronowski, John Wain & Craig Winstanley

University of Liverpool, Faculty of Medicine, Dept of Medical Microbiology and GUM, 8th Floor Duncan Building, Daulby Street, Liverpool L69 3GA

Non-typhoidal *Salmonellae* (NTS) are a major cause of morbidity and mortality in sub-Saharan Africa, where they are a leading cause of septicaemia in children. Phenotypic and genotypic analysis suggests that these NTS differ significantly from classic gastroenteritis strains. We used suppression subtractive hybridization (SSH) to identify novel genes present in representative strains of invasive NTS from Africa. SSH is a technique which allows the identification of sequences present in one strain but absent from another. The following four subtractions were carried out using African NTS isolates of different serovars: *S.* Heidelberg, *S.* Bovismorbificans and *S.* Typhimurium against *S.* Typhimurium LT2; *S.* Enteritidis against a genome sequenced *S.* Enteritidis. Serovar-specific sequences were identified in *S.* Heidelberg, *S.* Bovismorbificans. *S.* Typhimurium subtracted sequences were mostly bacteriophage- and plasmid-related, whilst *S.* Enteritidis produced few subtracted sequences, indicating limited variation in genomic content between the two strains used. Data from PCR and DNA:DNA hybridization assays, applied to a panel of African and UK NTS isolates, suggested that some subtracted sequences were distributed according to geographical source.

MI 18 Discovery of high level invasion of epithelial cells *in vitro* by Biotype 1A *Yersinia enterocolitica* using a novel 3d-tissue culture model

James Collins[1], Angus Best[1], Roberto La Ragione[1] & **Alan McNally**[2]

[1]Dept for Food and Environmental Safety, Veterinary Laboratories Agency, Woodham Lane, Surrey KT15 3NB; [2]School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS

Previous work using classical plate-count based, *in vitro* cell invasion assays have suggested that Biotype 1A *Y. enterocolitica* are invasive, but at greatly reduced levels compared to those observed for Biotype 1B and Biotype 2–5 strains. Using a 3d-tissue culture model and confocal fluorescence microscopy, we demonstrate the Biotype 1A isolates invade cultured porcine epithelial cells at levels equal to or greater than high pathogenic biotype strains. This work may have important implications in the use of invasion assays on standard epithelial cells, in monolayer cultures, as a marker for pathogenic potential of bacterial species.

MI 19 A novel signalling mechanism in *Paracoccidioides brasiliensis* for controlling the morphological switch to the pathogenic yeast form

Thamarai K. Janganan, Inês Borges-Walmsley & Adrian R. Walmsley

Centre for Infectious Diseases, Queen's campus, University of Durham, TS17 6BH

Paracoccioides brasiliensis is the causative agent of the disease Paracoccioidomycosis (PCM), which is one of the most prevalent systemic mycoses in Latin Amercia (Borges-walmsley et al, 2002). *Paracoccioides brasiliensis* is a thermally dimorphic fungi, which can under go morphological changes from a mycelial form at 26°C (environment) to a pathogenic yeast form at 37°C (human body) after inhalation of spores, and/or mycelium fragments, into the lungs of a human host (Nemecek J C et al, 2006). The cAMP pathway controls this morphological transformation in several fungi (Rocha C R C et al, 2001, Borges-walmsley et al, 2002, kronstad et al, 1998).

G proteins are Guanine nucleotide (GDP or GTP) binding proteins that are generally associated with the cytoplasmic side of the plasmamembrane. They receive signals from G-protein coupled receptors (GPCR) (Lanier SM et al, 2004). Adenylate cyclase acts down stream of these G-proteins. Gα subunits are required to regulate the activity of adenylate cyclase (AC), which controls the level of cellular cAMP (Ivey D F et al, 2004). Protein Kinase A (PKA), which is activated by cAMP, is required for morphogenesis and virulence (Durrenberger F et al, 1998&t Staudohar M et al, 2002).

The cAMP pathway in *P.brasiliensis* is poorly understood. However, recently the genes encoding a number of the components of the cAMP pathway have been cloned in our lab: these include the genes encoding three Gα proteins, Gpa1-3, a Gβ protein, Gpb1; a Gγ protein, Gpg1; Ras; adenylate cyclase, Cyr1; and the catalytic subunit of PKA, Tpk1. Two-hybrid analyses confirmed that Gpa1 and Gpg1 interact with Gpb1. These data indicate the formation of a Gαβγ trimer complex. A GST pull-down assay confirmed that Gpa1 and Gpb1 interacted with the N-terminus of adenylate cyclase. Our hypothesis is that Gpa1 and Gpb1 modulate the activity of the AC/Tpk1 signalling pathway. Consistent with this hypothesis, we found changes in intracellular cAMP levels during the mycelium to yeast transformation that correlated with changing transcript levels of the signalling genes (D.Chen,Thamarai K. Janganan, G.Chen *et al* 2007)

We have established that Tpk interacts with the N-terminus of adenylate cyclase, the G-protein β-subunit, Gpb1, and with the co-repressor TupA by both two-hybrid and GST pull-down analyses. The two-hybrid assays were confirmed by both α-galactosidase and β-galactosidase colony lift and ONPG assays. This suggests that Tpk activity is required for feedback regulation of AC to reduce cAMP levels. Our hypothesis is that Gpb1 is a substrate of Tpk, being phosphorylated, whilst TupA regulates the activity of Tpk; because Gpb1 binds to the catalytic C-terminal domain of Tpk, whislt TupA binds to the N-terminal domain.

P. brasiliensis Tpk–C-ter$^{226–583}$ GFP complements the growth defect of a *S. cerevisiae* tpk2 tempertaure sensitive mutant strain SGY446. *P. brasiliensis* Tpk $^{226–583}$–GFP fusion protein induced the formation of pseudo-hyphae in the *S. cerevisiae* tpk2 mutant diploid strain

XPY5a/α. Tpk$^{226-583}$ has been over expressed in *E. coli* and *in vitro* PKA activity was measured by ProFlour PKA assay (Promega Cat. No. V1240).

MI 20 Factor affecting the success or failure of meticillin-resistant *Staphylococcus aureus* (MRSA) decolonization protocols

Deirdre Gilpin[1], Shaunagh Small[2], Paddy Kearney[2], Anne Gardiner[2] & Michael Tunney[1]

[1]School of Pharmacy, Queens University Belfast, 97 Lisburn Road, Belfast; [2]Antrim Area Laboratory, United Hospitals Trust, 45 Bush Road, Antrim

Meticillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial infection. Colonization, often the precursor to infection, can prove difficult to eradicate with many patients remaining MRSA positive despite repeated attempts at decolonization. This study attempts to analyse factors, which may influence the success or failure of MRSA decolonization protocols currently in use in the Northern Health and Social Care Trust in Northern Ireland. Restriction enzyme digestion, followed by pulsed field gel electrophoresis (PFGE) was used to determine any strain differences in isolates from successfully and unsuccessfully decolonized patients. Successfully decolonized patients are currently being re-screened at 6 and 12 months. If positive, PFGE will be used to determine whether the patient has been persistently colonized with the same strain, or has been re-colonized with a new strain. To date, 296 patients have been recruited to the study, of which 127 have been removed due to death, a worsening of their underlying medical condition or non-compliance. Sixty patients have failed decolonization. Eighty one patients were successfully decolonized, of which 31 have been re-screened at 6 or 12 months. Of these, ten were found to be MRSA positive. Analysis of these isolates in terms of antibiotic resistance, PFGE profiles and patient characteristics (age, medical history, length of stay in hospital) is currently ongoing.

MI 21 *Mycoplasma pneumoniae* infection in a paediatric population: analysis of soluble immune markers

J. Hassan, F. Irwin, S. Dooley & J. Connell

National Virus Reference Laboratory, Ireland

Epidemiological and clinical evidence suggests that respiratory tract infection with *Mycoplasma pneumonia* is implicated in the initiation and exacerbation of asthma.

This study examines the incidence and frequency of *M. pneumoniae* infection in children and evaluates the cytokine profile and total IgE levels of patients with clinical presentation of either upper (URTI) or lower respiratory tract infections (LRTI). Serum samples were tested over a 6 year period. Specific IgM anti-*M. pneumoniae* and total IgE levels were measured by EIA. 12 cytokines were measured using the Linco multiplex cytokine assay. The cyclical incidence of *M. pneumoniae* infection was confirmed, however, the peak age of highest incidence in the most recent epidemic fell to 3–4 years. A high incidence was also observed in the 6–7 year age group. Children presenting with LRTI had higher serum levels of the proinflammatory cytokines; IL-1α , IL-6 and Th2-type cytokines; IL-4 and IL-10 when compared to those patients presenting with URTI. IL-8 levels were also higher in the LRTI group. IL-5, although not significant, was the only cytokine consistently higher in the URTI group. Th1-type cytokines; IFN-γ, IL-2 and IL-12 were low in all patients. IgE levels were higher in the LRTI group. Our findings show an increased proinflammatory and Th2-type cytokine response in all patients although patients with LRTI show a more heightened response indicative of an aggressive host response.

MI 22 Identification of novel peptides from a random display library for bacterial targeting

Rebecca Vince, Tim Paget, Martin Todman & John Greenman

Medical Research Laboratory, Wolfson Building, University of Hull, Hull HU6 7RX

MRSA is an organism increasingly reported within the microbial flora of venous ulcers and has an adverse effect on wound healing and infection. The incidence of venous ulcers necessitates alternative modes of identification and targeting of the relevant pathogenic organisms. Peptides (12-mer) were selected from a bacterial display library (FliTrx™) through panning against MRSA; synthesized and their binding evaluated by flow cytometry. No specific binding of the peptides was observed against MRSA. This method is costly and labour intensive allowing analysis of only a few colonies at a time. Therefore, a high-throughput method of screening clones is under development whereby peptide sequences of interest are cloned into a GFP/His tag vector for soluble expression in *E. coli* for assessment by flow cytometry, confocal microscopy and ELISA. Peptides, conjugated to photosensitizers will be used as delivery vehicles for photodynamic therapy. These peptide conjugates have potential both in treatment of wounds and as candidates for rapid diagnosis of the wound environment without the need for culture.

MI 23 Identification and expression of putative membrane acive toxins of *Burkholderia pseudomallei* and *Burkholderia mallei*

M. Bokori-Brown & R.W. Titball

University of Exeter, School of Biosciences, Exeter EX4 4QD

The closely related pathogens *Burkholderia pseudomallei* and *Burkholderia mallei* are Gram-negative bacteria found predominantly in Southeast Asia and northern Australia and they are the causative agent of melioidosis and glanders in both humans and animals, respectively. Currently, there is no licensed vaccine available for protection against these versatile bacteria and infections are difficult to treat with antibiotics. The organisms are also potential bioterrorism agents due to the severity of infection they cause through inhalation, as well as their ability to contaminate food and water. Several potential virulence factors of *Burkholderia pseudomallei* have previously been suggested to play a role in disease including putative membrane active toxins such as phospholipases C (PLC) and haemolysin. However, their detailed molecular modes of action have not been elucidated and their precise role in the pathogenesis of *Burkholderia pseudomallei* remains unclear. We have identified a range of putative membrane active toxins from the genome sequenced *Burkholderia pseudomallei* reference strain K96243 and expressed selected genes in *E. coli* for characterization and to evaluate their potential use as vaccine candidates.

MI 24 Identification of protein glycosyltransferases in *Burkholderia* spp.

Andrew Scott[2], Jo Prior[2], Rick Titball[1] & **Stephen Michell**[1]

[1]School of Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter, EX4 4QD; [2]Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ

The continued reports on the presence of protein glycosylation systems in numerous pathogenic bacteria raise several interesting questions, such as; by what mechanism is this post-translational modification established and what is its role in pathogenicity? Both O- and N-linked glycosylation systems have been characterized in several bacterial species. Whilst the mechanism of N-linked glycosylation is well characterized, exhibiting similarity to the conserved eukaryotic system, the same is not evident for bacterial O-glycosylation. To date, bacterial O-glycosylation has predominantly been reported as a modification of surface and secreted proteins including type IV pili, with different bacteria using different mechanisms of glycosylation. Here we demonstrate the presence of glycosylation by *Burkholderia* and the identification of a gene encoding a putative protein glycosyltransferase. PCR was used to screen strains of *Burkholderia* for the presence of this gene. Further characterization of these enzymes will allow us to address the role of this post-translational modification in pathogenesis.

MI 25 Development of *Burkholderia thailandensis* as an expression system for vaccine candidates

A.E. Scott, T.P. Atkins, S.V. Harding, S.L. Michell, R.W. Titball & J.L. Prior

Biomedical Sciences Dept, Dstl Porton Down, Salisbury, Wilts SP4 0JQ

Burkholderia pseudomallei is the causative agent of melioidosis, a debilitating disease with mortality rates often exceeding 40% despite appropriate antibiotic treatment. This, combined with a relatively low infectious dose through the aerosol route and its presence on the CDC list of bio-weapons has led *B. pseudomallei* to be considered a potential biological warfare agent. Development of an efficacious protein vaccine against *B. pseudomallei* requires a suitable expression system to ensure that the recombinant proteins are immunologically similar to the wild type proteins and can thus raise an appropriate immune response. *Burkholderia thailandensis* is closely related to *B. pseudomallei* and is generally considered avirulent. As such, *B. thailandensis* represents a good host in which to express potential vaccine proteins without the complications of expression in a virulent host or the loss of immunologically relevant post-translational modifications. To this end, we have developed an efficient method to transform *B. thailandensis,* constructed a vector capable of replication in *B. thailandensis* and identified promoter sequences to allow high level expression of the recombinant protein.

MI 26 Characterization of superoxide dismutase C (SodC) in *Yersinia pseudotuberculosis*

Olivia L. Champion[1], **Sarah James**[1], Donna Ford[2], Melanie L. Duffield[2] & Richard Titball[1]

[1]University of Exeter, Biosciences, Stocker Road, Exeter EX4 4QD; [2]Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ

Superoxide dismutases (SODs) are enzymes that catalyse the reduction of superoxide anions to hydrogen peroxide. Through BLAST searches a *sod*C homologue was identified in the genome of *Yersinia pseudotuberculosis*. We tested the hypothesis that *sod*C facilitates intracellular survival of *Y. pseudotuberculosis* by conferring superoxide anion resistance to the bacteria. A *sod*C knockout (Δ*sod*C) was constructed in *Y. pseudotuberculosis* and compared to the wild type strain in environmental stress assays. A significant survival defect was observed in Δ*sod*C when exposed to exogenous superoxide anions. However, no significant differences were observed between WT and Δ*sod*C for pH, temperature, osmolarity, acid and hydrogen peroxide sensitivity. Macrophage survival assays comparing intracellular survival of *Y. pseudotuberculosis* WT and *sod*C knockout are currently being carried out in our laboratory. Taken together these data indicate that *Y. pseudotuberculosis* expresses SodC in the presence of exogenous superoxide anions. We hypothesize that this enzyme aids intracellular survival of this pathogen by protecting the bacteria from harmful superoxide anions produced in the phagosome of professional phagocytic cells during the respiratory burst.

MI 27 Survival of *Campylobacter jejuni in vivo* is Nramp-1 dependent

Olivia L. Champion[1]†, Yanet Valdez[2]†, Lisa Thorson[2], Julian A. Guttman[2], Erin C. Gaynor[2] & Brett B. Finlay[2]

[1]University of Exeter, EX4 4QD; [2]University of British Columbia, Michael Smith Building, Vancouver, Canada, V6T 1Z4 (†These authors contributed equally to this work)

Campylobacter jejuni is an important human food and water-borne pathogen, with a poorly characterized mechanism of pathogenesis. We tested the hypothesis that host resistance to *C. jejuni* depends on natural resistance-associated macrophage protein 1 (Nramp1). Nramp1 – and ++ mice received approx. 10^8 c.f.u./mouse *C. jejuni* by intra peritoneal infection. The majority of the infection occurred in the liver, with *C. jejuni* primarily associated with Mac1 positive cells in the liver sinusoids. Nramp1 deficient mice were impaired in clearing *C. jejuni* with no significant reduction of bacterial load seen in the liver by 8 days post infection. In contrast, *C. jejuni* numbers in the liver decreased significantly in Nramp1 ++ mice by day 4 post-infection. The chemokine MCP-1 was significantly raised in Nramp1 – mice one day post infection compared to Nramp1 ++ mice. Taken together these data indicate that *C. jejuni* survival is Nramp1 dependent and *C. jejuni* survives *in vivo* associated with Mac1 positive cells.

MI 28 Cellular adaptation to ciprofloxacin challenge in *Pseudomonas aeruginosa*

Hannah G. Stickland & Martin Welch

Dept of Biochemistry, University of Cambridge, Cambridge CB2 1QW

The incidence of bacterial resistance to the billion-dollar drug ciprofloxacin is increasing. Much transcriptomic data has been collected on the bacterial response to ciprofloxacin treatment. We have complemented this research with proteomic data using 2-dimensional Fluorescence Difference in-Gel Electrophoresis (2D-DiGE) to investigate the response of *Pseudomonas aeruginosa* to ciprofloxacin and to characterize the proteomes of two clinically relevant spontaneous ciprofloxacin-resistance phenotypes. Treatment with two sub-inhibitory concentrations results in a concentration-dependent increase in the number of proteins modulated. Additionally, the proteome of a target site mutant (GyrA(T83I)) shows negligible changes from that of untreated wild type, confirming that the response to ciprofloxacin is a direct result of gyrase inhibition. In contrast, an efflux pump regulator mutant (Δ*nfxB*) shows global proteomic modulations, highlighting the fundamental role of these pumps in cellular function. The pleiotropic effect of the *nfxB* pump regulator mutation and up-regulation of the MexCD-OprJ pump has been further investigated. This combined approach provides insight into the mechanisms by which *P. aeruginosa* responds to the drug and adapts to accommodate resistance mechanisms.

MI 29 The impact of gene flux on *Citrobacter rodentium* evolution

Nicola K. Petty[1,2], George P.C. Salmond[1] & Nicholas R. Thomson[2]

[1]Dept of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW; [2]Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA

Citrobacter rodentium is a natural mouse pathogen and the model organism for infections caused by the important human pathogens, enteropathogenic and enterohaemorrhagic *Escherichia coli*. All these bacteria are members of the attaching and effacing pathogen family, a phenotype encoded on the locus for enterocyte effacement (LEE). In addition to the LEE, through annotation of the *C. rodentium* genome, we have identified a number of other genomic islands encoding factors likely to be important for host adaptation and pathogenicity, for example, a number of fimbral biogenesis operons, effector proteins and adhesins, as well as toxins, Type I and II secretion systems and a restriction/modification system. Also, six prophage-like elements have been identified, several of which are predicted to have disrupted core host functions, for example prophage insertion into flagella operons may be associated with *C. rodentium*'s lack of motility. The *C. rodentium* genome highlights the crucial role of horizontal gene transfer in the evolution, virulence and overall diversity of this bacterial pathogen.

MI 30 *Salmonella*: host adapted to harbour porpoises (*Phocoena phocoena*)

Jana Haase

Welcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA

Since 1991 over 120 *Salmonella enterica* isolates have been described from harbour porpoises caught or found around the coast of Scotland.

The antigenic formula of these strains was 4,12:a:– and typing at the Pasteur Institute Paris identified them as *Salmonella* serovar Fulica.

We have shown by MLST (Mutli Locus Sequence Typing) that *S.* Fulica and the study strains do not belong to the same clonal group (variation at 5 loci). However *Salmonella* serovar Bispebjerg (antigenic formula 1,4,[5],12:a:e,n,x) is a single locus variant with the study strains, which implicates a clonal relationship.

The growth rate of the 'porpoise strains' is decreased (2fold) in comparison to other *Salmonella* of Group B and further more they have lost the ability for several biochemical reactions compared with *S.* Bispebjerg (API20E and API 50CH). Those are typical characteristics for host adapted *Salmonella.*

We propose that the study strains are variants of *Salmonella enterica* subspecies *enterica* serovar Bispebjerg which are host adapted to the harbour porpoise.

MI 31 Effect of IncHI1 plasmids on the interaction of *Salmonella* Typhi with human macrophages

Silvia Pinero, Fernanda Schreiber, Minh Duy Phan & John Wain

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

IncHI1 plasmids are associated with multidrug resistance in *S.* Typhi. These stable elements cause minimal disruption and increase bacterial pathogenicity. Variants of these plasmids seem to compete with each other for colonization in natural populations of *S.* Typhi.

Our work is focused on the effect of different IncHI1 plasmids in the invasion and intracellular replication of *S.* Typhi in human cells.

THP1 cells were infected with *S.* Typhi BRD948 (Ty2 Δ*aroC aroD htrA*) and strains carrying plasmids (pHCM1 and pSTY7) to a MOI of ca 50 c.f.u. per cell.

We found significantly lower numbers of intracellular bacteria of BRD948/pSTY7 strain compared with BRD948 at 30 min, 2 and 4 h after infection. At 30 min, the percentage of intracellular BRD948 was 5% of the total cell-associated bacteria, while in BRD948/pSTY7 it was only a 0.45%. In contrast, the replication of BRD948/ pSTY7 from 30 min to 2 h is increased over 5 times compared with BRD948. BRD948/pHCM1 was not significantly different from the control strain.

MI 32 Oxygen stress induces biofilm formation in *Campylobacter jejuni*

M. Reuter, A. Mallett, **A.H.M. van Vliet** & B.M. Pearson

Institute of Food Research, Norwich NR4 7UA

Under laboratory conditions, *Campylobacter* requires strict growth conditions with respect to atmosphere and temperature. Yet, paradoxically, *Campylobacter* is widespread in the environment and can rapidly spread through a broiler house. Recent reports have postulated that *Campylobacter* may survive in the environment within a biofilm.

We have investigated biofilm formation in *Campylobacter* in physiologically relevant conditions using plasmid-containing and non-plasmid strains.

Biofilm formation was increased under atmospheric conditions compared to microaerobic growth for all strains tested. Biofilm formation under atmospheric conditions was detected after three hours incubation and increased over time. Biofilm-adapted cells showed phenotypic changes as judged by colony morphology and autoagglutination. However, biofilm-adapted cells did not show any increase in biofilm formation compared to WT strains under microaerobic and atmospheric growth conditions.

These data suggests that biofilm formation is stimulated under atmospheric conditions, and may represent a survival mechanism for this organism in the wild. Phenotypic adaptation within a biofilm may give rise to a population which are more able to survive in unfavourable conditions or better adapted to colonization in an avian host.

MI 33 Norepinephrine enhances iron uptake in *Campylobacter jejuni*

N. Shearer[1], R.D. Haigh[2], C.A. Perrett[3], A.H.M. van-Vliet[1], T.J. Humphrey[3], J.M. Ketley[2] & B.M. Pearson[1]

[1]Institute of Food Research, Colney Lane, Norwich; [2]University of Leicester, University Road, Leicester; [3]University of Bristol, Langford, Bristol

Norepinephrine (NE) has been found to enhance growth and virulence factor expression in several bacterial pathogens, including *Campylobacter jejuni*. We have previously demonstrated that NE alleviates growth-restriction of *C. jejuni* in serum-containing chelex-MH medium (CMH; Gut 2007;56:1060). While transcriptomic analysis of CMH-grown *C.jejuni* indicates that addition of NE has no direct effect on gene expression, NE does act synergistically to potentiate the effect of iron supplementation on gene expression. This implies that NE acts to facilitate iron-uptake, and that the growth restriction in CMH is mediated by iron-restriction. Comparison of the growth response of *C. jejuni* strains to NE indicates that there are strain-dependent differences, linked to differences in iron-uptake systems, including the CfrA enterochelin receptor. Mutation of the *cfrA* gene almost completely eliminates the NE growth response of NCTC11168. In conclusion, NE stimulates growth of *C. jejuni* through siderophore-mediated iron-uptake via CfrA.

MI 34 Identification of novel pathogenic bacterial adhesins

V.M. Pullabhatla & E.G. Hutchinson

School of Biological Sciences, University of Reading, Reading RG6 6AS

Bacteria remain the causative agents of many dreadful infections and diseases throughout the world. The process of bacterial colonization and adherence to the host is the initial and most crucial step in the infection. Adherence helps the bacteria to colonize a particular tissue, which is followed by invasion or release of virulence factors. The proteins involved in adherence may be potential candidates for drug and vaccine development. This project aims at identifying novel bacterial adherence factors at the genomic level using bioinformatics approaches. A dataset of 203 known bacterial adhesins were used as an initial step towards understanding adhesins. SPAAN, a bioinformatics tool which can predict adhesins at a genomic level, was evaluated using the dataset. Though SPAAN does well, it missed out 17.2% of known adhesins. Hence we used a combination approach of SPAAN, domain information and subcellular localizations to improve the prediction process. The combination approach has identified 94.6% of known adhesins decreasing the proportion of false negatives to 5.4%. Hence, this method has improved prediction and can be used at a genomic level for identifying novel adhesins.

MI 35 The role of AcrA in antibiotic resistance and pathogenicity of *Salmonella enterica* serovar Typhimurium

Jessica Blair, Roberto La Ragione, Martin Woodward & L.J.V. Piddock

Division of Immunity and Infection, The Medical School, Birmingham University, Vincent Road, Edgbaston, Birmingham B15 2TT

AcrAB-TolC is a tripartite efflux pump of *Salmonella* Typhimurium, whose over-expression is associated with multiple antibiotic resistance. This laboratory showed that AcrB and TolC are important in colonization and persistence in poultry – the major reservoir for this food borne pathogen. As *acrA* is co-transcribed with *acrB*, it has been assumed that inactivating *acrA* would produce a phenotype indistinguishable from that of the *acrB* mutant. In this study *acrA*

was inactivated and the phenotype determined. There was no significant difference in the growth kinetics of the mutant compared to the wild type (SL1344). *acrA*::*aph* was hypersusceptible to antibiotics, dyes and detergents and had an impaired ability to adhere to and invade human intestine cells (INT) and mouse macrophages (RAW) compared with the parent strain. However, the phenotype of *acrA*::*aph* was distinct to that of *acrB*::*aph*. These data confirm a key role for AcrA in the function of this pump.

MI 36 Integrons in environmental bacteria

W.H. Gaze[1], N. Abdouslam[1], K.G. Bailey-Byrne[1,2], J. Royle[1], L. Calvo-Bado[1], P. Hawkey[3] & E.M.H. Wellington[1]

[1]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL; [2]Dept of Plant & Microbiology, University of California, Berkeley, CA 94720, USA; [3]University of Birmingham, Division of Immunity & Infection, Birmingham B15 2TT

Integrons are recombination and expression systems that capture genes as part of a genetic element known as a gene cassette (Recchia and Hall, 1995). Most cassettes of known function confer antibiotic or quaternary ammonium compound (QAC) resistance, and these mobile genetic elements (MGEs) have been implicated in the spread of antibiotic resistance in pathogenic bacteria.

Culture dependent methods were use to investigate class 1 and class 2 integron prevalence in bacteria from agricultural and QAC contaminated soils. A wide range of bacterial species were found to carry integrons, including clinically important pathogens, some of which displayed a multi-antibiotic-resistant phenotype. Amplification of cassette genes from soil total community DNA, followed by cloning and sequencing revealed a range of cassette genes, some of which may be novel antibiotic resistance genes. Biocide resistance genes including *qacE* and *qacEΔ1* were also detected in isolates and clones. Novel IS element – promoter insertions were observed in isolates and clones from QAC contaminated soil, suggesting that biocide exposure selects for class 1 integrons, and therefore co-selects for antibiotic resistance.

MI 37 Quorum sensing in *Burkholderia cepacia* 2a and 2,4-D metabolism

W.B. Morris, A. Sebastianelli & I.J. Bruce

University of Kent, Giles Lane, Canterbury, Kent CT2 7NJ

Burkholderia cepacia strain 2a was isolated for its ability to utilize 2,4-Dichlorophenoxyacetic acid (2,4-D) as its sole source of carbon. The genes responsible for the metabolism of 2,4-D (*tfd*) are resident on a defective transposon (Tn*5530*). Tn*5530* is lost completely when grown on non selective media (absence of 2,4-D). This is believed to be due to its hybrid insertion sequence (IS*1071*::IS*1471*) . The mechanism of loss is not fully understood but is might be *via* homologous recombination between the two flanking IS elements.

B. cepacia 2a contains the *lux* homolog *cep* which is responsible for the production and detection of the quorum sensing (QS) molecules, homoserine lactones (HSL). *In silico* studies have revealed several putative CepR binding sites within IS*1071*::IS*1471,* which suggests that QS might play a role in the maintenance of Tn5530.

Work is currently being carried out to identify the mechanism by which the loss of Tn*5530* is prevented by QS using EMSA and DNaseI footprinting.

MI 38 Characterization and functionality of *Streptococcus equi* superantigens

R. Paillot, C. Robinson, K. Stuart, Z. Mitchell & A. Waller

Animal Health Trust, Centre for Preventive Medicine, Lanwades Park, Newmarket, Suffolk CB8 7UU

Streptococcus equi is the causative agent of strangles, the most frequently diagnosed infectious disease of horses worldwide. The disease is characterized by abscessation and swelling of the lymph nodes of the head and neck, which can literally strangle the horse to death. *S. equi* possesses four phage-associated bacterial superantigens (SeeH, SeeI, SeeL and SeeM) that share homology with the mitogenic toxins of *S. pyogenes*. However, little is known about their activity in horses.

The aim of this study was to produce recombinant *S. equi* super-antigens and to characterize their activity *in vitro* in order to better understand their role in pathogenicity. Each of the superantigens was successfully cloned and soluble protein produced in *E. coli*. Three of the superantigen toxins (SeeI, SeeL and SeeM) induced a strong dose dependent proliferative response in equine T lymphocytes and synthesis of IFNγ after only a few hours in culture. In the horse, superantigens are natural targets of the immune response as specific antibodies that neutralized *in vitro* mitogenic activity were present in sera from infected horses.

We propose that these immuno-modulatory proteins play an important role in *S. equi* pathogenicity by stimulating an overzealous and inappropriate T cell response.

MI 39 Characterization of the conjugative transfer of ICESt1 and ICESt3 from *Streptococcus thermophilus*

Xavier Bellanger[1], Adam P. Roberts[2], Peter Mullany[2], Bernard Decaris[1] & Gérard Guédon[1]

[1]Laboratoire de Génétique et Microbiologie, UMR INRA 1128, IFR110, Faculté des Sciences et Techniques, Nancy-Université, Boulevard des Aiguillettes, BP239, 54506 Vandœuvre-lès-Nancy, France; [2]Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, University College London, London WC1X 8LD

An integrative and conjugative element (ICE) is a genomic island that excises by site-specific recombination, self-transfers by conjugation and integrates in the genome of the recipient bacterium. Whereas only few ICEs have been characterized, *in silico* analyses recently revealed numerous putative ICEs in sequenced bacterial genomes suggesting that many genomic islands are ICEs or elements deriving from them. The current investigation shows the intraspecific conjugative transfer of the first ICEs described in *Streptococcus thermophilus*, ICE*St1* and ICE*St3*, and their transfer to other Firmicutes *Streptococcus pyogenes* and *Enterococcus faecalis*. Moreover the presence of ICE*St3* in the recipient bacterium confers conjugation immunity, i.e. strongly decreases the recipients capacity to acquire another copy of the element. Furthermore, preliminary data suggest that DNA damage produced by mitomycin C induces ICE*St3* conjugation.

MI 40 Insight into the highly diverse pool of Integrative and Conjugative Elements (ICEs) in Group B *Streptococcus* (GBS)

Mathieu Brochet[1], Elisabeth Couvé[1], Philippe Glaser[1], Gérard Guédon[2] & **Sophie Payot**[2]

[1]Genomics of Microbial Pathogens, URA-CNRS2171, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris cedex 15, France; [2]Laboratoire de Génétique et Microbiologie, Nancy-Université, INRA, Boulevard des Aiguillettes BP239, 54506, Vandœuvre-lès-Nancy, France

We systematically searched for ICEs and related elements in 8 GBS genome sequences. This allowed us identifying 34 such elements belonging to 9 families. Eleven elements correspond to putative ICEs, while 20 other could be *cis*- or *trans*-mobilized. These elements are extremely diverse and most of them are composite, indicating multiple accretion events. Functional analysis of coding sequences revealed that these elements carry genes that could be involved in stress adaptation, heavy-metal tolerance and virulence. We got insight into the mobility of these elements. Circular forms were detected by PCR in 4 families. The intra-species distribution of the 9 families was assessed by DNA-array hybridizations and suggested that most of them are horizontally

disseminated within the species. These results indicate that ICEscontribute to the GBS genome plasticity and might allow this species colonizing various niches.

MI 41 An ICESt1-related element found in commensal streptococci can transfer macrolide resistance genes to *Streptococcus pyogenes*

Jia Sun, Maria Jönsson & **Göte Swedberg**

Dept of Medical Biochemistry and Microbiology, Uppsala University, Sweden

The material came from a study on the effects of *Helicobacter* treatment in Sweden (the DalaHp study) and included a total of 98 consecutive patients with endoscopy-verified peptic ulcers and verified *H. pylori* infection. The subjects received triple therapy, omeprazole 20 mg, clarithromycin 250 mg and metronidazole 400 mg twice daily during one week. Commensal streptococci resistant to macrolides were isolated from throat samples.

Conjugation experiments were done on filter papers on the surface of agar plates to detect transfer from throat isolates of streptococci to *Streptococcus. pyogenes* BM137. The frequency of transfer of the *mef(E)* gene was generally low but reproducible. Nucleotide sequence determinations outside the *mef(E)* gene established that the gene is carried by the mega element. Some sequences outside the mega element were related to a transferable element (ICE*St*1) previously found in *Streptococcus thermophilus*. Similarities to *int* and *xis* genes were also detected, however these sequences were not identical to those of ICE*St*1, but showed roughly 50% sequence similarity to sequences in *Streptococcus agalactiae*.

MI 42 Regulation of expression and secretion of NleH, a new non-LEE-encoded effector in *Citrobacter rodentium*

Víctor A. García-Angulo & José L. Puente

Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico & Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada

Together with enterohemorrhagic (EHEC) and enteropathogenic *Escherichia coli* (EPEC), *Citrobacter rodentium* is a member of the 'attaching and effacing' (A/E) family of bacterial pathogens. A/E pathogens use a type III secretion system (T3SS) to translocate an assortment of effector proteins, encoded both within and outside the locus of enterocyte effacement (LEE), into the colonized host cell leading to the formation of A/E lesions and disease. Here we report the identification and characterization of a new non-LEE encoded effector NleH in *C. rodentium*. NleH is conserved among A/E pathogens and shares identity with OspG, a type III secreted effector protein in *Shigella flexneri*. Downstream of *nleH*, genes encoding homologues of the non-LEE-encoded effectors EspJ and NleG/NleI are found. NleH secretion and translocation into Caco-2 cells requires a functional T3SS and signals located at its amino terminal domain. Transcription of *nleH* is not significantly reduced in mutants lacking the LEE-encoded regulators Ler and GrlA; however, NleH protein levels are highly reduced in these strains, as well as in *escN* and *cesT* mutants. Inactivation of Lon, but not ClpP, protease restores NleH levels even in the absence of CesT. Our results indicate that the efficient engagement of NleH to active secretion is needed for its stability, thus establishing a post-translational regulatory mechanism that co-regulates NleH levels with the expression of LEE-encoded proteins. A *C. rodentium nleH* mutant shows a moderate defect during the colonization of C57BL/6 mice at early stages of infection.

MI 43 'Gently rough': vaccine potential of *Salmonella enterica* lipopolysaccharide mutants

Gábor Nagy[1], Harald Kusch[2], Ulrich Dobrindt[3], Susanne Engelmann[2], Levente Emődy[1], Tibor Pál[1] & Jörg Hacker[3]

[1]Dept of Medical Microbiology and Immunology, University of Pecs, Hungary; [2]Institute for Microbiology, University of Greifswald, Germany; [3]Institute for Molecular Biology of Infectious Diseases, University of Wuerzburg, Germany

Mutants of lipopolysaccharide (LPS) synthesis have traditionally been considered over-attenuated and hence inappropriate as live vaccine strains against enterobacterial infections. Here we show that multiple oral immunizations with *Salmonella enterica* serovar Typhimurium LPS mutants could elicit immune protection in the mouse model of typhoid. Unlike structural LPS mutants, the regulatory mutant lacking RfaH finely balanced between safety and immunogenicity and its vaccine potential in these respects was comparable to that of the well-characterized *aroA* mutant. The favorable vaccine potential of the *rfaH* mutant originated from a partial down-regulation of LPS synthesis. RfaH is a transcriptional antiterminator loss of which results in heterologous length of LPS chains, designated here as the 'gently rough' phenotype. Furthermore, we give evidence that the rough phenotype enhances immunogenicity of minor antigens that may improve cross-protection to heterologous bacteria. A panel of conserved antigens shared by members of *Enterobacteriaceae* was detected by immune sera raised upon vaccination with the *rfaH* mutant. The nature of these antigens was identified by a proteomic approach.

MI 44 The *pilT* gene of *Dichelobacter nodosus* is required for protease secretion

Xiaoyan Han[1,2], Ruth M. Kennan[1,2], John K. Davies[1,2], Leslie A. Reddacliff[3], Om P. Dhungyel[4], Richard J. Whittington[1,4], Lynne Turnbull[2], Cynthia B. Whitchurch[2] & Julian I. Rood[1,2]

[1]ARC Centre of Excellence in Structure and Functional Microbial Genomics; [2]Dept of Microbiology, Monash University, Australia; [3]Elizabeth Macarthur Agricultural Institute, Camden, Australia; [4]Faculty of Veterinary Science, University of Sydney, Camden, Australia

Type IV fimbriae are required for protease secretion in *Dichelobacter nodosus*. We investigated the role of twitching motility-related genes, *pilT* and *pilU*, in protease secretion and virulence. Mutation in *pilT* led to reduced protease secretion and adhesion to epithelial cells, whereas *pilU* mutants had wild-type levels of extracellular protease secretion and adherence. These data provided evidence that PilT was required for the type IV-fimbriae dependent protease secretion pathway. It was postulated that sufficient fimbrial retraction must be occurring in *pilU* mutants to allow protease secretion to take place, which was supported by evidence that aberrant motion was detected in an equivalent *pilU* mutant of *Pseudomonas aeruginosa*. Both *pilT* and *pilU* mutants were avirulent in sheep, providing evidence that twitching motility, not cell adherence or protease secretion, is essential for virulence.

MI 45 Study of peripheral blood neutrophil's biological response to *Helicobacter pylori* stimulation using flow cytometry technique

Saman Maleki-Vareki, Hamid Emami, Hamid Zarkesh-Esfahani, Mandana Lak & Arash Babaei

Dept of Biology, University of Isfahan, Isfahan, Iran

Helicobacter pylori is a gastric pathogen which evades the immune response. *H. pylori* causes gastritis, gastric lesions, gastric cancer and it causes the greatest microbial infection worldwide. The host immune response to *H. pylori* infection comprises both innate and adaptive immunity. Neutrophils are one of the most stimulated important

immune cells in the innate immune response toward *H. pylori*. These cells are common in type B gastritis which is recognized by massive neutrophil infiltration at Lamina propria. Although neutrophils are present in the site of *H. pylori* infection, they are unable of eradication of bacteria. One possible explanation could be the lower stimulation of neutrophils by *H. pylori* compared to other bacteria. Adhesion molecules like CD11b which are expressed during neutrophil activation are responsible for neutrophil migration to adjacent tissues. CD11b has several important roles in neutrophil physiology and could be measured as a marker for neutrophil activation. In this study the level of CD11b expression was compared in neutrophils stimulated by *H. pylori* or *E. coli* respectively as measured using flow cytometry. CD11b expression was lower in neutrophils stimulated by *H. pylori* compared to *E. coli*. We can conclude that *H. pylori* is less stimulating for neutrophils compared to *E. coli*.

MI 46 Frequency of *Helicobacter pylori* infection in dyspeptic patients who were referred to Shahid Mofatteh Clinic of Yasouj with UBT (14C)

O. Ilami & A.M. Khosravani

Yasouj University of Medical Sciences, Iran

Background Dyspepsia is belonging to a heterogenic group of disorder which appears as pain in upper part of abdominal. *Helicobacter pylori* (*H. pylori*) infection plays an important role in the cancer. Therefore the diagnosis of H. pylori infection is very important. The aim of the study was to validate a frequency of *H. pylori* infection in patient's referred to Shahid pathogenesis of chronic gastritis, peptic ulcer disease, MALT lymphoma and gastric Mofateh Clinic of Yasouj with UBT test.

Methods Among a population of patients with symptoms suspect for peptic ulcer referred to Shahid Mofateh Clinic of Yasouj 330 people were selected as random which included of 102 men and 228 women undergoing to the 14C-urea breath test (143C-UBT). The results then analyses by chi-square test via SPSS ($p<0.01$).

Discussion The frequency of *Helicobacter pylori* infection in dyspeptic patients has no significant differences compare to the public population. It seems that the *Helicobacter pylori* infection has not important role in dyspepsia. The rate of *Helicobacter pylori* infection in men with family history of peptic ulcer is higher than others. The reason for this is the exposure of men to the environment factors and the other members which is common in yasouj culture *Helicobacter pylori* infection.

MI 47 Antimicrobial effect of triterpenoid extract of *Ganoderma* on *Candida albicans* and *Proteus mirabilis*

A. Khosravani, A. Sharifi & B. Sharifi

Dept of Microbiology, Yasouj University of Medical Sciences, Iran

Due to emergence of resistance to antibiotics amongst micro-organisms, investigations for novel antimicrobial agents have always been one of the major preoccupations of medical society.

In the present study antimicrobial activity of *Ganoderma Reishi* that is Mushroom belong to Basidiomycete family and scientists reported anticancer, antialergy, anti-inflamatory and anti HIV properties of this mushroom. The crude Triterpenoid extract of *Ganoderma Reishi* was active against *Candida albicans* (MTCC 1637) & *Proteus mirabilis* (MTCC 1429) while, showed the zone of inhibition 22 & 25mm and MIC 32 and 64 in case of *C. albicanse* & *P. mirabilis* respectively.

The antimicrobial potential of Triterpenoid extract with commercial antifungal and antibacterial were compared and that was acceptable. It can be used in future as an antibiotic against micro-organisms.

Keywords *Ganoderma*, triterpenoid, antimicrobial activity

MI 48 Hypo-osmotic shock experiments with the mechanosensitive channel triple deletion mutant – a single-cell study

Marcel Reuter[1], Nicolas Hayward[2], Samantha Miller[2], Ian Booth[2] & David Dryden[2]

[1]School of Chemistry, West Mains Road, University of Edinburgh, Edinburgh EH9 3JJ; [2]School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Ashgrove Road West, Aberdeen AB25 2ZD

Escherichia coli MJF465 cells in which the three major mechanosensitive channel genes are deleted (Δ*mscL*, Δ*mscS*, Δ*mscK*) show only 10% cell viability upon hypo-osmotic shock (minimal medium + 0.5 M NaCl into distilled water) compared to 90% viability of the wild-type strain (FRAG1). Using optical tweezers combined with micro fluidic devices enables to observe single cell behaviour upon hypo-osmotic shock and unravels intra-population diversity. Phase-contrast imaging of MJF465 cells subjected to hypo-osmotic shock revealed different features of lysis, i.e. bursting and fainting cells (the latter leaking DNA and protein from the cytoplasm). Fluorescence microscopy of hypo-osmotically shocked GFP-expressing MJF465 cells showed either bursting of cells or fast leakage of GFP indicating strong cell wall ruptures. As a crucial step for observing cell lysis in the micro fluidic experiments the *E. coli* cells have to be supplied with oxygen (using 50 µM hydrogen peroxide as additive) which prevents early cell starvation. Statistical analysis of the generated data sets is presented.

MI 49 The Ard antirestriction proteins and their role in the spread of antibiotic resistance in bacterial populations

Gareth A. Roberts, John H. White, Bansi Sanghvi, Dimitra Serfioti-Mitsa & David T.F. Dryden

School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ

Horizontal gene transfer (HGT) by transduction, transformation and conjugation is extremely common within and between bacterial species and is responsible for the spread of antibiotic resistance genes. HGT occurs despite the presence of the restriction-modification (RM) systems that function to protect the host cell from invasion by foreign DNA. So why are the RM systems so variable in effectiveness against HGT? We suggest that it may be due to the presence of anti-RM genes on the mobile elements, such as phage, plasmids and transposable elements, which have been acquired and maintained by the host organism. The occasional activation of such genes weakens or negates the RM defence system allowing further HGT.

Ard (ArdA and ArdB) proteins are very commonly found on conjugative plasmids and transposons in a range of prokaryotes. However, very few Ard proteins have been characterised at a genetic or biochemical level. We have conducted sequence analysis and secondary structure predictions on 12 such putative antirestriction proteins from medically important bacteria. *In vivo* tests against Type I RM systems (IA, IB, IC and ID) show them all to display antirestriction activity. The ArdA proteins display antimodification activity against some subtypes of Type I RM system. By contrast, ArdB proteins are largely ineffective against modification.

MI 50 Biophysical characterisation of the anti-restriction protein Ocr

Augustinos Stephanou, Gareth A. Roberts, Andrew R. Thomson, Mark Tock, Emily Pritchard & David T.F. Dryden

School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ

The anti-restriction protein, Ocr (overcome classical restriction), is the product of gene 0.3 of bacteriophage T7 and is the first protein to be

expressed as the T7 phage enters a bacterium. Ocr interacts directly with Type β restriction-modification enzymes to inhibit their function. Structural analysis of Ocr reveals that the protein mimics the dimensions and charge of DNA. In particular, phosphate groups of B-form DNA can be directly superimposed onto amino acid side chain carboxyl groups upon the surface of Ocr. A range of mutations and chemical modifications to these amino acids have therefore been created in order to better characterise the inhibitory activity of Ocr.

MI 51 The ORF18 gene product from conjugative transposon Tn916 is an ArdA antirestriction protein inhibiting type I DNA restriction-modification systems

Dimitra Serfioti-Mitsa[1], Gareth Roberts[1], John H. White[1], Garry Blakely[2] & David T.F. Dryden[1]

[1]School of Chemistry, [2]School of Biology, University of Edinburgh, The King's Buildings, Edinburgh EH9 3JJ

In the right half of the conjugative transposon Tn*916* from the bacterial pathogen *Enterococcus faecalis*, gene *orf18* codes for a putative ArdA (alleviation of restriction of DNA) protein. Conjugative transposons are generally resistant to DNA restriction upon transfer to a new host. ArdA from Tn*916* may be responsible for the apparent immunity of the transposon to restriction systems following its introduction into a new host and for ensuring that the transposon has a broad host range. ArdA was successfully cloned, overexpressed and purified. The protein appears to exist as a dimer in nanomolar concentrations but can form hexameric polymers at micromolar concentrations. Fluorescence quenching studies suggest that the two tryptophan residues are located on the surface of the protein. Antirestriction and antimodification assays revealed that ArdA can efficiently inhibit restriction and modification by all four classes of Type I restriction-modification enzymes.

Posters

Physiology, Biochemistry & Molecular Genetics Group

PBMG 01 Notification of a new on-line transposable element registry

Adam P. Roberts

Division of Microbial Diseases, Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD

Currently there is no universal system used to name newly discovered transposable elements. This has led to a confusing array of systems being used to name transposons. Following discussions among the community a return to the previous nomenclature is proposed whereby transposons are designated a sequential Tn number. Here an on-line transposon registry is introduced (http://www.ucl.ac.uk/eastman/tn/) whereby investigators can request Tn numbers and log details of the newly discovered elements. The rules governing designation of a Tn number to an element are discussed and comments and suggestions from the community are welcomed.

PBMG 02 Diversity of putative transposons in *Streptococcus pneumoniae*

S.K. Henderson-Begg[1], A.P. Roberts[2] & L.M.C. Hall[1]

1Barts & the London School of Medicine and Dentistry, London; 2UCL Eastman Dental Institute, University College London

Pneumococcal resistance to tetracycline, chloramphenicol, erythromycin and clindamycin is often attributed to carriage of conjugative transposons of the Tn*916* family and the less well studied Tn*5252*. Here, carriage of Tn*5252* integrase (int_{5252}), Tn*5252 umuC* and *umuD* homologues and Tn*916* integrase (int_{916}) was examined among 60 antibiotic-resistant clinical isolates.

The int_{5252} gene was found in 14/60 isolates with diverse resistance patterns, nine of which also contained int_{916}, *umuC* and *umuD*. int_{5252} nucleotide sequences from five of these isolates showed up to 2% divergence from the published sequence, although the element appeared to be inserted at the site previously described. One int_{5252}/*umuC*/*umuD* carrier lacked int_{916} but its resistance profile differed from that described for Tn*5252*. Four isolates carried int_{5252} and int_{916} alone, 11 carried int_{916} only, two carried *umuC* and *umuD* only and 31 had none of these genes; resistance patterns were diverse in all groups.

The overall variability among resistance phenotypes and transposon-associated genotypes is high. Tn*5252*-associated genes appear more frequent than previously thought and warrant further investigation.

PBMG 03 The identification and characterization of *tet(32)* genes encoding tetracycline resistance from the human oral microbiota

Philip Warburton, Adam P. Roberts, Elaine Allan, Helen Ewles, Holli Lancaster, Lorna Seville & Peter Mullany

Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, University College London, London WC1X 8LD

Since its general introduction in the 1950's, tetracycline and its derivatives have been extensively used to treat bacterial infections because of their broad efficacy combined with the absence of any major adverse side-effects. However, this widespread use has resulted in the development, persistence and transfer of resistance. Tetracycline-resistant bacteria are common in the oral cavity and many of these have acquired genes encoding ribosomal protection proteins. In this study we describe tetracycline resistance genes from the oral microbiota which appear to be variations of the archetypal *tet*(32) gene. The predicted tetracycline resistance proteins over the entire 639 amino acids, shows 69% identity to both Tet(M) and Tet(O). The regions immediately flanking the gene were sequenced and analysed, and the regions were found to have similarities with mobile elements, in particular CTn*2* from *Clostridium difficile*, the ATE-*3* element from *Arcanobacterium pyogenes* and TnB*1230* from *Butyrivibrio fibrisolvens*.

PBMG 04 Characterization of a novel GH3 *N*-acetylglucosaminidase of *Haemophilus influenzae*

F. Chang-Pi-Hin, J. Pratten, M. Wilson & S.P. Nair

Division of Microbial diseases, UCL-Eastman Dental Institute, University College London, WC1X 8LD

Haemophilus influenzae is one of the major causes of acute otitis media and can cause sinusitis, meningitis and pneumonia. Genome sequencing of *H. influenzae* Rd identified a putative ORF, HI0959, predicted to code for an *N*-acetyl-β-glucosaminidase. This class of enzymes has an important role in cell wall turn-over and polysaccharide metabolism. To confirm the prediction of HI0959 as an *NAG* and to provide experimental evidence of its substrate specificity, the gene coding for this putative enzyme was cloned and the activity of recombinant protein characterized.

Characterization of the specific activity of HI0959 was carried out using different *p*-Nitrophenyl saccharides. The measured activities confirmed that HI0959 is a β-*N*-acetylglucosaminidase. Furthermore product inhibition studies demonstrated that *N*-acetylglucosamine is a competitive inhibitor.

In conclusion we have characterized a novel β-*N*-acetyl-glucosaminidase of *Haemophilus influenzae*. Since this class of enzymes play an important role in cell wall turnover determining their enzymatic mechanisms may lead to the development of inhibitors that could have potential use in treating infections with this organism.

PBMG 05 Cloning and characterization of the α-1,3-glucanase from the filamentous fungus *Penicillium purpurogenum*

Gil Shalom, Jonathan Pratten, Michael Wilson & Sean P. Nair

Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD

Extracellular polysaccharides produced by oral streptococci play an important role in the formation of biofilms (dental plaque) on tooth surfaces. A major component of these biofilms is mutan which is composed of mainly α-1,3-glucans. Mutanase (α-1,3-glucanase) has been shown to aid the suppression of dental plaque formation in humans. In this study the mutanase gene from *Penicillium purpurogenum* was expressed in the yeast *Pichia pastoris*. The molecular mass of the secreted protein was 102 kDa. The activity and specificity of the enzyme were determined using mutan and other glucan substrates. Activity was only detected towards mutan. Synthetic compounds were also tested as substrates to assess whether the mutanase has an *exo*- or *endo*-type mechanism of hydrolysis. The results suggest an *endo*-hydrolytic mode of action. The type of mechanism was confirmed since mutanase activity was not suppressed in the presence of inhibitors of *exo*-type enzymes. To conclude, the mutanase enzyme could potentially be used as an aid in disrupting dental plaque.

PBMG 06 Cypemycin: a peculiar lantibiotic

Jan Claesen & Mervyn Bibb

Dept of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH

Lantibiotics are ribosomally synthesized peptide antibiotics that are extensively post-translationally modified. They are produced by a variety of Gram-positive bacteria and their mechanisms of action include disruption of cell wall biosynthesis and membrane permeabilization in a lipidII-binding dependent manner. The occurrence of the unusual amino acid lanthionine (Lan) in the mature peptides, lent the name 'lantibiotics' to this class of antibiotics. In addition to Lan, a number of post-translationally modified residues are typically found, including methyl-lanthionine (MeLan), didehydroalanine and didehydrobutyrine (Dba). A range of less common modifications has also been reported, stimulating research into unveiling the novel enzymatic catalytic mechanisms resulting in these unusual amino acids.

Cypemycin is a peculiar lantibiotic produced by *Streptomyces* sp. OH-4156. While the mature peptide does not contain Lan or MeLan, it does contain four Dba residues and an *S*-aminovinyl-D-cysteine, typical of lantibiotics. Cypemycin also contains a number of unique post-translationally modified residues, including two *allo*-isoleucines and an N-terminal *N,N*-dimethylalanine. Functional analysis of the cypemycin biosynthetic gene cluster will lead to a greater understanding of the lantibiotic and its highly unusual chemical modifications.

PBMG 07 Analysis of a novel lantibiotic gene cluster from *Microbispora corallina*

Lucy Foulston & Mervyn Bibb

Dept of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH

Lantibiotics are ribosomally synthesized, post-translationally modified peptide antibiotics. They are characterized by lanthionine and methyl lanthionine bridges between cysteine and dehydrated serine and threonine residues, respectively, giving lantibiotics their characteristic conformations and stability. Lantibiotics are encoded by a structural gene (LanA) whose product is post-translationally modified by enzymes that include dehydratases, cyclases and leader peptidases. Additional proteins are required for export of the lantibiotic and for host resistance. The genes encoding these proteins are generally found within clusters, and are co-expressed.

A newly identified actinomycete species, *Microbispora corallina*, produces a potent lantibiotic with a high degree of modification including a chlorinated tryptophan and a dihydroxyproline, in addition to (methyl)-lanthionine bridges and an aminovinyl-cysteine residue. This project aims to identify and characterize the corresponding biosynthetic gene cluster via genome scanning, employing the rapid sequencing power of the Solexa System. This will be followed by heterologous expression of the cluster in *Streptomyces lividans* with deletion analysis and knock-out mutagenesis to determine the minimal gene set for lantibiotic production and the functions of individual genes.

PBMG 08 The Ser-Thr-Tyr phosphoproteome of *Streptomyces coelicolor*

Jennifer Parker[1], Mike Naldrett[2], Alex Jones[3] & Mervyn Bibb[1]

[1]Dept of Biological Chemistry; [2]Dept of Molecular Microbiology John Innes Centre, Norwich NR4 7UH; 3Sainsbury Laboratory, Norwich NR4 7UH

Phosphorylation by serine (Ser), threonine (Thr) and tyrosine (Tyr) protein kinases (STPKs) is well established as an important regulatory mechanism in eukaryotes. More recently its significance has been discovered in a number of complex bacteria, including the identification of 34 STPKs in *Streptomyces coelicolor* A3(2). Thus far only a handful of these kinases have had their functions elucidated including AfsK and RamC, with studies showing their involvement in secondary metabolism and morphological differentiation, respectively.

To date, 20 kinase deletion mutants have been generated. Phenotypic analysis using various complex and minimal solid media reveals variations in the onset of antibiotic production, growth and morphological differentiation compared to the wild type. Mutants in liquid cultures reveal subtle variations in growth when compared with M600.

Immobilized metal ion affinity chromatography (IMAC) has been used to enrich for phosphorylated peptides from *S. coelicolor* strain M600 cell lysates. These enriched samples were then subjected to analysis by mass spectrometry (LC-MS/MS) resulting in the identification of a number of novel proteins phosphorylated on serine, threonine and tyrosine residues.

PBMG 09 Regulation of transcription by the *Escherichia coli* nitric oxide sensor norr

Nicholas P. Tucker[1], Benoît D'Autréaux[3], Faridoon Yousafzai[1], Shirley Fairhurst[1], Stephen Spiro[2] & Ray Dixon[1]

[1]Dept of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH; [2]Dept of Molecular and Cell Biology University, of Texas at Dallas PO Box 830688 Richardson, Texas 75083-0688 USA; [3]Service de Biochimie et de Genetique Moleculaire, DBCM, DSV, CEA-Saclay, Gif-sur-Yvette,91191 Cedex, France

The NorR regulatory protein senses nitric oxide (NO) to activate genes required for NO detoxification under anaerobic and microaerobic conditions in *Escherichia coli*. NorR belongs to the σ^{54}-dependent family of transcriptional activators and contains an amino-terminal regulatory GAF domain that controls the ATPase activity of the central AAA+ domain to regulate productive interactions with σ^{54}. Binding of NO to a non-heme iron centre in the GAF domain results in the formation of a mononitrosyl-iron complex and releases intramolecular repression of the AAA+ domain to enable activation of transcription. In this study, we have further characterized NorR spectroscopically, and have substituted conserved residues in the GAF domain. This analysis, in combination with structural modelling of the GAF domain, has identified five candidate ligands to the non-heme iron, and suggests a model in which the metal ion is co-ordinated in a pseudo-octahedral environment by three aspartate residues, an arginine and a cysteine.

PBMG 10 Development of the *Campylobacter* genetic toolbox

D.J.H. Gaskin, B.M. Pearson & **A.H.M. van Vliet**

Institute of Food Research, Norwich NR4 7UA

Campylobacter research suffers from having few genetic tools. Specific gene knockout construction is straightforward. Other tools like reporter constructs, conditional or unmarked mutations and gene complementation are not widely employed. The available techniques are often cumbersome and limited, relying on the use of specific strains.

We have developed vectors providing reporter gene and gene complementation technologies. These utilize insertion into a pseudogene (NCTC11168 Cj0046) conserved across the sequenced strains. Promoter sequences have been cloned upstream of GFP, β-galactosidase or GUS reporter genes in pCREP vectors, allowing studies on gene expression. The pCOMP vectors are similar, into which genes and either the native promoter for the gene or, non-native constitutive or controlled promoters can be cloned allowing conditional or non-conditional gene complementation. Several selection markers are available allowing complementation of existing gene knockouts. Finally, we have developed unmarked mutation construction, potentially avoiding polar effects of the mutation.

Applications of this system include the insertion of protein purification tag sequences, fixing variable sequences ie G-tracts, and mutant complementation. Allowing these approaches will undoubtedly further our understanding of *Campylobacter* biology.

PBMG 11 TonB-dependent iron acquisition in *Helicobacter mustelae*

J. Stoof[1], E.J. Kuipers[1], J.G. Kusters[1] & A.H.M. Van Vliet[1,2]

[1]Erasmus MC, Rotterdam, The Netherlands; [2]Institute of Food Research, Norwich NR4 7UA

Helicobacter mustelae colonizes the gastric mucosa of ferrets. Iron-restriction at mucosal surfaces is a non-specific host defence mechanism against bacterial pathogens, and conversely iron acquisition is considered to be an important virulence factor. Iron-acquisition in Gram-negative bacteria is mediated by outer membrane proteins that require energy transduced by the TonB system. Here we describe the characterization of the role of the two *H. mustelae tonB* homologs in iron acquisition.

Wild-type *H. mustelae* was able to utilize hemoglobin, hemin and ferric citrate as sole iron source. A *tonB1* mutant was unable to utilize hemin as sole iron source, but was unaffected in growth on the other tested iron sources. In contrast, mutation of the *tonB2* gene resulted in reduced growth with ferric citrate and hemoglobin. Transcription of *tonB1* was iron-independent, whereas transcription of *tonB2* was iron-repressed.

In conclusion, the two TonB homologs of *H. mustelae* have differential roles in iron acquisition, and this is likely to assist *H. mustelae* in over-coming host-mediated iron restriction at the gastric mucosal surface.

PBMG 12 Adaptation of carnivore-colonizing *Helicobacter* species to the diet of their host

J. Stoof[1], S. Breijer[1], R.G.J. Pot[1], E.J. Kuipers[1], J.G. Kusters[1] & A.H.M. Van Vliet[1,2]

[1]ErasmusMC, Rotterdam, The Netherlands; [2]Institute of Food Research, Norwich NR4 7UA

Gastric *Helicobacter* species use the nickel dependent urease enzyme (encoded by *ureAB*) to mediate acid resistance. While nuts, seeds and vegetables are relatively rich in nickel, meat only contains low amounts of nickel. As a consequence *Helicobacter* species colonizing obligate carnivores are likely to be subjected to nickel-restriction. Recently a second urease gene cluster (*ureA2B2*) was detected in these *Helicobacter* species. Here we report the characterization of both urease enzymes in the ferret pathogen *Helicobacter mustelae*.

Both ureases were active and were expressed in nickel-restricted defined medium. In contrast to the nickel-induced UreAB, expression of UreA2B2 was iron-induced and repressed by nickel supplementation. The UreA2B2 urease did not require activation by urease accessory proteins, and both urease enzymes allowed survival of severe acid shocks in the presence of urea.

In conclusion, carnivore-colonizing *Helicobacter* species express a second urease system (UreA2B2) which is only expressed under nickel-restricted conditions, and may represent an adaptation of these *Helicobacter* species to the limitations of the diet of their carnivorous host.

PBMG 13 Characterization and adaptation of plasmids in *Lactobacillus salivarius*

Fang Fang, Douwe van Sinderen & Paul W. O'Toole

Dept of Microbiology & Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland

Background and Objectives *Lactobacillus* is one of the organisms most commonly used as a probiotic. About 38% of the species in this genus harbor plasmids. In order to investigate and analyse biologically relevant characteristics of probiotic lactobacilli, gene expression vectors, expression monitoring and gene mutagenesis systems need to be developed. As limited numbers and types of replicons can replicate in some strains of this genus, characterization of endogenous plasmids from *Lactobacillus* is of great interest.

Methods Pulsed-field gel electrophoresis and Southern blot were used to detect plasmids in 27 *L. salivarius* strains. Gene cloning and transmissible cloning vectors for probiotic lactobacilli were constructed by cloning either the replication region or the mobilization and the replication region from a *L. salivarius* plasmid pSF118-20 into an *E. coli* cloning vector.

Results and Conclusions Endogenous plasmids of various sizes were detected in *L. salivarius* strains. A gene cloning and expression vector (pLS203) and a transmissible cloning vector (pLS208) were constructed based on plasmid pSF118-20 from *L. salivarius* UCC118. pLS203 is quite stable in lactobacilli in the absence of antibiotic selection and may thus be used as a gene cloning and expression vector. pLS208 can be mobilized from *L. plantarum* to *L. fermentum* with the help of a conjugative plasmid. pSF118-20 derivatives showed broad host range, high segregational stability and ability for horizontal gene transfer. Therefore, vectors that were derived from lactobacillus plasmids have the advantage to be developed into various genetic tools to monitor and understand behavior of probiotic lactobacilli *in vivo*.

PBMG 14 Palatinose and cellodextrin metabolism in *Bifidobacterium breve* UCC2003

Karina Pokusaeva, Mary O'Connell-Motherway & Douwe van Sinderen

Dept of Microbiology & Alimentary Pharmabiotic Centre, University College Cork, Ireland

Bifidobacteria represent one of the dominant bacteria of the intestinal microflora of humans and animals. In recent years, bifidobacteria have been the subject of increasing interest due to their perceived positive role in the maintenance of gastrointestinal health through, among others, their effect on cholesterol and carbohydrate metabolism in the colon.

For the above reason, bifidobacteria are frequently added to the diet in probiotic foods. Another way to increase the number of indigenous bifidobacteria is through the consumption of prebiotics, which are mostly complex carbohydrates that escape digestion by the host and are specifically metabolized by probiotic bacteria.

The aim of this study was to investigate carbohydrate metabolism of *Bifidobacterium breve* UCC2003, an isolate from nursling stool, whose genome was shown to dedicate approximately 9% towards sugar metabolism. *In silico* genome analysis and subsequent experimental characterization allowed the identification of gene clusters responsible for palatinose and cellodextrin metabolism.

PBMG 15 Comparative and functional genomics of *Photorhabdus* species

M. Sanchez-Contreras[1], I. Eleftherianos[1], A. Dowling[2], P. Wilkinson[2], S.E. Reynolds[1], R.H. Ffrench-Constant[2] & N.R. Waterfield[1]

[1]Dept of Biology and Biochemistry, University of Bath, BA2 7AY; [2]Dept of Biological Sciences, University of Exeter, TR10 9EZ

Photorhabdus asymbiotica ATCC43949 is a recently sequenced model emerging human pathogen able to infect both insect and human hosts. Whole genome comparison with the strict insect pathogen *Photorhabdus luminescens* TT01 allows the identification of aquired genes potentially involved in human pathogenicity. In parallel to the *in silico* analysis and to assist with the annotation of *P. asymbiotica* we developed an approach termed Rapid Virulence Annotation (RVA). RVA involves the screening of cosmid libraries from pathogenic bacteria against three model invertebrates to identify functional virulence factors. In the case of *P. asymbiotica* an additional screen against a

macrophage cell line was employed, which identified toxins potentially involved in mammalian infections. The comparison of both RVA datasets revealed the commonalities of functional virulence factors for invertebrate pathogenicity in the two *Photorhabdus,* adding value to the whole genome sequence comparison. Furthermore, the RVA analysis highlights virulence factors likely to be involved in mammalian interactions providing an insight into the mechanisms for cross-species pathogenicity. The implications for understanding the emergence of bacterial diseases are discussed.

PBMG 16 Plasmids of the IncHI1 group and the competition within: a global replacement

Minh-Duy Phan, Keith Turner & John Wain

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

IncHI1 plasmids (180–220 kb) are important vectors of multiple-antibiotic resistance in *Salmonella enterica* subspecies *enterica* serovars Typhi and Paratyphi A, which cause enteric fever. Microarray and long-range overlapping PCRs were used to compare the presence and location of genes among IncHI1 plasmids from *S.* Typhi. This showed that IncHI1 plasmid maintenance functions are relatively conserved and arranged co-linearly whilst antibiotic resistance determinants are sited within regions of greater variation.

A plasmid multi-locus sequence typing (PMLST) scheme is also described, which defined 8 sequence types among 37 IncHI1 plasmids from UK, South East Asia, Japan, Mexico, India, Jordan and Pakistan between 1961 and 2004. Their evolutionary relationship, calculated using the eBURST program, revealed two main lineages. Lineage 1 includes plasmids from 1972–1996 whilst lineage 2 contains more recent plasmids from 1993–2004. The latter, however, are not directly descended from the former. These data suggest that the two separate lineages of IncHI1 plasmids co-existed in *S.* Typhi during 1993–1996 before plasmids of lineage 2 became predominant, and spread to *S.* Paratyphi A.

PBMG 17 A predicted ArsR/SmtB family transcriptional repressor, PigS, regulates prodigiosin production in *Serratia* sp. ATCC 39006

T. Gristwood, P.C. Fineran & G.P.C. Salmond

Dept of Biochemistry, University of Cambridge, Cambridge CB2 1QW

Serratia sp. ATCC 39006 (39006) is a Gram-negative enterobacterium which produces a carbapenem antibiotic, pectinases, cellulases and an intracellular red pigment, prodigiosin. Prodigiosin has been reported to have immunosuppressive and anti-cancer properties although the physiological role in 39006 is not yet known. Regulation of exoenzyme and secondary metabolite production involves numerous factors that form a complex hierarchal regulatory network. PigP, a master regulator of in 39006 with homologues in a very limited group of Enterobacteriaceae, modulates transcription of multiple genes involved in secondary metabolite production, including *pigS*. A mutation in *pigS* results in decreased production of prodigiosin. Unexpectedly, overexpression of *pigS in trans* in the wild-type also results in decreased prodigiosin production. PigS shares homology with the ArsR/SmtB of family of metalloregulatory transcriptional repressors. However, adjacent to the *pigS* gene are genes predicted to encode a YeeE/YedE family putative membrane protein (*mp1*) and a metallo-β-lactamase (*blh*). PigS autoregulates via direct binding to the *pigS* promoter, and may also regulate transcription of *mp1* and *blh*. Therefore, *pigS* appears to fall into the newly proposed BigR subfamily of ArsR/SmtB proteins.

PBMG 18 Use of antisense peptide nucleic acid (PNA) for species-specific bactericide

Madhav Mondhe, Anthony O'Donnell & Jem Stach

School of Biology-IRES, Devonshire Building, University of Newcastle, Newcastle upon Tyne NE1 7RU

Regulation of gene expression is endogenous to prokaryotes and eukaryotes, which ensures sequence-specific down regulation of the target gene. Synthetic DNA/RNA binding mimics/oligonucleotides, in particular, peptide nucleic acid (PNA) have increasingly been used to modulate the expression of desired genes for various medical and microbiological applications. Recent studies show that antisense PNAs can used to regulate bacterial growth, in both Gram-positive and negative species, when targeted to the gene essential for survival. The main objective of this study is to exploit the specificity of antisense PNAs in selective isolation or inhibition of bacterial species in mixed culture system.

In this study we examined the bactericidal activity of some essential-gene specific unique antisense peptide-PNAs against *Escherichia coli* BL21, *Salmonella enterica* (*Typhimurium*), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (PAO1). Upon treatment we found that antisense PNAs are indeed bactericidal at low micromolar concentration (except PAO1) and did not inhibit non-target species when treated in mono or mixed culture. This species-specific bacterial selection strategy, through antisense PNAs, can also be applied for studying the role of particular species in community structure and function, in addition to the antimicrobial use.

PBMG 19 Natural polymorphism in the *Helicobacter pylori* vacuolating cytotoxin signal sequence affects toxin production

C.G. Masters, D.P. Letley & J.C. Atherton

Institute of Infection, Immunity and Inflammation, University of Nottingham, NG7 2RD

Background The *H. pylori* vacuolating cytotoxin gene, *vacA*, is naturally polymorphic, particularly in the signal region (type s1 or s2). Heterogeneity in VacA levels exists, and type s1 (tox+) and s2 (tox-) signal sequences differ in the cleavage recognition site at positions –3 and –6: (s1; Ser, Pro, s2; Leu, Gly). We aimed to characterize the effect of polymorphic differences on VacA production. *Methods* Isogenic mutant strains of 60190 (s1), in which serine at position –3 was replaced by leucine S(–3)L, and proline at –6 was replaced by glycine P(–6)G were prepared. Reciprocal changes L(–3)S G(–6)P were made in strain Tx30a (s2). VacA levels were determined in broth culture supernatants by immunoblotting and ELISA.

Results For strain 60190, mutation S(–3)L did not significantly reduce VacA production. However mutations P(–6)G and P(–6)GS(–3)L significantly reduced VacA production ($p<0.05$). For Tx30a, mutations L(–3)S, G(–6)P, and G(–6)PL(–3)S significantly reduced VacA production ($p<0.005$).

Conclusions These results indicate that differences in the VacA signal sequence affect VacA production. We speculate that naturally occurring differences affect signal sequence processing efficiency.

PBMG 20 Methylglyoxal detoxification in enteric bacteria

Ertan Ozyamak, Camila de Almeida, Samantha Miller & Ian Booth

Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD

Bacteria live in dynamic environments and have to survive a multitude of challenges. A particular challenge is the exposure to electrophilic compounds such as methylglyoxal (MG). MG can be produced endogenously during unbalanced sugar metabolism and kills via damage to macromolecules. The glutathione dependent glyoxalase system, consisting of glyoxalase I and II, provides the main route for detoxification of MG. Intermediates in this detoxification pathway activate the potassium channels KefB and KefC. Channel activation limits the toxicity of the electrophile by lowering cytoplasmic pH. This project investigates the importance of and balance between glyoxalases and channels for cell survival upon MG stress. Our studies on glyoxalase II demonstrate that protection from MG and its detoxification is intimately linked to the activation of KefB and KefC.

ChIP-chip analysis was used to investigate global transcriptional changes upon MG stress in *Escherichia coli*. The observed expressional changes did not comprise components of the MG detoxification pathway, suggesting that regulation does not occur at a transcriptional level.

PBMG 21 Magnetosome formation in marine vibrio MV-1

D. Trubitsyn, C.E. French & F.B. Ward

Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JR

Marine Vibrio MV-1 is a magnetotactic bacterium capable of aligning its cell in response to the Earth's magnetic field. This ability is due to the presence of chain-like structures comprising magnetosomes, magnetite particles enclosed in a lipid membrane with associated proteins. Strain MV-1 produces magnetosomes that are unique in shape and present in higher numbers than other better-characterized strains of magnetotactic bacteria. Multiple potential biotechnological applications for magnetosomes have been reported.

With the availability of genomes of several magnetotactic bacteria it has been shown that many of the genes associated with magnetosome formation are clustered in one region termed the 'magnetosome island'. However, corresponding knowledge for MV-1 is lacking. Here we report the sequence of magnetosome island genes for MV-1 together with analysis of mass spectrometry for the corresponding magnetosome associated proteins. These results are consistent with the view that the magnetosome formation system in MV-1 is similar to those previously described for other magnetic Alphaproteobacteria bacteria.

PBMG 22 The StoMP research network: stochastic dynamical modelling for prokaryotic gene regulatory networks

Rosalind J. Allen[1], Dov Stekel[2] & A. Jamie Wood[3]

[1]SUPA School of Physics, Edinburgh University, James Clerk Maxwell Building, The King's Buildings, Mayfield Road, Edinburgh EH9 3JZ; [2]School of Biosciences, University of Birmingham, Birmingham B15 2TT; [3]York Centre for Complex Systems Analysis, Dept of Biology (Area 17), University of York, PO Box 373, York YO10 5YW

StoMP is a network of mathematical modellers and microbiologists, funded by BBSRC under the 'Mathematical tools for Systems Biology' initiative. The network began in September 2007 and will run for 3 years. StoMP's aim is to strengthen the UK community at the mathematics/physical science/microbiology interface, in the area of stochastic dynamical modelling of gene regulation. This will involve organizing a series of research and dissemination-focused workshops, running a website and mailing list and providing funding for collaborative research visits between network members. We hope that this will lead to new research collaborations. More information is available on the StoMP website: www.stompnet.org. New members are always welcome.

PBMG 23 Molecular investigation of the bundle-forming pili (Bfp) of Aeromonas veronii bv. sobria: role in colonization and biofilm formation

N. Hadi[1], M. Tabei[2], S. Kirov[3] & J.G. Shaw[1]

[1]Academic unit of infection and Immunity, Medical school, University of Sheffield; [2]Dept of Biochemistry, Shiraz University of Medical Sciences, Iran; [3]Dept of Pathology, University of Tasmania, Australia

Bfp is thought to be the major adhesin of *Aeromonas* spp. We believe this appendage is required for the colonization of host cells and for the attachment to abiotic surfaces to form biofilms. Investigation of this hypothesis was done by isolating the genes of this structure, creating a series of isogenic mutants, testing whether they were essential for the formation of the pilus structure and for adherence to various adhesion models. The genes that encode the pilus structural proteins were isolated by PCR using degenerate primers and isogenic mutants were created by the insertion of a kanamycin cassette within the genes (*mshA* and *mshB*) and allelic exchange. The *mshA* and *mshB* mutants had reduced adhesion to HEp-2 cells and did not form biofilms as readily as the wild type strain. The complementation analysis was done with the copies of the wild type *mshA* and *mshB* genes expressed on plasmids introduced into the defective *Aeromonas* strains and investigation of the adhesion properties of the bacteria. The collective data suggested that the Bfp plays a major role in *Aeromonas* adherence.

PBMG 24 Spontaneous deletions within the *icaADBC* operon of clinical *Staphylococcus epidermidis* isolates; the possible role of LexA in phenotypic variations

Titik Nuryastuti[1], Henny C. van der Mei[2], Henk J. Busscher[2], Roel Kuijer[2], Abu T. Aman[1] & Bastiaan P. Krom[2]

[1]Dept of Microbiology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia; [2]Dept of Biomedical Engineering, University Medical Center Groningen, The Netherlands

Biofilm formation is governed by an interplay of cell surface characteristics. Here, the role of genotypic changes within the *icaADBC* operon on phenotypic variations of *S. epidermidis* is investigated.

Biofilm formation was detected by microtiter plate assay, streaking on CRA plate and CLSM. To determine the presence of ica operon, PCR and Realtime PCR were employed. Sequence analysis of *lexA* were performed to find out the genetic recombination.

Five *S. epidermidis* isolates demonstrated phenotypic variation. Black colonies displayed bi-modal electrophoretic mobility distributions at pH 2, but it was absent in red colonies. All red colonies had lost their *icaADBC* genes and realtime PCR targeting *icaA* indicated a reduction in gene copy number within cultures exhibiting phenotypic variation.

This is the first report of *S. epidermidis* irreversibly switching from biofilm-positive to biofilm-negative phenotype by spontaneous deletion of *icaADBC* genes. Amino acid substitution in *lexA* could be responsible for the increased incidence of genetic rearrangements.

PBMG 25 The evolutionary history of Group B *Streptococcus* (GBS), a bacterial genome shaped by DNA conjugation

Mathieu Brochet[1], Elisabeth Couvé[1], Christophe Rusniok[1], Patrick Trieu-Cuot[2], Frank Kunst[1] & Philippe Glaser[1]

[1]Genomics of Microbial Pathogens, URA-CNRS2171; [2]Unité Biologie des Bactéries Pathogènes à Gram-Positif, URA-CNRS2172 Institut Pasteur, 28 Rue du Dr Roux, 75724 Paris cedex 15, France

Bacterial populations are subject to complex processes of genome diversification that involve horizontal DNA transfer events, mediated by three main mechanisms: transformation, transduction and conjugation. It has been previously observed that the resulting horizontal exchanges lead to bacterial genomes being pocked by small chromosomal replacements from other related lineages. We used single-nucleotide polymorphism patterns among related isolates as a powerful tool to study DNA transfer events. By this means, we analysed the genetic flux among 8 genome sequences representative of the diversity of clinical isolates of GBS, the leading cause of neonatal infections. This allowed us to reconstruct the evolutionary history of these strains in which a specific ecotype has recently emerged and subsequently diversified by transfer of large DNA fragments. This scenario was supported by the demonstration of conjugative transfers of large chromosomal DNA fragments between different GBS isolates.

PBMG 26 Comparison of the next-gen sequencing technologies for genome sequencing

Kerstin A. Stangier & Christopher Bauser

GATC Biotech AG, Konstanz, Germany

The next-gen sequencing technologies, embodied by the GS FLX, the Illumina Genome Analyser and the SOLiD System, generate huge amounts of sequence information. The Paired-End method for all technologies allow repetitive regions to be sequenced and assembled with accuracies approaching the Sanger method. GATC presents data of genomes that has been sequenced using all next-gen technologies.

For the longer reads of the 454 technology algorithms for *de novo* assembly of smaller genomes are available. The Newbler assembler provides a *de novo* assembly that can be imported into other assembly programs and assembled with ABI3730 data.

The high coverage of the Solexa and SOLiD technology is ideal for resequencing to find SNPs and other differences between strains or patients. The availability of a reference genome allows mapping of reads. The mapping functions of these technologies allow a visualization and analysis of SNPs between the genome of interest and the reference genome using a standard genome browser web interface.

GATC introduces its cross-platform tagging system for parallel sequencing with an unlimited increase in the number of samples. The technique is highly efficient, resulting in 99.9% of sequences successfully tagged.

PBMG 27 Characterization of the pathway and enzymes involved in pilin O-glycosylation in *Neisseria meningitidis*

Amirreza Faridmoayer, Messele A. Fentabil & **Mario F. Feldman**

Alberta Ingenuity Centre for Carbohydrate Science, University of Alberta, Edmonton, Canada

Protein glycosylation is an important posttranslational modification that occurs in all domains of life. Pilins, the structural components of type IV pili, are O glycosylated in *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and some strains of *Pseudomonas aeruginosa*. We have characterized N. meningitidis MC58 O glycosylation pathway in *Escherichia coli*. We show thet pilin glycans are transferred en bloc by an oligosaccharyltransferase named PglL. PglL has relaxed glycan specificity and is sufficient for glycosylation. Translocation of the undecaprenol-pyrophosphate-linked oligosaccharide substrates into the periplasm is required for glycosylation, indicating that PglL activity is located to this compartment. PglL is able to transfer diverse oligo- and polysaccharides to pilin, including the glycans usually found in *C. jejuni* N-glycoproteins and O-antigens from *Pseudomonas*, *Salmonella* and several *E. coli* strains. To our knowledge, this is the first report describing recombinant O glycoproteins synthesized in *E. coli*. This kind of glycoproteins may have an application in the synthesis and design of conjugate vaccine.

PBMG 28 Phenotype microarray analysis of the metabolism of *Helicobacter pylori*

Xiang-He Lei & **Barry Bochner**

Biolog, Inc, 21124 Cabot Blvd, Hayward, CA, USA 94545

Because of its role as a causative agent of stomach ulcers, *Helicobacter pylori* was one of the first bacteria to have a completed genome sequence published. In the annotation of that genome the authors used *in silico* data analysis tools to predict its carbon metabolism. We used Phenotype MicroArrays to culture and test the carbon metabolism of a strain of *H. pylori* with 190 diverse carbon sources. We found that the *in silico* predictions were correct as far as they went, but were incomplete in that they failed to predict a large number of carbon sources utilized and they failed to predict which carbon sources were most preferred for rapid utilization. The Phenotype MicroArray data indicated that the preferred carbon sources of *H. pylori* are not sugars, but instead are some organic acids and amino acids. These carbon sources were part of a core set of carbon sources utilized, however *H. pylori* also could use additional carbon sources when serum albumin was added to the culture medium. We speculate on the biological role of this serum effect.

PBMG 29 *In vivo* resolution of conflicting *in vitro* results: synthesis of biotin from dethiobiotin does not require pyridoxal phosphate

Ahmed M. Abdel-Hamid[1] & John E. Cronan[1,2]

[1]Microbiology and [2]Biochemistry, University of Illinois, Urbana, Illinois 61801, USA

BioB from *E. coli* is a homodimer of 76 kDa. The catalysis of BioB is dependent on *S*-adenosyl-methionine (SAM). The source of the biotin sulfur atom remains a contested point in studies of biotin synthase (BioB) *in vitro*. Recent reports that BioB has an intrinsic pyridoxal phosphate (PLP)-dependent cysteine desulfurase activity were tested by depleting *Escherichia coli* cells of PLP. The *E. coli* host strain was blocked in the synthesis of pyridoxal (due to a null deletion/insertion mutation of *pdxH* which encodes pyridoxine 5`-phosphate oxidase as well as biotin (due to deletion of the *bioABFCD* operon). Biotin synthesis was measured by the covalent attachment of biotin to the 87-residue biotinoyl domain of *E. coli* AccB (AccB-87) catalysed by the *E. coli* BirA biotin protein ligase. The BioB source was an N-terminally hexahistidine-tagged protein produced from an arabinose-inducible *araBAD* promoter on a multicopy plasmid. The N-terminally hexahistidine-tagged AccB87 biotinoyl domain and BirA ligase were expressed from the powerful IPTG-inducible T7 promoter on a compatible multicopy plasmid. Biotin synthesis was detected by western blotting with a streptavidin-enzyme conjugate or by separation of the holo- and the apo domain on native PAGE and western blotting against anti-pentahistidine antibody. Biotinylation was also assayed by measuring incorporation of radioactivity into protein (holo Acc87 plus AccB) in cultures supplemented with radioactively labeled DTB. The results showed that essentially the same levels of conversion of DTB to biotin occurred in the presence or absence of biotin suggesting that BioB reaction does not require PLP *in vivo*.

PBMG 30 Chromosomally located integrons in *Pseudomonas* belong to a single family that has been repeatedly acquired

N.L. Wilson[1,2], S. Tetu[1], M.R. Gillings[2], H.W. Stokes[3] & **A.J. Holmes**[1]

[1]School of Molecular and Microbial Biosciences, University of Sydney, Sydney, NSW, Australia 2006; [2]Dept of Biological Sciences; [3]Dept of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, NSW 2109, Australia

Integrons facilitated the emergence and spread of multiple antibiotic resistance. Broader impact on bacterial evolution is suggested by the frequent presence of chromosomal integrons in disparate bacterial lineages. Here we explored the impact of chromosomal integrons in *Pseudomonas* as a model system. Phylogenetic analyses indicated all integrons were related (*Pseudomonas* Chromosomal Integrons, PCIs), but that different strains had independently acquired PCIs on one or more occasions. Most PCIs included deletions predicted to result in loss of recombination functions, but not gene cassette expression functions. All gene cassette arrays were distinct, including those at the same locus in closely related strains, suggesting deletions occurred after integron acquisition. Numerous IS elements were associated with the integrons, including a new family that specifically targets attC sites and frequently caused truncation of the gene cassette array. We conclude that integrons are inherently acquired elements, derived from the 'mobilome'. Distinct integron lineages show significant correlation to bacterial phylogenetic groups and are frequently integrated into bacterial chromosomes where they form associations of varying stability that influence the genome evolution of that lineage. The fate of integron-assembled arrays, and potential for re-mobilization of the integron are likely to be influenced by specific interactions with other genetic elements.

PBMG 31 Molecular analysis of two chromate-resistant *Synechocystis* species

Saiqa Razi & Shahida Hasnain

Dept of Microbiology and Molecular genetics, University of the Punjab, Lahore-54590, Pakistan

The effect of gamma radiation on the characterization of two locally isolated Cr-resistant *Synechocystis sp.* AHZ-HB-MK (DQ381960) and AHZ-HB-P2A (DQ398589) were investigated. Both strains were able to tolerate up to 200 µg ml^{-1} Cr (VI). These strains were irradiated with gamma rays after 5, 15 and 30 days of incubation. Doses ranged from 0–20 Gys. Mutants were selected from each dose for further characterization. Colony morphology was changed after irradiation but there was no effect on the cell morphology. Growth was measured in terms of O.D, Dry weight and chlorophyll *a* concentration. An enhancement in growth was observed at 1, 2, 5 and 10 Gys in cultures irradiated after 5 days of incubation in both strains. Cr reduction potential was enhanced in all of the selected mutants with few exceptions. Soluble protein content was increased in AHZ-HB-MK cultures irradiated after 5 and 15 days of incubation, whereas no enhancement was observed in case of AHZ-HB-P2A. There was a significant increase in peroxidase and auxin content in AHZ-HB-P2A cultures irradiated after 5 and 15 days of incubation at 1, 2, 5 and10 Gys. No auxin production was observed in AHZ-HB-MK. Protein profile of all mutants was studied on SDS-PAGE. Variation in protein profile and the expression of stress proteins was observed at 5, 10 and 20 Gys in both strains as compared to control. Gamma irradiation enhanced the Cr reduction potential in both strains without causing effect on their other properties, thus these mutants could be used in future for the bioremediation of Cr-contaminated waste water and soils.

Posters

Plenary

PS 01 Energy transduction in the bacterial pre-protein translocase

Alice Robson, Vicki Gold, Tony Clarke & Ian Collinson

Dept of Biochemistry, University of Bristol, BS8 1TD

In bacteria, the SecYEG complex employs the cytosolic ATPase SecA to couple the energy of ATP binding and hydrolysis to the mechanical force required to push polypeptides through the membrane. The molecular basis of this energy transducing reaction is poorly understood. A fluorescent reporter on SecYEG has been exploited to characterize a long-distance conformational change that connects the nucleotide-binding fold of SecA to the transmembrane polypeptide channel in SecY. These movements are driven by binding of non-hydrolysable ATP analogues to SecA in association with the SecYEG complex. We also determine that interaction with SecYEG simultaneously decreases the affinity of SecA for ATP and inhibitory magnesium, favouring a previously identified active state of the ATPase. Pre-steady-state kinetic analysis of the ATPase activity of SecA unravels the molecular details of the ATP hydrolysis reaction. The rate-limiting step during ATP turnover is release of ADP, implicating this as the major regulation point for the ATPase. The activation of the ATPase by SecYEG, lipids and preprotein substrate has been characterized, shedding light on the molecular consequences of these interactions.

PS 02 Projection structure of *yidC*: a conserved mediator of membrane protein assembly

Mirko Lotz[1], Werner Kühlbrandt[1] & **Ian Collinson**[2]

[1]Max-Planck-Institute of Biophysics, Frankfurt, Germany; [2]Dept of Biochemistry, University of Bristol, BS8 1TD

Bacteria, mitochondria and chloroplasts harbour related factors that facilitate the insertion, folding and assembly of membrane proteins. In *E. coli*, yidC is required for membrane insertion, acting in both a Sec-dependent and Sec-independent manner. There is an expanding volume of biochemical work on its role in this process, but none on its structure. We present the first of this class of membrane protein determined by electron cryo-microscopy in the near native-like state of the membrane. YidC forms dimers in the membrane and each monomer has an area of low density that may be part of the path trans-membrane segments follow during their insertion. Upon consideration of the structures of yidC and SecYEG we speculate on the nature of the interfaces that facilitate the alternative pathways (Sec-dependent and independent) of membrane protein insertion.

PS 03 The ATP hydrolysis cycle of SecA and its consequence in protein translocation

Vicki A.M. Gold, Alice Robson, Anthony R. Clarke & Ian Collinson

Dept of Biochemistry, University of Bristol, BS8 1TD

Molecular motors have evolved to interconvert chemical energy and mechanical work. They have been exploited to drive a number of processes that are central to cell biology, such as protein translocation. In bacteria, the SecA motor ATPase associates with a ubiquitous channel SecYEG where it drives the post-translational secretion of pre-proteins across the plasma membrane. In spite of the recent progress within the field, there are several disputed and outstanding aspects of the reaction mechanism. By steady-state analysis, we have previously determined an inhibitory allosteric binding site for magnesium, distinct from that associated with hydrolysis of ATP. Alleviation of this inhibition and full activation of SecA was achieved by the presence of SecYEG presented in the context of cardiolipin containing membranes. Maximum activity was however reached in the presence of such proteoliposomes and pre-protein substrate proOmpA, the latter of which altered the SecA steady-state complex. This information was then used to build a model of pre-protein translocation through the SecYEG channel by the SecA motor ATPase.

PS 04 Structural and functional properties of chimeric EspA-FliCi filaments of EPEC

Valerie Crepin[1], Eric Martinez[1], Rob Shaw[2], Gad Frankel[1] & **Sarah Daniell**[3]

[1]Imperial College London; [2]University of Birmingham; [3]University of Bristol

Enteropathogenic *E. coli* (EPEC) utilize a filamentous type III secretion system (fTTSS) to translocate effector proteins into host gut epithelial cells. The primary constituent of the extracellular component of the fTTSS is EspA. This forms a long flexible helical conduit between the bacterium and host and has a structure almost identical to the flagella filament. We have inserted the D3 domain of FliCi (from *Salmonella Typhimurium*) into the outer domain of EspA and have studied the structure and function of modified filaments when expressed in an EPEC *espA* mutant. We found that the chimeric EspA-FliCi were biologically active as they supported protein secretion and translocation [assessed by their ability to trigger actin polymerization beneath adherent bacteria (FAS test)]. The expressed filaments were recognized by both EspA and FliCi antisera. Visualization and analysis of the chimeric filaments by EM after negative staining showed that, remarkably, EspA filaments are able to tolerate a huge protein insertion without a significant effect on their helical architecture. This is crucial for their development as an epitope display technology.

PS 05 Complete 3D-reconstruction of the *Shigella flexneri* T3SS needle complex base

Julie Hodgkinson[1], Ashely Horsley[1], David Stabat[1], Martha Simon[2], Joe Wall[2] & **Ariel Blocker**[1]

[1]University of Oxford, Sir William Dunn School of Pathology, Oxford OX1 3RE; [2]Biology Dept, Brookhaven National Laboratory, NY 11973-5000, USA

Type III secretion systems (T3SSs) are widely distributed virulence determinants of Gram-negative bacteria. They translocate proteins into host cells during infection. T3SSs are composed of a cytoplasmic secretion machinery and a base spanning both bacterial membranes into which a hollow needle is embedded, protruding from the bacterial surface. The latter two parts are termed the needle complex (NC). To ascertain the composition of the NC, we recently purified it by affinity. We then assessed the stiochiometry of NC parts by scanning transmission electron microscopy (STEM) studies of the mass of its subdomains. We also collected further low dose transmission electron microscopy NC images after negative staining. Processing of these images allowed determination the symmetries within and then reconstruction of the entire base of the NC (inner and outer membrane domains and connector region). This reconstruction is consistent with our STEM mass measurements. Finally, fitting of available crystal structures into the new map was carried out.

PS 06 Building the *Shigella flexneri* type 3 secretion system pore

Steven Johnson, Janet E. Deane & Susan M. Lea

Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE

More than 1 million people die per annum as a result of T3SS-expressing enteropathogenic bacteria such as *Shigella flexneri,* the causative agent of human bacillary dysentery. The T3SS is composed of a basal body, which traverses both bacterial membranes, and an external needle through which effector proteins are secreted in a partially folded state. During *S. flexneri* infection, contact of the tip of the needle with a eukaryotic host cell triggers insertion of a pore in the host cell membrane, in a process which is dependent on proteins located at the tip of the needle. Our studies have addressed the structure/function relationships of the pore proteins in the presence and absence of their shared chaperone, including their ability to bind other components of the T3SS. These results have revealed different modes of chaperone binding. In combination with our ongoing structural work on other components of the T3SS basal body, these studies are increasing our understanding of the construction of these complex machines.

PS 07 Regulating type III secretion: components and complexes

Janet E. Deane, Pietro Roversi, Miriam Friede, Carole King, Steven Johnson & Susan Lea

Sir William Dunn School of Pathology, South Parks Rd, University of Oxford, OX13RE

Many Gram-negative pathogenic bacteria use a complex macromolecular machine, the Type Three Secretion System (T3SS), to deliver into host cells the effector proteins required for invasion. The T3SS comprises structural components of the apparatus, secreted effectors, chaperones and regulators. Secretion is regulated by cytoplasmic and inner-membrane proteins that recognize the substrates to be exported. These secretion regulators control the substrate specificity of the T3SS and respond to specific signals in order to ensure that structural components (needle subunits and pore proteins) are secreted first and that virulence proteins are not secreted before contact with a eukaryotic cell. We have characterized several of the cytoplasmic and inner-membrane proteins of the *Shigella flexneri* T3SS in order to better understand the roles of these components in substrate switching and regulation of effector secretion. Our recent structure of MxiC, the homolog of YopN and TyeA from *Yersinia* sp., has revealed significant domain re-arrangement suggesting that signalling via conformational changes might be important for the function of T3SS secretion regulators.

PS 08 Secretion and virulence in *Pseudomonas aeruginosa* biofilms

Helga Mikkelsen[1], K. Nishikawa[2], M.E. Skindersoe[3], M. Givskov[3], K.S. Lilley[1] & M. Welch[1]

[1]Dept of Biochemistry, University of Cambridge, Cambridge CB2 1QW; [2]Dept of Traumatology and Critical Care Medicine, National Defense Medical College, 3-2 Namiki Tokorozawa, Saitama, 359-9513, Japan; [3]Biocentrum-DTU, Technical University of Denmark, DK-2800 Lyngby, Denmark

Pseudomonas aeruginosa is an opportunistic pathogen that causes chronic infections in immunocompromised individuals. These infections are notoriously hard to treat, partly due to the high intrinsic tolerance of the bacterium to clinically used antibiotics, and partly due to the formation of biofilms. Biofilm tolerance has often been attributed to starvation and low metabolic activity, and gene expression in biofilms has been shown to resemble that of planktonic cells in stationary phase. Furthermore, biofilms in chronic infections are often considered to be passive reservoirs for more virulent planktonic cells. However, our proteomic and transcriptomic investigations show that this is not always the case. Biofilms in our system are more similar to fast growing cells than to slow growing cells. They are active structures that produce a different spectrum of secreted virulence factors than do planktonic cells. Importantly, the type III secretion system is induced in the biofilm mode of growth, indicating that biofilms may play a more active role in virulence than previously thought.

PS 09 Rhamnolipids have pleiotropic effects on the membrane proteome of *Pseudomonas aeruginosa*

Bianca Price & Martin Welch

Dept of Biochemistry, University of Cambridge, Cambridge CB2 1QW

Pseudomonas aeruginosa (PA) is a Gram-negative bacterium which inhabits a wide range of biological niches. It is commonly found in soil and can readily form biofilms on biotic and abiotic surfaces. PA is also a well-known opportunistic human pathogen and is responsible for causing a range of infections, particularly among immune-compromised patients. Rhamnolipids are detergent molecules produced exclusively by Pseudomonads consisting of one or two rhamnose head groups attached to a fatty acid tail (mono- or di-rhamnolipids respectively). These molecules have a variety of suggested functions including development and maintenance of biofilm structure, aiding swarming motility, in killing other bacteria and as a virulence factor in human infection. A mutation in the *rhlA* gene of *Pseudomonas aeruginosa* PA01 causes a defect in rhamnolipid production. The membranes of wild-type PA01 and of a *rhlA* mutant were harvested and the proteome was profiled using LC-MS/MS. Changes in the protein profile correlated with subsequent phenotypic assays. Pleiotropic effects have been observed linking the absence of *rhlA* not only with rhamnolipids but also with motility, pyocyanin production and iron uptake.

PS 10 Ordered export of flagellar subunits via a type III pathway in *Salmonella typhimurium*

P. Dhillon, G. Stafford, L.D.B. Evans, S. Ahmed, G. Fraser & C. Hughes

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

Motility is a fundamental attribute in prokaryotes, commonly aiding bacterial pathogens to colonize host cell surfaces. In *Salmonella*, swimming and swarming is enabled by peritrichous flagella, which are assembled sequentially from the inner to the outermost structures. The strict order of assembly is dictated by a temporally expressed gene hierarchy and by a subunit-specificity switch in the flagellar type III export machinery, which delivers subunits to the nascent flagellum. Our aim is to dissect the series of events and transient interactions within this system.

We have shown that chaperones play a role in piloting cognate subunits to the cell surface where they interact with the export machinery. By manipulating the pathway *in-vivo*, we have demonstrated that a membrane-associated ATPase forms a common docking site for chaperoned and unchaperoned subunits, which are subsequently sorted and ostensibly accepted for export, or rejected. Subunit sorting at the level of the export machinery is crucial in maintaining ordered assembly in a bacterium with multiple cell flagella at different stages of biogenesis.

The degree to which subunits themselves influence the processes of substrate recognition and discrimination has not yet been elucidated. We have precluded the influence of bound chaperones as flagellar sorting signals and have generated data indicating a role for *N* termini subunit signals in promoting the ordered export of flagellar proteins.

PS 11 Detection and partial characterization of lipoprotein shaving factor from *Streptococcus uberis*

Emma Denham[1], Lawrence Hunt[1], Andrew Gill[1], Phil Ward[2] & James Leigh[3]

[1]Institute for Animal Health, RG20 7NN; [2]Nuffield Dept of Clinical Laboratory Sciences, OX3 9DU; [3]School of Veterinary Medicine and Science, University of Nottingham, LE12 5RD

Alternative processing of the signal peptides of lipoproteins has been reported for several species of Gram-positive bacteria including *Bacillus subtilis* and *Staphylococcus aureus*. In the absence of the known processing enzyme (Lgt) *Streptococcus uberis* was shown to cleave lipoproteins between the invariable cysteine residue and the C-terminal portion of the remaining protein; thus releasing lipoproteins from the bacterial cell. The protein responsible for this activity is unknown. To address this question, a FRET peptide based on the lipobox motif and the 4 amino acids on the C-terminal side of the *S. uberis* lipoprotein MtuA was used to characterize this activity. A peptidase that was active against the peptide was shown to be present within the cell wall, membrane and cell contents of the bacteria. Metallopeptidase inhibitors were shown to inhibit the activity of ShvU.

PS 12 Biochemical and biophysical characterization of the highly repetitive region of the *Helicobacter pylori cag*-pathogenicity island protein, CagY

Rob Delahay[1], Graham Balkwill[2], Wayne Edwards[2], Karen Bunting[3], John Atherton[1] & Mark Searle[2]

[1]Institute of Infection, Immunity and Inflammation; [2]Structural Biology & Biophysics Group, Centre for Biomolecular Sciences; [3]Institute of Genetics, University of Nottingham, Nottingham NG7 2RD

Helicobacter pylori utilize a type IV secretion system (T4SS) to mediate delivery of the CagA effector protein to gastric epithelial cells. The T4SS extends into an extracellular filament elaborated on the surface of the bacterium which contacts the host cell surface and unusually, is sheathed by a processed form of the secreted CagY protein largely encoded by an extensive region of highly repetitive sequence. We show this repetitive region to comprise tandem arrays of two α-helical repeat motifs, termed A and B. Each array consists of 1–5 repeats of the A motif flanked by a single copy of the B motif. We demonstrate homo- and hetero-oligomeric interactions between isolated motifs consistent with modular assembly of the large CagY repeat structure, and reveal a remarkable structural and functional tolerance for duplication and deletion of discrete motif modules in CagY proteins from different *H. pylori* strains.

PS 13 TolA and the proton motive force are required for immunity protein release upon cell entry of colicin E9

M.N. Vankemmelbeke, C.N. Penfold & R. James

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

Nuclease colicins bind their receptor in the outer membrane of sensitive cells as a high affinity complex with their cognate immunity proteins. Upon cell entry the immunity protein is lost from the complex and the colicin uses members of the *tol* operon for its translocation into the cytoplasm.

We have developed a fluorescence assay to study the molecular requirements for the release of Im9 from the colicin E9/Im9 complex bound to *E. coli* cells. We have found that the first eighty residues of the colicin molecule are required for Im9 release. Im9 release was only partially affected after binding of the colicin E9/Im9 complex to *E. coli* strains with deletions in either the *tol*B, *tol*Q or *tol*R genes. In contrast, Im9 release from the complex bound to an *E .coli tol*A deletion strain was nearly completely abolished. We also show that collapsing the proton motive force of the inner membrane prohibits Im9 release. Together these results suggest that energy transduced from the cytoplasmic membrane to the outer membrane by TolA is the driving force behind immunity protein release.

PS 14 Involvement of the Tat system in *Bdellovibrio predation*

C.-Y. Chang, L. Hobley, M. Capeness, W. Burtt & Liz Sockett

Institute of Genetics, School of Biology, University of Nottingham Medical School, Nottingham NG7 2UH

Bdellovibrio bacteriovorus, a Gram-negative bacterium, preys upon other Gram-negative bacteria including several human pathogens. During predation *Bdellovibrio* have a requirement to transport many proteins and enzymes into their prey for growing within and consuming prey. Genomic analysis revealed a set of genes encoding a *Bdellovibrio* twin-arginine translocation (Tat) system which includes a pair of genes encoding TatC and TatB proteins and two separately located genes encoding TatA and TatE proteins. In order to test the functions of these potential Tat proteins, four Tat proteins from *Bdellovibrio* were expressed from pTrc99A-derived plasmids in different Tat mutants of *Escherichia coli*. Cross species complementations have shown that induced $TatA_{Bd}$ and non-induced $TatC_{Bd}$ complement the TatA and TatC mutants of *E. coli* respectively but not $TatB_{Bd}$ and $TatE_{Bd}$. Interestingly, over expressed $TatB_{Bd}$ and $TatC_{Bd}$ repressed the growth of *E coli*. To further investigate the role of Tat system in *Bdellovibrio* an insertional inactivation mutant of $TatE_{Bd}$ has been constructed and shown to have a delayed predation process by luminescent prey assay and small plaques on bacterial prey lawns. Ten potential Tat substrate proteins have been predicted to be encoded from the *Bdellovibrio* genome. Reverse transcriptase PCR (RT-PCR) showed that the expression of two of the genes encoding potential Tat substrates was also delayed during the predatory process in the *tatE* mutant of *Bdellovibrio*. This suggests that TatE in *Bdellovibrio* plays a key role in transporting proteins during predation.

PS 15 Intracellular triggering of inflammation by the Gram-negative extracellular bacterium *Pseudomonas aeruginosa*

Cecilia S. Lindestam & Thomas J. Evans

Division of Immunology, Infection & Inflammation, Glasgow University, University Place 120, Glasgow G12 8TA

Microbes can trigger inflammation by interaction with intracellular sensors such as the nucleotide oligomerization domain (NOD) family proteins, independent of interaction with Toll-like receptors. This activation can trigger NF-κB activation as well as caspase-1 activation and processing of IL-1β. We set out to study how *Pseudomonas aeruginosa* activates these pathways, and how the host regulates this process. We found that infection of cells with *P. aeruginosa* does not result in pores allowing direct entry of extracellular markers. However, using a luciferase reporter gene to follow NF-κB activation, we have found that *P. aeruginosa* can activate NF-κB independently of TLRs by a mechanism dependent on a functional bacterial type III secretion system. This suggests that a potential NOD-stimulatory molecule is introduced directly from the bacteria into the host cell through the type III secretion pore. The NOD-protein Nod1 was not responsible for this as DN-Nod1 was shown to be without effect on NF-κB activation.

P. aeruginosa can also directly activate caspase-1 following infection, leading to IL-1β cleavage and release. We have shown that this activation is dependent on a functional type III secretion system. High extracellular potassium concentration abrogated the caspase-1 activation after infection with *P. aeruginosa*. As has been shown for other triggers of caspase-1 activation, this suggests membrane damage leading to K^+ efflux is essential. However, given the lack of detectable pores following infection, the mechanism of this effect is unclear. Together these results suggest that a functional type III secretion system is needed for triggering of inflammation via intracellular detection pathways by *P. aeruginosa*.

PS 16 Proteomics analysis of salmonellae secretomes

A. Sherry[1], D. Fraser-Pitt[2], A. Stevenson[1], N. Ingles[2], P. Everest[1], D.G.E. Smith[1,2] & M. Roberts[1]

[1]Institute for Comparative Medicine, Faculty of Veterinary, University of Glasgow; [2]Moredun Research Institute, Pentlands Science Park, Edinburgh

Salmonella spp. are major food borne pathogen of worldwide economic and health importance. Bacterial pathogens often sense the host environment and respond by expressing products required for adaptation to that niche. Exported proteins, principally secreted proteins of *Salmonella* were examined to identify any that could significantly influence interaction between this pathogen and its hosts. From media known to induce secretion of bacterial proteins (mainly but not exclusively via type three secretion system(s) – TTSS) and in pre-conditioned medium (pcm) incorporating epithelial-derived stimuli to assess stimulation by epithelial-derived components, bacterial secretomes were monitored and characterized. In addition to assessing the influence of environment on secretomes, serotype heterogeneity was monitored to define putative strain or serovar-specific markers. Contact with epithelial cells/derived signals have discernable effects on *Salmonella* secretomes. Growth in PCM resulted in different secretome content from that in conventional media. Secretome profiles of Salmonellae show variability between different isolates; this may be of epidemiological relevance and may influence host-pathogen interactions.

PS 17 Cross-talk between O-islands and the LEE in enterohaemorrhagic *Escherichia coli*

Jai J. Tree[1], Allen Flockhart[1], Andrew J. Roe[2] & David Gally[1]

[1]Centre for Infectious Diseases, University of Edinburgh; [2]Division of Infection and Immunity, University of Glasgow

Recent sequencing of two Enterohaemorrhagic *E. coli* (EHEC) genomes has shown that EHEC has 177 distinct insertions into a common backbone shared with commensal *E. coli*. Many of these insertions (O-islands) encode virulence determinants that are required for pathogenicity, including type III secretion (TTS). Regulatory cross-talk between these O-islands and TTS has been demonstrated for OI115, OI43, OI47, and OI48. To identify novel OI encoded regulators of TTS we have screened a library of defined OI deletions for effects on TTS and have shown that deletion of OI50 increases TTS. Repression of TTS was shown to be encoded by the AraC family regulator z1789 encoded by OI50 and the closely related regulator z2104 encoded by OI57. Further analysis has shown that at least seven related AraC type regulators are encoded by OIs including the previously characterized *eivF* encoded by OI115 (ETT2). These related AraC family regulators may consititute a conserved family of regulators that mediate cross talk between OI genes and TTS.

PS 18 Reactivation and chaperone binding of a 'remnant' Tat signal sequence

Sarah J. Coulthurst[1], Bérengère Ize[2], David J. Richardson[2], Tracy Palmer[1] & Frank Sargent[1]

[1]Molecular and Environmental Microbiology, University of Dundee; [2]School of Biological Sciences, University of East Anglia

The twin arginine translocase (Tat) exports folded proteins, often oligomeric and/or cofactor-containing, across the bacterial cytoplasmic membrane. Substrate proteins are directed for Tat-dependent export by an N-terminal signal sequence containing a conserved twin arginine motif. In *Escherichia coli*, the majority of Tat substrates are involved in varied respiratory pathways and are known or predicted to bind redox cofactors. Recently, a family of cytoplasmic chaperone proteins has been discovered, exemplified by TorD, whose role is to ensure that cofactor integration and complex assembly is completed before Tat export occurs. The NarGHI nitrate reductase of *E. coli* is a redox cofactor-containing cytoplasmic enzyme which depends on a TorD-like protein, NarJ, for proper assembly. NarJ binds a Tat signal-like sequence at the N-terminus of NarG. This 'remnant' signal sequence has been reactivated for Tat export competence by point mutation and the impact of this reactivation on NarJ binding and chaperone function has been examined. Interestingly, the NarGHI enzyme of *Archaeoglobus fulgidus* is apparently exported and has a Tat-active signal sequence on NarG. The hypothesis that the corresponding NarJ homologue plays a chaperone role in assembly and export has been investigated. It appears that the biosynthetic mechanisms of certain exported and non-exported enzymes are evolutionarily related.

PS 19 Tat pathway and virulence of *Escherichia coli* K1

Bérengère Ize[1], Naveed Khan[2] & Tracy Palmer[3]

[1]School of Biological Sciences, UEA, Norwich; [2]Dept of Biology, Birkbeck College, London, [3]Division of Molecular & Environmental Microbiology, University of Dundee

The Tat pathway is a protein transport system found in the cytoplasmic membranes of many bacteria. It transports folded proteins that are targeted by N-terminal twin arginine signal peptides. A growing number of pathogenic bacteria show attenuation of virulence when the Tat pathway is knocked out. *Escherichia coli* K1 is the leading Gram-negative organism causing neonatal meningitis and results in 50,000 deaths per year worldwide. In 2000, Microscience filed a patent reporting that a *tat* mutant of *E. coli* K1 is avirulent. Previous work showed that *E. coli* K12 *tat* strains are defective in the integrity of their envelope. We demonstrated that the pleiotropic cell envelope defect of the *tat* mutant was due to mislocalization of two amidases, AmiA and AmiC, which were Tat substrates. We want to ascertain whether the loss in virulence of the *E. coli* K1 *tat* mutant is due to the inability to export AmiA and AmiC. We have constructed a strain that is deleted for *tatC* and a strain that is deleted for *amiA* plus *amiC*. We are testing their virulence in the locust model. Moreover we have used a combination of phenotypic and bioinformatic approaches to define the *Escherichia coli* K1 Tat substrates.

PS 20 Mapping subunit interactions in the *Escherichia coli* Tat translocase

B. Maldonado[1], S. Grahl[1], C. Punginelli[3], R. Jack[3], B. Berks[2] & T. Palmer[1]

[1]University of Dundee, Dundee; [2]Dept of Biochemistry, University of Oxford; [3]John Innes Centre, Norwich

The Tat (twin arginine translocation) system is a protein export pathway found in the cytoplasmic membranes of many bacteria and in plant thylakoids. This transport machine moves fully folded proteins, which are often metal cofactor-containing and sometimes oligomeric, across energy-conserving membranes. Protein substrates are targeted to the Tat machinery by N-terminal signal peptides that contain an S/T-R-R-x-F-L-K 'twin arginine' consensus motif. The two arginine residues are almost invariant, are critical for export and give the system its name.

The *Escherichia coli* Tat transporter comprises of multiple copies of three proteins, TatA, TatB and TatC. These form two types of complex, a complex that is made up of TatB and TatC proteins that functions as the substrate receptor, and a separate, heterogeneous complex comprising homo-oligomers of TatA that probably functions as the protein transport channel.

The TatBC complex contains TatB and TatC in a 1:1 ratio, and each are present in multiple copies. Previous work from our group has involved the use of cysteine scanning mutagenesis and cysteine cross-linking to the dimerization interface of TatB, the results suggesting that TatB forms a homomultimeric inner ring in the TatBC complex [1]. As part of my studies I have use a similar approach to undertake mapping

studies aimed at defining interaction interfaces between TatC proteins. The results are consistent with the idea that TatC is arranged as an outer ring in the BC complex [2]. I have further gone on to use this approach to characterize the site(s) of interaction between TatB and TatC proteins. My latest results will be presented.

The cysteine cross-linking study of TatB, coupled with protein purification experiments of TatBC complexes harbouring doubly cysteine-substituted TatB proteins showed that at least 4 copies of TatB are present in the TatBC complex. However, the exact number of TatBs and TatCs in the complex is not clear [1]. In order to ascertain the number of TatC proteins present in the TatBC complex, I have devised a strategy whereby I can construct covalent fusions of TatC proteins *ad infinitum*. To date I have constructs that carry 2, 3, 4 and 5 TatC units in the fusion protein. My results show that a fused dimer of TatC is almost completely functional, whereas progressively larger fusions of TatC give lower levels of Tat transport function. My latest data on the fusion studies will be presented and my results will be discussed in relation to models for the structure and function of TatBC.

References [1] Lee *et al.* (2006) Cysteine-scanning mutagenesis and disulfide mapping studies of the conserved domain of the twin-arginine translocase TatB component. *J Biol Chem* **281**, 34072–34085 / [2] Punginelli *et al.* Cysteine scanning mutagenesis and topological mapping of the *Escherichia coli* twin-arginine translocase TatC component (submitted).

PS 21 Biochemical and biophysical studies of *Escherichia coli* TorD: a twin-arginine signal peptide binding protein

David Guymer, Julien Maillard & Frank Sargent

College of Life Sciences, University of Dundee, Dow Street, DD1 5EH

The export of proteins across the cytoplasmic membrane is an essential function of all bacterial cells. In *Escherichia coli*, most proteins are exported via the general secretory pathway, however a subset of extracellular enzymes are transported into the periplasm by the twin-arginine translocation (Tat) apparatus. Such proteins contain distinctive N-terminal signal peptides exhibiting a tripartite structure comprising a polar n-region, a relatively hydrophobic h-region, a polar c-region, and a highly conserved SRRxFLK twin-arginine motif. One of the most heavily exploited bacterial Tat signal peptides is that of the trimethylamine *N*-oxide reductase (TorA) of *E. coli*. Prior to export, the TorA signal peptide (ssTorA) is bound tightly by a dedicated cytoplasmic chaperone (TorD), possibly to prevent export until the maturation of TorA is complete in a process described as Tat Proofreading. TorD was recently reported to bind weakly to the guanosine moiety of GTP and the role of GTP in Tat proofreading is under investigation. Additionally a facile expression system has been developed allowing the purification of ssTorA as a fusion with maltose binding protein. Interactions between the MalE:ssTorA fusion protein and monomeric and multimeric forms of TorD have been assessed using liquid chromatography and calorimetric techniques. This work has provided new insights into the biological significance of the higher order oligomers of TorD.

PS 22 Targeting to Caf1A usher N-terminal domain

S. Vasiljevic, A. F. Pudney, Z. Duck & S. MacIntyre

School of Biological Sciences, University of Reading, Reading RG6 6AJ

The F1 antigenic capsule of *Yersinia pestis* is a homopolymer assembled by the two-component chaperone-usher system; a terminal branch of the general secretory pathway. A periplasmic chaperone (Caf1M) is responsible for folding and capping of monomeric Caf1, whilst preserving it in an energy competent conformation for subsequent fibre formation at the cell surface most likely catalysed by the outer membrane usher, Caf1A. To investigate the specificity of targeting to, and binding of chaperone:subunit complex with usher we centred on the functional significance of the N terminus. The N-terminal domain of Caf1A usher was expressed and purified functional activity was demonstrated by co-purification with chaperone:usher complex. We are now using mutagenesis to investigate critical residues involved in protein:protein interactions within this FGL system.

PS 23 Mechanism of donor strand exchange during *Yersinia pestis* F1 fibre formation

L.J. Fooks[1], E. Moslehi[1], S.D. Knight[2], A.V. Zavialov[2] & S. MacIntyre[1]

[1]School of Biological Sciences, University of Reading, Reading; [2]Swedish University of Agricultural Sciences, Uppsala, Sweden

F1 antigen of *Yersinia pestis* is a polymer of Caf1 subunit, assembled via the chaperone:usher pathway into fibres surrounding the bacterial cell. During polymerization, an N-terminal β-strand extension of each Caf1 subunit replaces the chaperone donor strand, completing the Ig-like fold of the neighbouring subunit and thereby forming F1 polymer. Despite the common role of both chaperone (G1) and subunit (G_d) donor strands in complementing Caf1 structure an important difference between the two is the presence, in the G1 strand, of bulky hydrophobic residues that insert deep into the subunit cleft.

To investigate the role of chaperone in influencing the folding, stability and polymerization of Caf1, we performed convergence mutagenesis of the binding motifs of chaperone and subunit, and, using X-ray crystallography and calorimetry, analysed the effect on structure and stability of Caf1M:Caf1 and Caf1:Caf1 complexes. Our results suggest that chaperone hydrophobic binding motif is important in trapping early folding structural intermediates in an open distorted conformation, thereby facilitating fibre formation via the zip-in-zip-out donor strand exchange pathway.

PS 24 Characterization of novel type III effectors in EHEC O157

Sophie A. Matthews, Rob K. Shaw, Rasha Younis, Steve Dove & Mark J. Pallen

University of Birmingham, Edgbaston, Birmingham B15 2TT

Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 is an attaching and effacing (A/E) pathogen of the human gastrointestinal tract. EHEC possesses an arsenal of proteins termed 'effectors' that can be delivered directly into the host enterocyte cytoplasm through a needle-like type III secretion system (T3SS). The components of this system along with several effectors are encoded by genes within the locus of enterocyte effacement (LEE). These effectors have been shown to subvert normal host cell processes and functions such as the maintenance of tight junctions, ion transport and cytoskeletal rearrangement. Recently 39 effectors encoded outside the LEE were discovered – we aimed to discover the function of these novel effectors. Non-LEE encoded effector proteins were endogenously expressed in yeast and mammalian cells and their effects on cytoskeletal function and cell growth were studied. Six effectors including two members of the novel NleG family affected cell division and morphology of yeast. The effector EspM2, a homologue of the *Shigella* effector IpgB2, induced the formation of actin stress fibres in transfected HeLa cells suggesting that EspM2 may, like IpgB2, mimic Ras-like GTPases.

PS 25 Bioinformatic identification of lipoproteins of Gram-positive bacteria

Obaidur Rahman[1], Stephen P. Cummings[1], Dean J. Harrington[2] & **Iain C. Sutcliffe**[1]

[1]Northumbria University, Newcastle upon Tyne NE1 8ST; [2]University of Bradford, West Yorkshire BD7 1DP

Bacterial lipoproteins are translocated across the plasma membrane by virtue of their signal peptide sequences and then post-translationally modified with a lipid moiety that anchors these proteins to cellular membranes. As lipoproteins possess specialized signal peptides with a characteristic cysteine-containing 'lipobox', these sequences are highly

amenable to bioinformatic analysis. We present a revised and updated version of our G+LPP sequence pattern, derived from the signal peptide analysis of 73 experimentally verified lipoproteins from Gram-positive bacteria. This pattern can be used in ScanProsite pattern searches to accurately recover putative lipoprotein sequences encoded in Gram-positive bacterial genomes. We have also used an experimentally verified lipoprotein dataset and a 'false positive' dataset (34 sequences) to critically evaluate the precision (specificity) and recall (sensitivity) of five online bioinformatics tools which have recently been introduced for the identification of lipoprotein signal peptides. Recall for several tools is considered good whereas precision discriminates between these bioinformatic tools. LipoP (www.cbs.dtu.dk/services/LipoP/) performed best when considering both criteria.

PS 26 Protein secretion rates fine tune flagellar gene expression in *Salmonella*

J. Brown[1], S. Saini[2], C. Aldridge[1], J. Herbert[1], C. Rao[2] & P. Aldridge[1]

[1]Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne; [2]Dept of Chemical & Biomolecular Engineering, University of Illinois, Urbana, IL, USA

Flagellar gene expression is temporally regulated in response to the assembly state of the growing flagellum. The key mechanism for enforcing this temporal hierarchy in *Salmonella typhimurium* is the σ^{28}-FlgM checkpoint, which couples the expression of the late flagellar (P_{class3}) genes to the completion of the hook-basal-body. This checkpoint is triggered when FlgM is secreted from the cell. In addition to σ^{28}, two other regulatory proteins, FliT and FlgN, are regulated in response to the secretion of late proteins. In this work, we determined how these different flagellar regulators affect gene expression dynamics in mutants that are incapable of secreting late proteins or secrete them at rates greater than wild type. Dynamic analysis of flagellar gene expression identified a novel mechanism that negatively regulates P_{class2} promoters in response to assembly and secretion. Using a number of different approaches, we were able to show that this mechanism did not involve any known flagellar regulator. In addition, our data suggests that all three promoter classes are continuously regulated in response to assembly and that after completion of the first few flagella the regulation is no longer hierarchical.

PS 27 The characterization of the variation found in the flagellar filament of *Caulobacter crescentus*

Alex Faulds-Pain[1,2], Christopher Birchall[1,2], Giulia Grimaldi[1,2], Suhichi Nakamura[3], Tohru Minamino[3], Keiichi Namba[3] & Phillip Aldridge[1,2]

[1]Centre for Bacterial Cell Biology, University of Newcastle, Newcastle upon Tyne NE2 4HH; [2]Institute of Cellular and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne NE2 4HH; [3]Graduate School of Frontier Biosciences, University of Osaka, Suita, Osaka, Japan

The *Caulobacter* flagellar system belongs to a growing number of systems shown to utilize more than one unique flagellin to assemble its flagellar filament: six flagellins instead of one. Here we asked the question: what happens to the flagellar filament when multiple flagellin gene deletions are combined?

We have created a series of in-frame deletions of the six flagellin genes to determine their role in filament formation. All the mutants exhibit a range of phenotypes from non-motile, partially motile to motile. A subset of mutants, predicted from the literature to be non-motile, was surprisingly able to produce filaments and thus was motile. When filaments were produced they were physically very similar to wild type. The only change observed was that a subset of mutants possessed filaments shorter in length. The physical similarity of the mutant filaments was consistent with MALDI-TOF analysis that suggested isolated filaments were comprised of all remaining flagellins. The observed changes in the motility phenotypes could also not be ascribed to a change in swimming speed as this was only affected in a small subset of mutants.

In conclusion our data suggests that the *Caulobacter* flagellar system exhibits an expected level of redundancy. However, to observe the redundancy in the system a level of regulation underpinning filament assembly must be overcome.

PS 28 Role of a putative secretion system in the pathogenicity of the *Burkholderia cenocepacia*

Sravanthi Shastri & Mark S. Thomas

University of Sheffield

Rationale and Hypothesis Gram-negative bacteria use various secretion systems to export toxins and other proteins across their outer membrane. These secretion systems have been found to be extremely important during invasion of cells or intracellular survival by pathogenic Gram-negative bacteria. *Burkholderia cenocepacia*, an opportunistic pathogenic Gram-negative bacterium, also encodes several secretion systems. Recent studies aimed at identifying genes activated during cell invasion in Gram-negative bacteria such as *Burkholderia pseudomallei* (1), *Vibrio cholerae* (2), *Pseudomonas aeruginosa* (3) and *Edwarsiella tarda* (4) have revealed the presence of a novel putative secretion system known as the type VI secretion system (T6SS). Genes encoding a T6SS have also been identified in *B. cenocepacia*. The analysis of the *B. cenocepacia* T6SS gene cluster (termed the '*tss* unit') may provide an insight into the role of these genes in the pathogenicity of this bacterium.

Objectives of this study

- To identify the function of the *tss* unit in the virulence of *Burkholderia cenocepacia*.
- To identify the conditions under which these genes are expressed.
- To identify the protein-protein interactions which occur between each gene product encoded by the *tss* unit.

Methodology

- Construction of *Burkholderia cenocepacia* T6SS mutants by inactivating selected *tss* genes.
- To determine the affect of the *tss* knockout mutations on the phenotype of *B. cenocepacia* using host model systems such as *Dictyostelium discoideum*.
- Determination of promoter location and size of transcription units. Construction of reporter gene fusions and determination of promoter activity under various conditions, such as pH, temperature, media etc.
- Using bacterial or yeast two-hybrid system to investigate protein–protein interactions within the *tss* unit.
- Determine whether any *tss* gene products are secreted.

Findings We have successfully inactivated three of the genes in the *tss* unit and are in the process of characterizing the gene; also two of the promoters in the *tss* units has been located as we could identify its activity by constructing reporter gene fusions. Hence we are in the process of investigating its mode of regulation. In addition we are conducting experiments to check protein-protein interactions between the gene products in the *tss* unit to have a detailed insight on the interactions of the genes in this cluster.

PS 29 Probing PulD secretin structure using small artificial binding proteins

Martin Krehenbrink[1], Ingrid Guilvout[1] & Frédéric Pecorari[2]

[1]Institut Pasteur, 25 rue du Dr. Roux, Paris 75724 cedex 15, France; [2]Université de Nantes, 2 rue de la Houssinière, BP 92208, Nantes 44322 cedex 3, France

The secretin PulD from the Type II protein secretion system of *Klebsiella oxytoca* assembles into a dodecameric structure in the outer

membrane. The polypeptide itself is composed of two major domains; tryptic digest reveals a trypsin-sensitive N domain and a resistant C domain. While the C domain retains the ability to assemble into membrane-associated dodecamers, the N domain remains soluble when expressed on its own. This study uses artificial binding proteins based on a small (7 kDa) archaeal DNA-binding protein (Sac7d) and limited proteolysis to probe the structure and function of the N-terminal domain. Sac7d variants selected for their ability to bind to the N domain by ribosome display were selected from a combinatorial library of mutants. As the binding was found to be dependent on the structure of the PulD N domain, binding proteins were used to screen for detergents permitting purification of native PulD dodecamers for structural studies and to test the *in vivo* conformation. Co-expression of binding proteins and the Type II secretion system was also used to probe the role of the recognized epitopes in secretion and multimerization.

PS 30 PulD multimerization and membrane insertion

Ingrid Guilvout[1], Mohamed Chami[2], Catherine Berrier[3], Alexandre Ghazi[3], Andreas Engel[2], Anthony P. Pugsley[1] & Nicolas Bayan[1,3]

[1]Molecular Genetics Unit and CNRS URA2172, Institut Pasteur, Paris, France; [2]M.E. Müller Institute, Biozentrum, University of Basel, Basel, Switzerland; [3]CNRS UMR8619 , Université de Paris Sud, Orsay, France

The *Klebsiella oxytoca* secretin PulD is a 1 MDa dodecameric cylindrical complex through which the amylolytic enzyme pullulanase is secreted. PulD targeting to the outer membrane is dependent on the chaperone PulS, but its insertion into the outer membrane is independent of the general outer membrane insertion factor Omp85/YaeT. In the absence PulS, PulD still multimerizes but inserts into the inner membrane. Production of PulD without a signal peptide is lethal. Mutants that grow produce only monomeric PulD. PulD produced in a coupled transcription-translation system (Roche) in the presence of detergents or surfactant migrated as a full-length monomeric protein upon SDS-PAGE. PulD multimers were produced in the presence of liposomes and inserted into the liposome bilayer, as visualized by electron microscopy. OutD secretin from *Erwinia chrysanthemi*, which needs a PulS homologue for its targeting, behaved like PulD. In contrast, Omp85/YaeT-dependent secretin PilQ from *Neisseria meningitidis* was produced as a monomeric protein *in vitro*. These results suggest that chaperone dependent secretins (PulD and OutD) can multimerize and spontaneously insert into membranes in the absence of specific accessory factors. The precise role of PulS in PulD outer membrane targeting is still unknown.

PS 31 Outer membrane localization and insertion of the type II secretion system secretin

Séverine Collin, Ingrid Guilvout & Anthony P. Pugsley

Institut Pasteur, Molecular Genetics Unit, 25 rue du Docteur Roux, 75015 Paris

Secretins are outer membrane (OM)-spanning channel-forming proteins that allow exoprotein secretion (type II and type III secretion machineries), type IV pilus extrusion and filamentous phage release. They contain 12 to 14 subunits that form a complex of ca 1 MDa, the largest OM protein complexes known. Unlike classical OM proteins (such as LamB), the secretin PulD of the type II secretion machinery of *Klebsiella oxytoca* is independent of the OM β-barrel assembly complex Omp85/YaeT. To study the insertion/multimerization mechanism of this unusual protein further, we focussed our attention on the PulD-specific chaperone, PulS. PulS is a lipoprotein previously shown to prevent PulD degradation by periplasmic proteases and to be essential for PulD OM targeting. As an OM lipoprotein, PulS is localized to the OM by the Lol machinery. We hypothesize that PulS chaperones PulD monomers in the periplasm and that these two proteins together are recognized by the soluble component of the Lol machinery, LolA. Studies to detect the trimeric complex will be described. Furthermore, we are characterizing the interactions between PulS and secretin using site-directed mutagenesis and a bacterial two-hybrid system to identify regions of interaction involved in protection, piloting or both.

PS 32 Dissection of type II secretion pseudopilus structure and biogenesis

Manuel Campos[1], Michaël Nilges2 & Olivera Francetic1

1Unité de Génétique Moléculaire and [2]Unité de Bioinformatique structurale, Institut Pasteur, Paris

The type II secretion system of *Klebsiella oxytoca* is dedicated to pullulanase (PulA) secretion. According to the molecular piston model proposed in 1992, the inner membrane factors assemble pseudopilins into a filamentous structure that pushes PulA through the outer membrane secretin. In support of this hypothesis, overproduction of the major pseudopilin PulG leads to the assembly of pili on the cell surface. To test the piston hypothesis, high resolution models of the PulG pilus were calculated based on the crystal structure of the PulG soluble domain and the structural characteristics of purified filaments. We used site-directed mutagenesis of *pulG* to test these models *in vivo* by studying the impact of mutations on pilus assembly and PulA secretion. At least three classes of mutants of PulG were distinguished affecting (a) pseudopilin membrane insertion, (b) early steps of filament biogenesis and (c) pseudopilus stability. Observed differences in associated phenotypes could reflect at least two stages in pseudopilus biogenesis: pseudopilin interaction with the inner membrane platform and helical stabilization of pseudopilins within the filament. The high-resolution model tested *in vivo*, together with complementary approaches (interaction studies, suppressor analysis), will allow us to understand different steps of pseudopilus biogenesis and function.

PS 33 Peptide determinants of lipoprotein localization in *Borrelia burgdorferi*

Ryan J. Schulze, Ozan S. Kumru, & Wolfram R. Zückert

University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66061, USA

In contrast to most other species of bacteria, *Borrelia burgdorferi*, the Lyme disease spirochete, transports lipoproteins to its cell surface. The mechanisms responsible for the differential localization of lipoproteins within and between the *Borrelia* inner and outer membranes are unclear. We have previously demonstrated the critical nature of the unstructured N-terminus of the mature lipoprotein (the lipoprotein 'tether' region) for sorting an inert fluorescent reporter to the *Borrelia* cell surface. Here, we show that deletion of amino acid residues from a particular region within this tether results in the inefficient transport of a major surface lipoprotein (OspA) across the *Borrelia* outer membrane. The defect can be rescued by insertion of an alanine residue at an alternative location in the polypeptide. To better understand the determinants necessary for proper lipoprotein localization, we have designed a FACS-based assay that allows us to mutagenize and then reliably identify mislocalized borrelial lipoproteins. This assay allows us to quickly determine the functional consequences of altering the N-terminal primary sequence and the impact this has on lipoprotein trafficking within the *Borrelia* cell.

PS 34 Defining interactions in the accessory Sec system of *Streptococcus gordonii*, a dedicated export pathway for the platelet adhesin GspB

R. Seepersaud, B.A. Bensing & P.M. Sullam

San Francisco VA Medical Center and University of California, San Francisco, CA, USA

Bacterial binding to human platelets is an important step in the pathogenesis of infective endocarditis (IE). *S. gordonii* can mediate its

platelet attachment through a glycoprotein termed GspB ('Gordonii surface protein B'). GspB is encoded within an operon that also contains a seven component accessory sec system, containing two homologues of the general secretory pathway (SecA2 & SecY2) and five accessory Sec proteins (Asps1–5). The Asps show no homology to any proteins of known function but are essential for GspB export. We sought to elucidate the mechanism of GspB secretion by identifying interactions among components of this secretion system. A Yeast Two-Hybrid screen revealed novel interactions among the accessory Sec proteins (Asps1–3). Asp3 was shown to interact with Asp1, Asp2 and with itself. These interactions were also observed when assessed by *in vivo* cross-linking and co-purification analysis. In addition, Asp3 homo-multimers were detected by size-exclusion chromatography, further indicating that larger complex formation occurs. These findings suggest that unique Asp complex formations may be utilized for protein secretion of GspB.

PS 35 Interactions of *Escherichia coli* REMPs with their Tat-specific substrates revealed by three *in vivo* protein–protein interaction techniques

Haiming Li, Limei Chang & Raymond J. Turner

University of Calgary, 2500 University Drive NW, Calgary, AB, Canada T2N 1N4

Redox enzyme maturation proteins (REMPs) are involved in the assembly and targeting of many *E. coli* redox enzymes which are translocated by the Tat pathway. To build on our biochemical and biophysical investigations *in vitro*, various *in vivo* techniques are explored in this work. We employed either the bacterial two-hybrid (BACTH), the bimolecular fluorescence complementation (BiFC) and/or the fluorescence resonance energy transfer (FRET) techniques to study the interactions of DmsD, TorD and NarJ with their substrates as well as general chaperones (GroEL and GrpE) and enzymes involved in the molybdopterin synthesis pathway (MoeA, MoeB and MobB). While these REMPs were shown to associate with their cognate substrates by the three *in vivo* techniques, some degree of cross-interaction was revealed by the BiFC assay. The two fluorescence techniques also revealed the Tat dependency of the interaction between NarJ and NarG which only possesses a vestige twin-arginine motif at its N-terminus. The advantages and disadvantages of these approaches are compared.

PS 36 The effect of type three secretion systems of *Vibrio parahaemolyticus* on human epithelial Caco-2 cells

Ksenia Matlawska-Wasowska[1], Joshua Bell[1], Nicky O'Boyle[1], Eleanor Coffey[2] & **Aoife Boyd**[1]

[1]Dept of Microbiology, National University of Ireland, Galway, Ireland; [2]Aabo Akademi and Turku Centre for Biotechnology, Turku, Finland

Vibrio parahaemolyticus causes gastroenteritis in humans following the consumption of contaminated shellfish. The aim of this study was to investigate the effects of *V. parahaemolyticus* on human epithelial intestinal cell responses, in particular the role of the two Type Three Secretion Systems (TTSS). The Caco-2 human intestinal epithelial cell line was co-incubated with wild-type and TTSS mutant bacteria and alterations of various activities of the eukaryotic cells were determined. Gentamicin protection assays demonstrated that the bacteria invaded Caco-2 cells and that the TTSS had no effect on invasion. ELISA assays showed that the bacteria modulated the production of the chemokine IL-8. The results obtained using MTT and LDH assays indicated that *V. parahaemolyticus* were cytotoxic to Caco-2 cells in a concentration- and time-dependent manner. Immunoblotting revealed that *V. parahaemolyticus* induced JNK activation in human epithelial Caco-2 cells. The results provide evidence that the TTSS play an important role in the bacterium's interaction with human epithelial cells.

PS 37 Mutational studies of the autotransporter protein Hbp

Kaoru Nishimura, Isabel Kingston, Sam-Yong Park & Jeremy Tame

Yokohoma City University, Suehiro 1-7-29, Tsurumi, Yokohama, Japan

We have characterized a hemoglobin-degrading enzyme called Hbp (110 kDa) secreted by pathogenic *Escherichia coli* which causes peritonitis. This protein, a member of the autotransporter family, is implicated in bacterial synergy between *E. coli* and *B. fragilis* in mixed infections. This synergy is important for the development of abcesses in severe peritonitis. The crystal structure of Hbp, also called Tsh, has been published by us in 2005, suggesting the structure is made of three structural domains. The N terminal domain I is a serine protease of the trypsin family. Domain III is a long β helix, ending with a so-called autochaperone domain, believed to be involved in secretion across the outer membrane. Domain II is a small domain protruding from domain III with unknown function. We have carried out extensive mutagenesis of domain III in order to understand the role of the C terminal region in secretion, and the mechanism by which this process occurs. Our results suggest that the folding of domain III is involved in the transport process.

PS 38 Preprotein-controlled catalysis through two gates in the helicase motor of SecA

G. Gouridis[1,2], S. Karamanou[1], M. Koukaki[1] & A. Economou[1,2]

[1]IMBB-FORTH, Vassilika Vouton, 71110 Iraklio-Crete, Greece; [2]University of Crete

Protein trafficking is an essential process in cells from all three domains of life. Bacterial polypeptide translocase catalyses protein crossing through the plasma membrane. The translocase is an unusually dynamic nanomachine that comprises a SecYEG membrane-embedded protein-conducting channel and a processive molecular motor, the SecA ATPase which undergoes cycles of ATP hydrolysis and drives protein translocation. A novel mechanism used by secretory preproteins to control the catalytic cycle of the helicase 'DEAD' motor of SecA has been revealed (Karamanou *et al*, 2007). The central feature of this mechanism is a highly conserved salt-bridge termed Gate 1. Here we show that a second Gate in the 'DEAD' motor termed Gate 2 regulates ATP hydrolysis of the 'DEAD' motor. Gate 2 must be able to 'loosen' in order for the translocase to display membrane and preprotein-stimulated ATPase activity and preprotein translocation. Nevertheless Gate2 opening is not required for docking of the SecA motor to the membrane. We propose that loosening of Gate2 is essential for translocase priming and for preprotein-mediated full activation of translocase catalysis.

PS 39 The role of CesAB chaperone on T3S secretion

Vassilia Balabanidou[1], Charalampos Pozidis[1], Spyridoula Karamanou[1], Gad Frankel[2] & Anastassios Economou[1]

[1]Institute of Molecular Biology and Biotechnology (FORTH/IMBB), 711 10 Heraklion, Crete, Greece; [2]Centre for Molecular Microbiology & Infection, Dept of Biological Sciences, Imperial College London

Several pathogenic Gram-negative bacteria inject virulence factors into host cells through a type III secretion system (T3S) and specialized bacterial chaperones are required for their effective delivery. To characterize the early events of interaction between chaperones and substrates of Enteropathogenic *E. coli* (EPEC), we have purified the chaperone CesAB alone and in complex with its substrate EspA. Laser light scattering analysis showed that CesAB is dimeric and forms a 1:1 complex with EspA. Crystallography and limited trypsinolysis reveal that CesAB comprises an N-terminal domain and a flexible C-tail. Only the N-domain is necessary and sufficient for EspA binding. However CesABDC-tail fails to fully complement an EPECDCesAB strain for *in vivo* secretion. We conclude that CesAB has two distinct roles: it acts on EspA as a chaperone with its N-domain and it mediates an important as yet uncharacterized downstream event with its C-tail.

PS 40 Two novel secreted proteins from *Pseudomonas aeruginosa*: a patatin-like autotransporter and a type six secretion effector

R. Salacha, A. Hachani, R. Voulhoux, A. Filloux & **S. Bleves**

LISM–CNRS, 31 chemin J. Aiguier, 13402 Marseille cedex 20, France

The pathogenicity of *Pseudomonas aeruginosa* relies on its ability to secrete numerous proteins in the extracellular medium and to directly inject some toxins in the cytosol of the eukaryotic target cell.

Patatins, potato tuber storage glycoproteins, show PLA_2 activity, possibly as a defense against plant pathogens and stress. The presence of genes coding for patatin-like proteins (PLP) has been detected in several genomes of symbiotic or pathogenic bacteria, suggesting the importance of such domains in the bacteria-host interaction. We showed that PlpD of *P. aeruginosa* strain PAO1 posses the active dyad of the PLP family, and that it is secreted by an autotransporter mechanism.

T6SS is the latest secretion pathway identified in Gram-negative bacteria. Three T6SS loci are present in the PAO1 genome. We evidenced that the second locus is responsible for the secretion of a protein we called VgrG2. Moreover, we determined that while this T6SS locus is clearly is required for cytotoxicity toward macrophages, the VgrG2 protein is not implicated.

PS 41 Crystal structure of the major periplasmic domain of the bacterial inner membrane protein insertion and assembly facilitator YidC

David Oliver & Mark Paetzel

Dept of Molecular Biology and Biochemistry, Simon Fraser University, South Science Building, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6

Here we report the crystal structure of the *Escherichia coli* YidC major periplasmic domain ($YidC_{EC}P1$) at 2.5 Å resolution. This domain is present in YidC from Gram-negative bacteria and represents more than half the size of the full-length protein. $YidC_{EC}P1$ is made up of a large twisted β-sandwich protein fold with a C-terminal α-helix that packs against one face of the β-sandwich. The C-terminal α-helix and the β-sheet that it lays against are the most conserved regions of the domain. The region corresponding to the C-terminal α-helix was previously shown to be important for YidC's protein insertase function. A region of YidC that was previously shown to be involved in binding to SecF maps to one edge of the β-sandwich. Electrostatic analysis of the surface of this region reveals a predominantly charged surface which suggests that the SecF/YidC interaction may be electrostatic in nature. Intriguingly, $YidC_{EC}P1$ shares structural similarity with galactose mutarotase from *Lactococcus lactis*.

Posters

Systematics & Evolution Group

SE 01 Comparison of the evolutionary history of genes located in the core and arm regions of the *Streptomyces* genome

Paris Laskaris, Leonides Calvo-Bado & Elizabeth H.M. Wellington

University of Warwick, Gibbet Hill Road, Coventry CV4 7AL

Analysis of the *Streptomyces coelicolor* genome revealed a stable core region (essential genes) and two flanking arm regions (adaptive genes and those involved in secondary metabolism). It was suggested that this arrangement might enable more rapid evolution in the arms involving adaptive genes in addition to frequent gene acquisitions and loss. We examined the conservation of ten core genes (*16S, argH, atpD, efp, gyrB, ppc, recA, rplC, rpoB*) and four arm genes (*crtE, geoA, hopB, strA*) from streptomycete isolates. Amplified sequence fragments were used to generate neighbour-joining phylogenetic trees and the rates of synonymous (Ka) and nonsynonymous (Ks) nucleotide substitutions were compared. Tree morphologies of the core and the arm region genes are highly congruent, with the exception of the *strA* gene and the same applies to their substitution rate. This indicates that genes located in the arm regions can be equally stable as those from the core of the chromosome.

SE 02 Carbon dioxide fluxes in cyanobacterial soil crusts from the Kalahari

A.D. Thomas, S.R. Hoon & **P.E. Linton**

Dept of Environmental & Geographical Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD

In many desert regions photosynthetic cyanobacteria form soil crusts which are relatively rich in C and are important in determining CO_2 flux and sequestration. We report diurnal measurements of CO_2 fluxes for contrasting seasons from sandy soils in the Kalahari Desert, Botswana containing cyanobacterial crusts overlying heterotrophic bacterial subsoil populations. We find minimal CO_2 efflux during prolonged dry periods when the subsoil lacks moisture and easily utilized energy sources. We have observed natural sequestration rates of ~6 mg C m^2 h^{-1} without any added moisture after long periods of drought. A rapid increase in daytime CO_2 sequestration has, however, been observed even when only small amounts of moisture are present, up to ~75 mg C m^2 h^{-1} after 30 minutes, suggesting that surface cyanobacteria are readily activated by small amounts of water. In contrast, during or immediately after prolonged wet periods when the subsoil is moist, soils exhibit net CO_2 efflux of ~60 mg C m^2 h^{-1}. This is consistent with heterotrophic subsoil bacteria metabolizing leached soluble polysaccharides that are generated by (surficial) cyanobacteria.

SE 03 Population genetic structure of the *Staphylococcus intermedius* group: insights into its ancient and recent evolution

Jeanette Bannoehr[1], Nouri L. Ben Zakour[1], Andrew S. Waller[2], Luca Guardabassi[3], Adri H.M. van den Broek[4], Keith L. Thoday[4] & J. Ross Fitzgerald[1]

[1]Centre for Infectious Diseases, The Chancellor's Building, New Royal Infirmary, University of Edinburgh, Edinburgh EH16 4SB; [2]Centre for Preventive Medicine, Animal Health Trust, Newmarket, Suffolk CB8 7UU; [3]Dept of Veterinary Pathobiology, Faculty of Life Science, University of Copenhagen, Frederiksberg C, Denmark; [4]Dermatology Unit, Division of Veterinary Clinical Sciences, Royal (Dick) School of Veterinary Clinical Studies, Easter Bush Veterinary Centre, University of Edinburgh, Roslin, Midlothian EH25 9RG

Staphylococcus intermedius is the cause of pyoderma, an important skin infection, in dogs and other animals. Despite its clinical relevance, the population genetic structure of this animal pathogen is poorly understood. Using a multi-locus sequencing approach, we investigated the genetic diversity of isolates from different animal hosts in several different countries. Isolates phenotypically identified as *S. intermedius* are discriminated into three distinct species including *S. intermedius, S. pseudintermedius,* and *S. delphini*. We discovered that *S. pseudintermedius* and not *S. intermedius* is the common cause of canine pyoderma. We also investigated the allelic variation of *agrD*, which encodes the autoinducing peptide (AIP) of the *agr* quorum-sensing system. Four AIP variants were found including several which were represented in all three species suggesting that a common quorum sensing capacity has been conserved despite species differentiation in different hosts. Considerable clonal diversity exists within the *S. pseudintermedius* species including several meticillin-resistant clones which have evolved by recent acquisition of the *mecA* gene. Taken together, these data provide new insights into the classification and evolution of the '*S. intermedius* group' of animal pathogens.

SE 04 Genes for multilocus analysis of *Arthrospira*

Małgorzata Waleron[1], Magdalena Miklaszewska[1], Annick Wilmotte[2] & Krzysztof Waleron[1,2]

[1]Dept of Biotechnology, Intercollegiate Faculty of Biotechnology University of Gdansk & Medical School of Gdansk Poland; [2]CIP, Institute of Chemistry B6, University of Liege, B-4000 Liege, Belgium

The genus *Arthrospira* includes filamentous cyanobacteria with high nutritional value and therapeutic properties. *Arthrospira* PCC 8005 is a part of ESA project MELISSA (Micro Ecological Life Support System Alternative). This project aims to recover O_2, water and edible biomass from waste, CO_2 and minerals using light as the major energy source during long-term space missions.

The genomic diversity of *Arthrospira* strains belonging to different ITS clusters was studied by a multilocus approach based on the polymorphism of 5 DNA loci: *recA, gyrA, rpoC*1, *cpcBA*, *gvpCA*, and additionally one fingerprinting method (ERIC-PCR). A comparison of the resolution power of the different DNA loci with the data of the ITS sequences was carried out.

The new molecular markers and fingerprints have confirmed the existence of the 4 subclusters found on the basis of ITS sequences. The polymorphism of *recA* and *cpcBA* gene sequences, and the ERIC fingerprints show that the additional gene sequences allow to differentiate *Arthrospira* strains with higher resolution than ITS in the case of strains belonging to the ITS cluster II.A/B, but they do not seem to give a better resolution in the case of strains from cluster I. On the basis of the *gyrA* gene sequences, it was possible to differentiate only strains belonging to ITS clusters I and II. In case of *rpoC1* sequences, the difference between clusters I and II.B was clear, while it was very small between clusters I and II.A.

SE 05 Cyanobacteria from river biofilms as bioindicators of water quality: a morphological, molecular and physiological approach

Virginia Loza, Elvira Perona & Pilar Mateo

Dpto. Biología, Fac. de Ciencias, Univ. Autónoma de Madrid, 28049 Madrid, Spain

Cyanobacterial strains collected from river biofilms of several locations with different water quality, were isolated and grown as cyanobacterial monocultures. These strains were morphological and molecularly (16S rRNA) characterized. Additionally, they were physiologically characterized in order to evaluate their role as bioindicators of eutrophication. A series of competition bioassays was carried out in which cyanobacterial growth was measured in monocultures and in mixed cultures. Selected strains were subjected to a gradient of different concentrations of phosphate, nitrate and ammonium. Depending on the concentration of phosphorous and nitrogen either stimulation or inhibition of growth could be observed in monocultures of the studied cyanobacteria. Some strains of the mixed cultures dominated at low nutrient concentrations while other strains dominated at higher concentrations. It is concluded that ecological ranges of individual cyanobacterial populations can differ depending on nutrient concentrations, and that the persistence of several strains under low or high nutrient concentrations in the river could be explained on the basis of their different ecophysiological properties. This clarifies field observations published previously.

SE 06 The role of cyanobacterium *Nostoc calcicola* Bréb. in the development of microbial communities and soil formation in extra arid deserts

D.L. Golovanov[1], M.P. Verba[2], M.F. Dorochova[1] & L.B. Slobodkin[3]

[1]M.V. Lomonosov Moscow State University, Geographic Faculty, Moscow, Russia; [2]V.V. Dokuchayev Soil Science Institute, Moscow, Russia; [3]S.N. Vinogradskiy Institute of Microbiology, Moscow, Russia

The integrated soil and microbiological research, pioneered on extra arid desert soils on alluvial fans in Tsagan-Bogdo (Gobi, south of Mongolia) has shown that organic matter of the soil crust (0–2 cm) is produced entirely by Cyanobacteria *Nostoc calcicola Bréb* and attendant algae *Chlorella vulgaris Beijer* (Chlorophyta). These two make possible existing of various heterotrophic micro-organisms providing microbiological processes for organic matter destruction. The research has revealed the presence of specific micro-organisms capable of dissimilative reduction of Fe (III). This suggests actual mobilization of iron and development of short-term anaerobic conditions in the soil crust.

The possibility of participation of micro-organisms capable of sulfate reduction and those providing oxidation of reduced sulfur compounds in soil formation in extra arid desert is discussed.

SE 07 Detection of potential microcystin-producing cyanobacterium, *Chroococcidiopsis* sp. with mcyA molecular marker

R.P. Wanigatinge, D.T. Gnanakkan & D.N. Magana-Arachchi

Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka

Early detection of water blooms caused by potential toxin producing cyanobacteria is important in environmental monitoring. A study was conducted on the characterization of toxic cyanobacterial species, particularly on those able to produce microcystin in household drinking-water wells located at Sammanthurai, Sri Lanka after December 2004 tsunami disaster. Water samples, collected from different sites were inoculated into BG11 and BG11o medium. DNA extractions were carried out by Boom's method using silica particles and guanidium isothiocyanate. A method based on the PCR technique and cyanobacterial specific primers for the specific amplification of 16S rRNA gene were used to confirm the presence of cyanobacteria in cultured samples. The potential for microcystin production was determined by DNA amplification using self designed primers (McyAF19 and McyAR47) of the mcyA gene in the microcystin synthesis pathway. The sequenced PCR fragment for the 16S rRNA showed 99% of the nucleotide homology to the *Chroococcidiopsis sp.* gene for 16S ribosomal RNA. The presence of the unique amplified product of approximately 600 bp indicated that the *Chroococcidiopsis* sp. isolates have the genetic potential to produce microcystins.

Authors

Authors

Authors

Hot Topic Symposium

Influence of climate change on disease and microbial environmental processes: microbes and climate change

Predictive epidemiology, a case for cholera

Rita R. Colwell

Distinguished Professor, University of Maryland College Park and, Johns Hopkins University Bloomberg School of Public Health, Senior Advisor and Honorary Chairman Emeritus, Canon U.S. Life Sciences

An environmental source of cholera was hypothesized as early as the late nineteenth century by Robert Koch. Standard bacteriological procedures for isolation of vibrios from environmental samples, including water, between epidemics generally were unsuccessful because *Vibrio cholerae*, a marine vibrio, enters into a dormant, 'viable but nonculturable stage', when conditions are unfavorable for growth and reproduction. An association of *V. cholerae* with zooplankton, notably copepods, has been established. Furthermore, the sporadicity and erraticity of cholera epidemics have beencorrelated with El Niño. Since zooplankton harbor the bacterium and zooplankton blooms follow phytoplankton blooms, remote sensing can be employed to predict cholera epidemics from sea surface temperature (SST), ocean height (OH), chlorophyll, and turbidity data. Cholera occurs seasonally in Bangladesh, with two annual peaks in the number of cases. From clinical remote sensing data, it has been found that SST, OH, and blooms of phytoplankton and zooplankton are correlated with cholera epidemics. Thus, selected climatological factors and incidence of *V. cholerae* can be recorded, bringing the potential of predicting conditions conducive to cholera outbreaks to reality. A simple filtration intervention takes into account the association of *V. cholerae* with plankton, and has proven to be a simple solution to the age-old problem of controlling this water-borne disease for villagers in remote regions of Bangladesh.

Clinical Virology Group Session

Vaccines against viral infections from concept to practice

Japanese encephalitis virus

T. Solomon

University of Liverpool

Japanese encephalitis is one of the most important causes of viral encephalitis globally, causing an estimated 30-50,000 cases and 10–15,000 deaths. Although confined to the Asia-Pacific Region, this mosquito-borne flavivirus is spreading. Phylogenetic studies suggest the virus originated in the Indonesia-Malaysia region and spread from here. Recent work has shown the importance of seizures, raised intracranial pressure, and a poliomyelitis-like flaccid paralysis in the presentation features. The pathogenesis is incompletely understood, but recent evidence suggests the inflammatory response is critical.

Vaccines against Japanese encephalitis have been around for a long time. Formalin inactivated mouse brain vaccines were made by the Japanese in the 1930s, and subsequently developed into the vaccines which many travellers use today. However, their use has been controversial because of adverse events. In addition, the vaccines have not been available to the majority of people in rural Asia that need them, because of issues over cost and production. In the last decade there have been considerable advances in vaccines against Japanese encephalitis, not only in the laboratory, but also translating through to public health policy.

A live attenuated vaccine (SA-14-14-2) developed by the Chinese has been used extensively in China, and has the potential for wider use across Asia. A new inactivated vero-cell derived vaccine (based on SA-14-14-2) also has the potential for wider use, especially among travellers whilst a chimeric vaccine, based on the 17D yellow fever vaccine strain is also in advanced development. To help countries make decision about vaccine implementation they need better data on disease burden. This in turn requires enhanced surveillance, improved diagnostics, and a better way of measuring the disability caused by Japanese encephalitis. In addition to discussing the new vaccines, this talk will cover Liverpool's work in helping the WHO and the Bill and Melinda Gates-funded Japanese encephalitis project at PATH with vaccine implementation.

Virus Group Session

Virus modulation of host defences

T cells and murine cytomegalovirus: obsession and deception

Ann B. Hill, Kathy Cho & Chris Snyder

Oregon Health and Science University, USA

Persistent infection by murine cytomegalovirus drives a huge CD8+ T cell response that begins 'inflating' shortly after infection and is subsequently maintained at high levels. These cells closely resemble human CMV-specific cells which comprise a major component of the peripheral T cell compartment in most people. Despite the persistence of the virus and a phenotype that suggests extensive antigen-driven differentiation, MCMV-specific T cells remain functional throughout life and respond vigorously to viral challenge, though they respond poorly to homeostatic signals. Strikingly however, most inflated cells do not divide in chronically infected hosts and have a short life-span. Sporadic bursts of cell division do occur in the presence of viral antigen, but divided cells fail to accumulate. Instead, the overall population is maintained by recruitment of naïve cells that join this short-lived pool. These data suggest that T cell dysfunction is avoided by consistently replacing a short-lived T cell pool.

Virus Group Session

Control of virus gene expression

Targeting of the ubiquitin-proteasome pathway by adenovirus E1A

Mozhgan Rasti[1], Roger Grand[1], Ahmed Yousef[2], Joe Mymryk[2] & **Andy Turnell**[1]

[1]CR-UK Institute for Cancer Studies, The Medical School, The University of Birmingham, Edgbaston, Birmingham B15 2TT; [2]Depts of Oncology & Microbiology & Immunology, University of Western Ontario, London, Ontario N6A 4L6, Canada

Adenovirus (Ad) early region 1A (E1A) expression is essential for Ad replication and Ad-mediated transformation. E1A is expressed predominantly from two splice variant transcripts, 12S and 13S that in Ad2/5, give rise to protein products of 243 and 289 amino acids, respectively. These protein products differ by conserved region 3 (CR3), which is present only in the larger 13S species. Ad-regulated expression of early viral genes, and some cellular genes, is dependent upon the expression of the 13S E1A species. In this regard CR3 serves to recruit the basal transcriptional machinery, transcriptional mediator, transcriptional co-activators, components of the proteasome and potentially other transcriptional regulators to transcription factor-bound promoters in order to temporally co-ordinate Ad E1A-stimulated gene expression during viral infection. CR3 expression is however, dispensable for Ad-mediated cellular transformation. The N-terminal region, CR1, CR2 and CR4 of E1A target both transcriptional activators, and repressors, in order to promote cellular transformation. In this regard: the N-terminal region and CR1 bind CBP/p300; the N-terminal region, CR1 and CR2 bind proteasomal components; CR1 and CR2 bind the pRB family; and CR4 binds the CtBP family. Here, I will discuss how E1A targets components of the ubiquitin-proteasome pathway in order to regulate transcription programmes.