Contents

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

Plenary session

Micro-organisms and earth systems – advances in geomicrobiology

Novel approaches for study of diversity and function of microbial communities

Michael Wagner

Dept of Microbial Ecology, University of Vienna, Althanstr 14, A-1090 Vienna, Austria (Email wagner@microbial-ecology.net)

The last 20 years in microbial ecology dramatically changed our perception of biodiversity within the three domains of life. *Bacteria* and *Archaea* are no longer viewed as groups of organisms which show relatively little diversification, but have now been recognized to harbour a perplexing number of novel phylogenetic lineages. This dramatic paradigm shift was only made possible by the development of cultivation-independent molecular approaches for surveying microbial diversity in nature. Due to these technological advances many novel and often numerically important prokaryotic species have been identified in various ecosystems. However, for most of these players their ecophysiology and contribution to the functioning of the ecosystem remains hidden.

Given the above, one of the biggest challenges in contemporary microbial ecology is to develop strategies which enable us to (i) directly investigate metabolic properties of defined but uncultured micro-organisms, and (ii) to identify those uncultured organisms which are responsible for defined processes within their natural environment. Both sides should be equally well served by the methods outlined in this presentation. Specifically, I will provide an overview of a group of recently developed methods which exploit the addition of isotope-labelled substrates to complex microbial communities in order to bridge the gap between microbial community structure and function.

Minerals, mats, pearls and veils: themes and variations in giant sulfur bacteria

Neil D. Gray & **Ian M. Head**

School of Civil Engineering and Geosciences, Institute for Research on the Environment and Sustainability and Centre for Molecular Ecology, University of Newcastle, Newcastle upon Tyne NE1 7RU

The biogeochemical cycling of sulfur has been at the heart of microbial ecology since the mid-19th century. This is due, at least in part, to the striking forms of many of the organisms involved in the transformation of reduced sulfur species. Giant sulfur bacteria were among the earliest micro-organisms to capture the interest of microbiologists exploring the links between geochemical cycling of the elements and the microbiota responsible. Sulfur bacteria play a pivotal role in the biogeochemical cycling of sulfur, carbon, nitrogen and possibly metals. Their activities also affect the precipitation and dissolution of minerals. For these reasons, they can serve as models to establish links between environment, physiology and evolutionary diversification that may be extrapolated more widely. Some of the adaptations in the energetics and biochemistry of giant sulphur bacteria have consequences for ecosystem-scale processes. These are discussed in the context of the geochemical features of habitats dominated by conspicuous sulfur bacteria.

Trace metal geochemistry in contaminated waters: biomineralization and community interactions within biofilm communities

Lesley A. Warren

School of Geography and Earth Sciences, GSB 309, McMaster University, 1280 Main St West, Hamilton ON L8S 4K1, Canada

Biofilms are commonly found at geochemically reactive environmental interfaces (e.g. sediment-water), where in a metabolically dependent manner, they can influence many geochemical processes relevant to metal behaviour. Contaminated systems such as acid mine drainage (AMD) where biofilms flourish, provide an opportunity to evaluate micro-organism-geochemical interactions involved in metal dynamics. Integrated, high-resolution, spatial and temporal investigation of AMD biofilms using multiple techniques to identify depth-resolved, biofilm biological and geochemical linkages [e.g. (1) microprofiling of biofilm geochemical gradients, (2) microscopies, e.g. CLSM, HR-TEM, ESEM, STXM, XAS, to characterize micro-organism – mineral assemblages, and (3) molecular biological techniques (16S RNA, FISH), to characterize biofilm community structure] at a Ni/Cu mine in Northern Ontario, has shown a novel and highly complex biofilm community driving biomineralization and associated metal sequestration. The developed model of biofilm biogeochemical architecture capturing the micro-scale linkages amongst geochemical gradients, metal dynamics and depth resolved micro-organism community structure, will illustrate how metabolism drives metal geochemistry and the utility of this integrated approach for environmental investigation.

Soil micro-organisms in Antarctic Dry Valleys: resource supply and utilization

D.W. Hopkins

School of Biological & Environmental Sciences, University of Stirling, Stirling FK9 4LA

The dry valleys of Antarctica are the harshest terrestrial environment on the planet, experiencing extremes of low temperature and available water. The land surface lacks a conspicuous community of autotrophs, yet the soils contain enduring populations of heterotrophic micro-organisms and invertebrates. Understanding the provenance of resources for soil heterotrophs in nutrient- and energy-poor polar deserts, requires consideration of a wider range of possible sources than in most other ecosystems. When the indigenous stocks of soil organic C and N are as low as occurs in the dry valleys, even very modest inputs from other sources may represent a significant subsidy. This presentation will evaluate various possible sources of spatial and temporal subsidies of resources in the soils of the Antarctic dry valleys, including (1) modern autotrophic activity *in situ*, such as the cryptoendolithic communities, mosses, cyanobacteria and heterotrophic algae in the soils, and autotrophic bacteria such as nitrifiers, (2) ancient *in situ*, or 'legacy', organic deposits from a time when the climate was warmer and conditions were wetter, and organic lake sediments accumulated on the surfaces which developed into the modern dry valleys, (3) spatial subsidies from the coastal regions where there is abundant marine and ornithogenic deposits carried into the dry valleys by aeolian dispersal, and (4) spatial subsidies from the margins of modern lakes, where microbial mats accumulate under favourable conditions and organic matter from the mats is subsequently dispersed onto the surrounding soils, representing relatively short-range aeolian dispersal.

New insights into bacterial cell wall structure and physicochemistry: implications for interactions with metal ions and minerals

Terry J. Beveridge

Dept of Molecular & Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

The rapid freezing to vitrify cells and cryo-transmission electron microscopy (cryoTEM) of *frozen hydrated thin-sections* from the cells are revealing true native cellular structure. This is especially true of the bacterial surface. Unlike other forms of TEM, frozen hydrated thin-sections cannot be contrasted by heavy-metal stains (such as U, Pb and Os) and their contrast is via the inherent density of the constituent molecules within the cells. Therefore, these frozen sections show the true mass distribution within the biomatter. Another cryoTEM technique, *freeze-substitution*, also produces thin sections, but these are plastic sections of heavy-metal stained cells. The heavy-metal ions of the stain bind to the available reactive sites of the biomatter. When such images are compared to those from the frozen hydrated sections, a clear interpretation of native structure and its metal reactivity can be made. At the same time, pH titrations of cells provides an indication of the ionization of metal-binding reactive groups and their isoelectric points, whereas zeta potential measurements determines the overall cellular charge density. Hydrophobic/electrostatic interaction chromatography, contact angle measurements and adhesion to selected inanimate surfaces together with structural NMR analysis of lipopolysaccharides suggest this macromolecule has a strong bearing on surface physicochemistry. These types of observations will be invaluable for the study of microbe-metal/mineral interactions.

Horizontal gene transfer of metal homeostasis genes and its role in microbial communities of the deep terrestrial subsurfaces

Jonna Coombs & **Tamar Barkay**

Dept of Biochemistry and Microbiology, Cook College, Rutgers University New Brunswick, NJ, USA

Evolution of metal resistance in the microbial world often proceeds by horizontal transfer, and such transfers in the subsurface may be essential to ensure the integrity, and thus activities, of the resident microbial communities. In our examination of the role of gene transfer in the evolution of resistance to lead, cadmium, and zinc via P_{IB}-ATPases efflux pumps, we have discovered four instances of phylogenetic incongruence among subsurface bacteria of the Costal Atlantic Plain, USA. Supporting evidence for horizontal gene transfer was found in three of these cases by the occurrence of unusual DNA sequence composition, i.e. %mol G+C content and presence of indels. This frequency was similar to the frequency commonly observed for P_{IB}-ATPases genes in the microbial world as revealed from sequenced microbial genomes. Plasmid DNA from *Comamonas* sp. B0173 was further analyzed utilizing a specialized DNA microarray, and results suggested that lead resistance was transferred on a broad host range plasmid belonging to incompatibility group P1β. Transfer by broad host range conjugal plasmids is one mechanism for the evolution of metal resistance in the subsurface. Thus, research focused on such plasmids in subsurface microbial communities should advance understanding of genetic interactions and evolution, and support strategies for bioremediation in the subsurface.

Biosilicification: the role of cyanobacteria in silica sinter deposition

Liane G. Benning[1], Vernon Phoenix[2] & Bruce W. Mountain[3]

[1]Earth and Biosphere Institute, School of Earth and Environment, University of Leeds; [2]Molecular and Cellular Biology, University of Guelph, Canada; [3]Institute of Geological and Nuclear Sciences, Wairakei Research Centre, Taupo, New Zealand (Email liane@earth.leeds.ac.uk)

Cyanobacteria play an important role in the formation of silica sinter structures in modern geothermal environments and in ancient fossil analogues. Many field, laboratory as well some molecular dynamic studies concluded that covalent bonds between silica and bacterial components are not favoured, that cyanobacteria play a negligible role in the initial silica polymerization and that silica nucleation is driven by purely inorganic poly-condensation. Nevertheless, a clear link between silica sinter structures/textures and micro-organisms exists. Ultimately silicification promotes the incorporation of micro-organisms into the sinter structure, and leads to the preservation of microbial colonies as fossils. However, the question remains, as to the exact role of the microbial surface in the processes that lead to silica precipitation. We argue here that the formation of silica sinters follows a multi-step process primarily governed by inorganically poly-condensation of silica monomers to the formation of silica nanoparticles, followed by microbially enhanced silica nanospheres aggregation. Cyanobacteria react to silica nanoparticle nucleation and aggregation by increasing the amount of EPS. Once silicification is advanced and thus unavoidable, the thicker polysaccharide sheath will boost the aggregation of the inorganically nucleated silica nanoparticles. This process is occurring while they are alive but with continual silicification leads to fossilization.

Microbial diversity and function in surface, subsurface and extreme environments: relevance to exobiology

K.H. Nealson

University of Southern California, Los Angeles, USA

Metabolic diversity has become a catch phrase for the wonders of the microbial world wonders that may help explain why the world is like it is through the energetic exploitation of the geosphere by the metabolism of the biosphere. A direct, but not entirely obvious, extension of this thinking is that it applies not only to understanding the evolution and distribution of life on our own planet, but to the possibility of life in abodes other than Earth: i.e. could we use this kind of 'logic' to distinguish a living from a dead planet? A quick perusal of the metabolic diversity of the prokaryotic life on our planet reveals that virtually every redox-related energetic niche is occupied. If there is fuel (an electron donor), and something with which to burn the fuel (an electron acceptor), then some microbe has learned how to exploit this energetic niche. This exploitation, in its simplest sense, defines the metabolic diversity with which we work microbes striving to make a living on whatever resources are available, and being remarkably successful in plying their trade. Given that such metabolic diversity is so dominant on our own planet,it is reasonable to expect no less of any other planet on which life has evolved. If so, then the search for life in all realms (surface and subsurface of the Earth, as well as potential extraterrestrial sites) should begin with considerations of energy types, levels, and fluxes: always with an eye to finding things those disequilibrium situations that should not be there the true connection of microbial diversity to exobiology. Thermodynamics tells us what to measure: to search for the right things. Kinetics the specific and extensive catalysis of these reactions – will be the signpost of life the geobiological indicator of microbial diversity at work.

Biogeochemical cycling in polar, temperate and tropical coastal zones: similarities and differences

David B. Nedwell

Dept of Biological Sciences, University of Essex

The coastal zone is an area of high primary production and biogeochemical cycling, compared to the open ocean However, different physico-chemical factors influence biological activity in the coastal zones at different latitudes, primarily associated with differences in seasonality of light and temperature. Polar regions have extremely short summer seasons with pulsed inputs of organic matter at constant low temperature; temperate regions have longer summer seasons and seasonal variation in both light and temperature; tropical regions have least seasonality with relatively little change in light or day legth at constant high temperature. These regional differences influence the degree to which microbial populations, and hence communities, can adapt physiologically to their environment. In polar, constant low temperature communities, and in temperate communities populations may be physiologically less well adapted

to environmental temperature than in tropical constant high temperature environments. These physiological differences may in turn influence their ability to sequester substrates in the different latitudinal coastal regions. The reasons for this will be discussed. Furthermore, is there any evidence that there are differences in the importance of the different functional groups of micro-organisms contributing to biogeochemical cycling in the different regions; or in the phylogenetic composition of these functional groups in the different regions?

Fungal roles and function in rock, mineral and soil transformations

Geoffrey M. Gadd, Ademola O. Adeyemi, Euan P. Burford, Karrie Melville & Marina Fomina

Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN

'Geomycology' can be defined as the impact of fungi on geological processes, including weathering of rocks and minerals, accumulation of metals and role in nutrient cycling. This presentation will highlight our recent work on the fungi inhabiting limestone, dolomite (and other rocks), and the plant mycorrhizosphere, particularly regarding mineral dissolution and transformation, and formation of secondary mycogenic minerals. For example, fungi can play a role in the transformation of limestone minerals through the formation of 'mycogenic' fabrics such as secondary calcite ($CaCO_3$) and whewellite ($CaC_2O_4.H_2O$): metabolism-dependent and -independent processes play integral roles. An important mechanism of metal mobilization from minerals is a combination of acidification and ligand-promoted dissolution: if oxalic acid is produced, the production of metal oxalate minerals may result. For ericoid mycorrhizal and ectomycorrhizal fungi in axenic culture and in mycorrhizal association, proton-mediated dissolution was the main leaching mechanism. However, other significant mechanisms of mineral dissolution were ligand-promoted (e.g. oxalate, citrate), and metal accumulation by the mycelium, where the biomass acted as a 'sink' for mobilized metal species. Mobilized zinc, copper and lead were oxygen-coordinated within fungal biomass to carboxylate and phosphate ligands. The roles of fungi as agents of biogeochemical change in the terrestrial environment will be emphasised.

The deep intraterrestrial biosphere

Karsten Pedersen

Deep Biosphere Laboratory, Dept of Cell & Molecular Biology, Göteborg University, Box 462, SE-405 30 Göteborg, Sweden

It was not more than 20 years ago, that exploration of the world of intraterrestrial microbes gathered momentum. Previously, it had generally been assumed that persistent life could not exist deep underground, out of reach of the sun and a photosynthetic ecosystem base. In the mid 1980s scientists started to drill deep holes, from hundreds to a thousand meters deep, in both hard and sedimentary bedrock, and up came microbes in numbers equivalent to those found in many surface ecosystems. The world of intraterrestrial microbes had been discovered. The environments of intraterrestrial microbial ecosystems occupy a special position, differing substantially in many aspects from those of most surface-based ecosystems. In many ways underground ecosystems must be approached quite differently from how surface ones would be approached. The intraterrestrial world is huge and diverse, and this talk will provide a brief overview of the following matters: Where do intraterrestrial environments begin? Variability of intraterrestrial environments. Strategies for exploring intraterrestrial environments. Organisms living in intraterrestrial ecosystems. Range of biomass in various intraterrestrial environments. Intraterrestrial species diversity. Energy sources for intraterrestrial life. Activity of intraterrestrial life forms.

Iron, nitrogen, phosphorus and zinc cycling and consequences for primary productivity in the oceans

John A. Raven[1], Karen Brown[1], Maggie Mackay[1], John Beardall[2], Mario Giordano[3], Espen Granum[4] & Richard C. Leegood[4]

[1]Plant Research Unit, University of Dundee at SCRI, Scottish Crop Research Institute, Dundee DD2 5DA; [2]School of Biology, Monash University, Clayton, VIC 3800, Australia; [3]Dept of Marine Science, Università Polytecnica delle Marche, 60121 Ancona, Italy; [4]Dept of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN

Marine primary productivity by phytoplankton is limited by the supply of a number of resources in different areas and at different times. Photosynthetically active radiation limits when there is deep mixing of the surface ocean. Nutrient elements limit (or co-limit) in different parts of the ocean and at different times in the decreasing order N, Fe, P and (possibly) Zn. The widespread N limitation of phytoplankton species that cannot fix N_2 is a result of restriction of the rate of biological N_2 fixation relative to other inputs of combined N to the ocean, and the processes removing combined N from the ocean. Restriction of N_2 fixation by marine cyanobacteria in the surface ocean is a result of limitation by Fe, P and/or light. Fe and/or P supply can also limit primary productivity using combined N in parts of the ocean. Restriction of primary productivity by lack of one of the four elements alters the characteristics of inorganic carbon concentrating mechanisms involved in photosynthesis by most phytoplankton. Growth limitation by N, P, Fe and Zn increases the extent of silica deposition by diatoms and N, P and Zn deficiency increase calcite deposition in coccolithophores, thereby potentially increasing sedimentation of these elements in organic particles due to the extra ballast.

New uses for old enzymes; mechanisms, environmental impact and biotechnological potential of microbial metal reduction

Jonathan R. Lloyd

Williamson Research Centre for Molecular Environmental Studies and School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL (Email Jon.lloyd@manchester.ac.uk; Tel 0161 275 7155; Fax 0161 275 3947)

The microbial reduction of metals has attracted recent interest as these transformations offer models for early life, play crucial roles in the cycling of both inorganic and organic species in a range of environments on modern Earth and, if harnessed, may offer the basis of a wide range of innovative biotechnological processes. These include the bioremediation of metal contaminated land and water, the oxidation of xenobiotics under anaerobic conditions, metal recovery in combination with the formation of novel biocatalysts, and even the generation of electricity from sediments. Under certain conditions, however, microbial metal reduction can also mobilise toxic metals with potentially calamitous effects on human health. Rapid advances over the last decade have resulted in a detailed understanding of some of these transformations at a molecular level, with added impetus expected from the imminent availability of complete genome sequences for key metal-reducing bacteria, in combination with genomic and proteomic tools.

New insights ito the physiology and regulation of the anaerobic oxidation of methane

Martin Krüger[1,2] & Tina Treude[2]

[1]Federal Institute for Geosciences and Resources (BGR), Stilleweg 2, D-30655 Hannover, Germany; [2]Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany

The anaerobic oxidation of methane (AOM) is one of the most important sinks for the greenhouse gas methane in marine ecosystems, consuming more than 80% of the methane produced in marine sediments prior to their emission into the atmosphere. After the discovery of AOM approximately 40 years ago and its verification and localisation via geochemical evidence, the identification of the

responsible micro-organisms became possible. In addition to biogeochemical methods, newly developed molecular tools have provided deeper insights into the size and composition of AOM communities under different environmental regimes. Furthermore, microscopy and stable isotope analysis allowed first conclusions about the physiology and regulation of this important process. However, only the very recent establishment of *in vitro* systems and the discovery of samples naturally enriched in AOM biomass enabled laboratory studies of the environmental regulation and enzymatic mechanism of AOM. This review gives an overview on the latest findings in the study of AOM, which contribute to the elucidation of this still largely enigmatic microbial process and to further estimates of the quantitative significance of AOM *in situ* in different environments.

Biogeochemical roles of fungi in marine and estuarine habitats

Nicholas Clipson[1], Eleanor Landy[2] & Marinus Otte[3]

[1]Dept of Industrial Microbiology, University College Dublin, Belfield, Dublin 4, Ireland; [2]School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH; [3]Dept of Botany, University College Dublin, Belfield, Dublin 4, Ireland

Marine ecosystems occupy around 70% of the global surface area and are strongly delineated by both proximity to land masses and by depth through the water column, from surface through to benthic and deep sea zones. The fungal component of marine biota has only been recognised relatively recently, and our knowledge of the role of fungi in the diversity of marine ecosystems remains scant. The review will define what differentiates marine from terrestrial fungal species, will consider the extent of marine fungal diversity and will identify environmental factors for which marine fungi require adaptation, together with the nature of those adaptations. Although little is known about processes mediated in marine ecosystems by fungi, an assessment of their known and likely roles in marine food webs is advanced together with their possible contributions to biogeochemical cycling processes.

Role of micro-organisms in karstification

Philip C. Bennett[1] & Annette Summers Engel[2]

[1]Dept of Geological Sciences, The University of Texas at Austin, Austin, TX 78712 USA; [2]Dept of Geology and Geophysics, Louisiana State University, Baton Rouge, LA 70803, USA

While chemolithoautotrophic microorganisms are found in nearly every environment on Earth, they are more abundant in dark habitats where competition by photosynthetic organisms is eliminated. Caves represent dark but accessible subsurface habitats where the roles of microbial chemolithoautotrophy to biogeochemical and geological processes can be directly examined. Hydrogen sulphide (H_2S)-rich springs discharge into some caves, and from observations of Lower Kane Cave (USA) in the 1970s, the original sulphuric acid speleogenesis model was proposed as a cave enlargement process. Hydrogen sulphide, however, is a rich energy source for chemolithoautotrophic microorganisms, and a surprisingly complex consortium of microorganisms, dominated by sulphur-oxidizing bacteria, is found in Lower Kane Cave springs and stream. Several evolutionary lineages within the class '*Epsilonproteobacteria*' dominate the biovolume of subaqueous microbial mats, and these microbes support the cave ecosystem through chemolithoautotrophic carbon fixation. The anaerobic interior of the cave microbial mats is a habitat for anaerobic metabolic guilds, dominated by sulphate-reducing and fermenting bacteria. Biological controls to speleogenesis had not been previously considered and it was found that cycling of carbon and sulphur through the different microbial groups directly affects sulphuric acid speleogenesis. Most of the H_2S entering the cave was found to be consumed by subaqueous, sulphur-oxidizing microbial communities in the cave stream, with little vapour-phase H_2S escaping to the cave atmosphere. '*Epsilonproteobacteria*' generate sulphuric acid as a metabolic by-product, rapidly dissolving limestone. This new recognition of microbial processes to geologic processes provides a better understanding of the causal factors for porosity development in sulphidic groundwater systems.

Cells & Cell Surfaces Group session

Cell polarity and compartmentalization

Cooperation between actin homologues during cell wall morphogenesis in *Bacillus subtilis*

R. Carballido-López, Alex Formstone, Mark Leaver & **Jeff Errington**

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

MreB proteins have recently been identified as bacterial homologues of actin. They are involved in cell morphogenesis in a wide range of organisms, and probably fulfil a variety of other cellular functions. Like actins MreB proteins polymerize to form linear filaments or cables, which have dynamic properties and can be rapidly turned over and remodelled. In rod-shaped cells MreB cables have been visualized as helical structures that coil around the long axis of the cell, probably associated with the inner surface of the cell membrane. By analogy with eukaryotic actins, MreB proteins probably interact with a range of effector proteins that subserve their various functions. However, so far, these effector proteins remain largely unknown. *B. subtilis* has three MreB homologues; MreB, Mbl (which stands for MreB like) and MreBH (MreB homologue). The first two of these have been implicated in cell wall (peptidoglycan) synthesis, and connections between these proteins and the peptidoglycan synthetic machinery are beginning to emerge. We have recently identified a function for the third actin homologue, MreBH, in cell wall maturation. MreB exerts control over this process by governing the localized export of a cell wall hydrolase LytE, with which it interacts directly.

Envelope stress sensing during *Myxococcus* multicellular development

M. Esmaeiliyan, J.J. Rivera & **H.B. Kaplan**

University of Texas Medical School, Houston, TX 77030, USA

Myxococcus xanthus is a Gram-negative soil bacterium that undergoes multicellular development upon starvation at high cell density. Expression of the early developmental gene *4445* requires starvation, high cell density, aeration, and at least 8 mM Mg^{++}. Random mutagenesis identified the regulators of *4445* expression to be a positive regulator, EcfA, and two negative regulators, ReaA and ReaB. This *M. xanthus* EcfA/ReaA/ReaB signal transduction system is encoded by one operon and is analogous to the *E. coli* sigmaE/RseA/RseB signal transduction system that is activated by certain envelope stresses.

Mutants containing *reaA* or *reaB* mutations express *4445* at levels more than three orders of magnitude greater than wild-type cells during growth and development. These same mutants overproduce EcfA during growth and development indicating that the *ecfA* operon is autoregulated. The expression of *4445* is increased three fold during growth by the addition of 0.5 M glycerol, which induces starvation-independent sporulation. In addition, mutations that alter LPS O-antigen biosynthesis bypass the high-density requirement for *4445* expression during starvation in an *ecfA*-dependent manner. These data indicate that the *ecfA* operon encodes the elements of a new regulatory pathway that integrates and transduces starvation and cell density cues during early *M. xanthus* development and also senses cell envelope alterations.

Directional motility and the role of specialized secretory organelles in apicomplexan protozoa

Fiona Tomley

Institute for Animal Health, Compton, Newbury RG20 7NN

The Apicomplexa comprise many parasitic protozoa that cause serious and widespread diseases of man and animals such as malaria (*Plasmodium* species), toxoplasmosis (*Toxoplasma gondii*), water-borne enteritis (*Cryptosporidium* species) and coccidiosis (*Eimeria* species). Apicomplexans are obligate intracellular parasites that share a common mechanism of invasion that is linked to their unique form of locomotion, directional gliding motility, and which is dependent on the release of material from specialised apical secretory organelles called micronemes and rhoptries. Micronemes store soluble and transmembrane proteins are secreted from the apical end of the parasite onto the parasite surface, where they interact with host cell ligands. Energy is provided by an actin–myosin motor that lies just under the parasite plasma membrane and which is linked to the parasite surface through dynamic multi-protein complexes that include C-terminal domains of surface-bound transmembrane microneme proteins. After binding host-cell ligands these surface-bound microneme proteins are rapidly redistributed backwards over the parasite surface and released by proteolysis as the parasite is propelled into the host cell. Coincident with parasite entry is the formation of a membrane-bound vacuole, within which the parasite resides in the host cell cytoplasm, and the secretion of proteins and lipids from the rhoptry organelles, which are believed to modify the vacuole so it is fusion-incompetent, thus preventing the parasite from being degraded by host cell lysosomes.

Subcellular sites for protein export in gram-positive bacteria

Girbe Buist, Nathalie Campo, Jan D.H. Jongbloed, Harold Tjalsma, Anja Ridder, Jan Kok & Oscar P. Kuipers

Dept of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

The secretome of a bacterium includes all components for the protein secretion machineries and the native secreted proteins. Microscopic analysis showed that multiple sites for protein export are present in the rod-shaped bacterium *Bacillus subtilis*. The core components of the major protein secretion machinery, SecA and SecY, are organised in a spiral-like structure along the cell. In the coccoïd bacterium *Lactococcus lactis* SecA was found to localise at the poles, the septum and throughout the cytoplasm while SecY was present in the membranes, although highest signals were observed at the poles. Substrates of the Sec machinery were localised at the septum and poles. The localisation of SecA is growth phase-dependent in both bacterial species. Upon secretion of some proteins by *L. lactis* they are covalently linked by sortases to the peptidoglycan. Electron- and immunofluorescent microscopic analyses showed that these proteins are primarily anchored at the septum, the site of newly synthesised cell walls. Components of the sorting machinery were located at the poles and septae.

Current research focuses on the localization of components of the Tat- (Twin-arginine translocation) machinery in *B. subtilis*, on co-localization of secretome components and on the dynamic nature of the protein translocation machineries.

Regulation of cell asymmetry in *Caulobacter crescentus*

Christine Jacobs-Wagner

Dept of Molecular, Cellular and Developmental Biology, Yale University, P.O. Box 208103, New Haven, CT 06520, USA

Cell polarity is one of the most basic principle in biology. The dimorphic bacterium *Caulobacter crescentus* provides a valuable, single-celled model system to probe the basic mechanisms of intrinsically-acquired cell polarity. In this crescent-shaped organism, cell polarity originates from no external cue and is morphologically apparent by the formation of appendages at a specific cell pole and

the asymmetric division that generates progeny of unequal size. These morphological manifestations of cell polarity are faithfully reproduced at each cell cycle. How polarity is achieved and re-established after cell division is largely unknown. We have discovered a global regulator of cell polarity, TipN whose normal function relies of its localization at the nascent poles during cell division.

Engineering a magnetic personality: biomineralization by magnetotactic bacteria

Dennis A. Bazylinski

Dept of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011, USA

Magnetotactic bacteria (MTB) are a diverse group of aquatic, motile prokaryotes whose swimming direction is influenced by the Earth's geomagnetic field. MTB biomineralize magnetosomes: intracellular, membrane-bounded, single-magnetic-domain crystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4) that are responsible for magnetotaxis. Magnetosomes appear to function as a means for the cell to efficiently locate and maintain an optimal position in chemically-stratified environments. MTB produce crystals of a specific size range, about 35-120 nm, which has physical significance. Crystals any smaller or larger than this size range show a reduction of magnetic remanence; thus the cell has maximized the magnetic remanence of the particle by producing crystals in this size range. Moreover, most MTB arrange magnetosomes in a chain, head-to-tail, along the long axis of the cell. In this motif, the chain behaves as a single magnetic dipole rather than a collection of individual dipoles thereby maximizing the magnetic remanence of the entire cell. Thus, the magnetosome is a masterpiece of microbial engineering. Little is known about the chemistry/biochemistry of magnetosome synthesis in MTB, the species-specific morphologies and compositional purity of the crystals and the precise arrangement of the crystals within the cell suggest that MTB synthesize magnetosomes via a biologically-controlled biomineralization process.

Bacterial membrane domains and cell cycle control

Itzhak Fishov

Dept of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

One of the most fascinating processes in a living cell, the cell division cycle, is tightly coordinated in space and time. In bacteria, the mechanism of the regulation of the cell cycle also directly involves the envelope, as the cells lack internal membranes and a cytoskeleton. It has recently become evident that specific DNA- and protein-membrane interactions are essential in the main bacterial cell cycle events – initiation of DNA replication, chromosome segregation and constriction/septation, leading to division. We consider membrane heterogeneity as the principal control in the regulation of bacterial cell cycle. Membrane domains created around rapidly replicating and transcribed DNA were proposed to serve for regulation of activity and assembly of key proteins at the right time and place. The advantage of this mechanism is that it provides a spatially localized signal for randomly distributed cytoplasmic proteins. In this talk, the existence, distribution and dynamics of membrane domains in bacteria will be reviewed in respect of both phospholipid and protein components. In addition, the role of membrane domains will be illustrated by the lipid-dependent properties of DnaA and MinD, proteins participating in temporal and spatial regulation mechanisms.

Secretion of autotransporter proteins at the bacterial pole

S.J. Jain, P. van Ulsen, I. Benz, M.A. Schmidt, R. Fernandez, J. Tommassen & **M.B. Goldberg**

University Park / Partners HealthCare, Cambridge, USA

Pathogenic bacteria interact with their surroundings via surface molecules. Autotransporter proteins are an extensive family of large virulence-associated proteins present in Gram-negative bacteria that are secreted to the bacterial surface. Secretion of such large proteins poses unique challenges to bacteria. We demonstrate that autotransporter proteins from a wide variety of rod-shaped Gram-negative pathogens are secreted at the bacterial pole. Further, an autotransporter of the spherically-shaped bacterium *Neisseria meningitidis* is secreted at distinct sites around the cell. Thus, secretion of large proteins likely occurs via specific pathways located at the pole of rod-shaped bacteria or at specific sites in spherically-shaped cells.

Raising the roof – aerial mycelium formation in *Streptomyces*

Mark J. Buttner[1], Marie A. Elliot[1,3], Shinya Kodani[2], Michael E. Hudson[3], Justin R. Nodwell[3] & Joanne M. Willey[2]

[1]Dept of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH; [2]Dept of Biology, Hofstra University, Hempstead, NY, USA; [3]Dept of Biochemistry, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada

A special feature of the developmental cycle of the filamentous soil bacterium *Streptomyces coelicolor* is the formation, at the start of differentiation, of an aerial mycelium. This aerial mycelium consists of specialized 'aerial hyphae' that grow upright out of the aqueous environment of the colony surface and into the air, imparting a characteristic white fuzzy appearance to the developing colonies. In order to break surface tension, *S. coelicolor* has to produce a surfactant peptide called SapB, and it has to coat its aerial hyphae in a hydrophobic sheath that is absent from the substrate hyphae. Although the existence of SapB and the hydrophobic sheath have been known for many years, until very recently both remained biochemically and genetically uncharacterized. We now know that the hydrophobic sheath is composed of 10 proteins, two 'rodlins' and eight 'chaplins', that assemble on the surface of the aerial hyphae into hydrophobic filaments that are covalently anchored into the cell wall peptidoglycan. Further, we have characterized SapB, shown that it is specified by the *ramCSABR* locus, and found that it is structurally and biosynthetically related to lantibiotics. Lantibiotics are ribosomally-synthesized oligopeptide antibiotics produced by Gram-positive bacteria that are translated as inactive prepeptides, which undergo extensive posttranslational modification before being cleaved to yield the mature peptide. This is the first example of a morphogenetic role for an antibiotic-like molecule.

The two flagella systems of *Aeromonas*: how does the polar flagellum know where to grow

Jonathan Shaw

Division of Genomic Medicine, University of Sheffield School of Medicine and Biomedical Sciences, Beech Hill Road, Sheffield S10 2RX

Aeromonads are aquatic bacteria that are able to cause disease in both poikilothermic animals and humans. Mesophilic *Aeromonas* strains swim in liquid through the action of a polar monotrichous flagella. However, upon contact with a solid surface, 60% of strains are able to produce an entirely distinct peritrichous-lateral flagella system for swarming over a surface. Approximately 45 genes encode the polar system and about 38 different genes encode the lateral system. The polar flagella system contains a number of genes not present in the lateral system these include *flhF*, *flhG* and *soj2*. The protein FlhF is a homologue of the GTPase signal recognition pathway protein FtsY, FlhG is similar to MinD an ATPase that oscillates from cell pole to cell pole during cell division and Soj2 shows homology to a series of chromosome partitioning ATPase proteins. All three genes are present in bacteria that place flagella at their poles. Very little is known about the role of these proteins, although in *Pseudomonas* an *flhF* knockout results in aberrantly placed flagella and a *flhG* mutant has multiple flagella, both proteins have been linked to flagella placement on the cell. Nothing is known about Soj2 in flagella biogenesis.

Subcellular organization in a minimal microbe: the attachment organelle of *Mycoplasma pneumoniae*

D.C. Krause

University of Georgia, USA

Mycoplasmas are cell wall-less prokaryotes considered to be among the smallest and simplest known cells capable of an independent existence. Their minimal genomes confer limited biosynthetic capabilities, necessitating a symbiotic relationship with a host species to meet their complex nutritional requirements. *Mycoplasma pneumoniae* is the leading cause of pneumonia in older children and young adults and a contributing factor in asthma. Despite a genome of only 816 kilobasepairs, *M. pneumoniae* possesses a complex, differentiated terminal organelle that functions in adherence to host respiratory epithelium, gliding motility, and cell division. This terminal organelle is characterized by an electron-dense core of twin parallel, flattened rods which are part of a mycoplasma cytoskeleton. Through mutant analysis proteins associated with this structure have been identified and localized, resulting in a model for binding partners and assembly sequence in terminal organelle duplication. This model lends itself to testing through analysis of recombinant green fluorescent protein fusions with terminal organelle components in *M. pneumoniae* by using digital time-lapse microcinematography. Despite the small size of mycoplasma cells it is possible to follow the incorporation of specific protein components during duplication of the attachment organelle and migration of nascent terminal organelles in dividing mycoplasmas.

Education & Training Group session

Working out? Bioscience placements and careers

Work experience – why bother?

Joy Perkins

School of Medical Sciences, Foresterhill, Aberdeen University, Aberdeen AB25 2ZD

Widening participation in Higher Education has resulted in an increase in the number of graduates seeking employment. It is now more important than ever for students to market themselves effectively. As employers recruiting graduates request an extensive list of skills and knowledge, those students with a CV that includes additional transferable skills obtained through work experience can really stand out from the crowd. These transferable skills include enhanced communication, numeracy, use of IT, group work and time management. Undertaking some form of work experience such as a sandwich placement as part of a degree also allows a student to experience workplace culture. Employers are increasingly pre-selecting students with prior work experience, but a period of work experience may become a student's passport to employability whether or not an employer considers a sandwich placement as central to their recruitment processes.

The relevance of work experience

Liz Rhodes MBE

Director, National Council for Work Experience

Mention the words 'work experience' and many people are dubious – having had variable experiences from both sides of the fence. However, there are those who have made it work for them. Speak to a graduate who has found a job following a rewarding work placement with a good employer and there is no doubt of the benefits to the individual as well as to the company. What is also becoming clear is that employers of all shapes and sizes are beginning to see offering work placement opportunities as an efficient means of recruitment: research conducted last year by the University of Manchester Careers Service shows that an average of 70% of work experience placements lead to a graduate job offer.

This session will look at the work NCWE has been doing with GSK to develop a Quality Mark for employers. It is intended that it will be awarded to employers who can demonstrate that they provide quality work experience/placements/internships in line with rigorous codes of practice and criteria. These have been carefully researched and confirmed by occupational and academic professionals to ensure that the standard is robust and that it contributes to positive developments in student learning, so contributing to their career and professional development.

Getting involved – a guide for academics

Martin Adams

University of Surrey

Running a professional training programme for undergraduate students can be a time consuming and occasionally exhausting task. It is also very rewarding, and when it comes to the various administrative jobs 'on offer' within an academic department it is arguably the most fulfilling. The transforming potential of a year of professional experience cannot be overstated and it is a privilege to see this at close hand year after year. Being a professional year tutor provides an opportunity to establish and maintain contacts with a wide range of industries and research institutes and this can help inform and influence both your teaching and research. It also allows you to get to know many students a lot better than you might otherwise have done.

Being an intermediary between employer and student it is not without its hazards. You need to perform a balancing act between securing placements and having enough good students to fill them. Student expectations of potential placements can often be wildly unrealistic and instilling a little realism rarely leads to popularity. Similarly it is occasionally necessary to encourage employers to take greater responsibility for the training part of their role. When it goes well, as it does in the vast majority of cases, it is unquestionably beneficial to all involved.

Enhancing the employability of bioscience students

Ian Hughes

Bioscience Centre, Higher Education Academy and School of Biomedical Sciences, University of Leeds, LS2 9JT

Employability is important because: it is a government priority; it reflects in university performance indicators/funding; it is of increasing significance to prospective students in their choice of course/university; and having employability enables our graduates to compete within the UK, EU and globally for graduate-level employment in the current transient and changing work environment. Employability should be embedded throughout a degree programme and should enable students to compete successfully for graduate jobs which may or may not be related to the discipline in which they are taking a degree. There is a variety of ways in which the employability focus of a degree programme can be increased. For example, surveys of employment needs as perceived by students or by recent graduates, employer involvement in course design, audits (of employability, placements, work related learning, ethics), benchmark statements, external examiners, card sorts and the use of employer appraisal materials for new graduate employees. These all provide ways of identifying and implementing employability enhancing changes to degree programmes and help is available to assist teachers to obtain and use these methods within their courses. Changes may be relatively easy to achieve, are not necessarily resource intensive and are in any case often simply good teaching.

ASET: work placement and employability in biosciences

Tony Waite

ASET Executive Committee; Work Placement Officer-Biosciences, Brunel University

ASET is the national body for work-based learning practitioners. Its mission is to promote and support the concept of higher education programmes that integrate periods of academic study with periods of relevant work experience in an employing organisation. The integration of work and learning and the development, promotion and implementation of best practice in this area is the primary focus of ASET.

The presentation will give an overview of ASET's work with particular emphasis on the expanding and changing work experience scene.

There will also be a specific focus on employability in Biosciences with a review of the benefits of work placements in this area at Brunel University.

A well established 12-month placement scheme has been in operation for many years for students following the sandwich course pattern with many employability benefits. The Department recognising the need to develop employability skills for students following the full-time course has recently introduced a 'credit-rated' summer vacation placement.

Human resources departments and selection processes

A.G. Buchanan

Eli Lilly & Co Ltd, Basingstoke

Abstract not received

Assessment of placement-based learning using a negotiated, reflective e-portfolio

Lynne Lawrance[1], Collette Allen[2], Elisabeth Thorne[2], Andrew Tuck[2], David Lush[1] & Stephen Gomez[1]

[1]Faculty of Applied Sciences (FAS), University of the West of England, Bristol BS16 1QY; [2]Health Protection Agency Laboratory, Southampton General Hospital, SO16 6YD

Sandwich placements within degree programmes have traditionally received only notional credits. FAS recently devised a novel scheme which awards 20 Level 3 credits for work-experience. Placement students must evidence their learning, and this is facilitated through the use of a web-based electronic portfolio (Profile: www.profile.ac.uk). Students negotiate their learning with work supervisors, formulate a learning agreement with their university tutor in terms of 'tasks' equivalent to final year knowledge and equating to 200 learning hours. Students upload files to the e-portfolio evidencing their learning and complete a reflective self-evaluation of the skills and knowledge gained in completing the task. Work supervisors and academic tutors confirm that tasks have been completed satisfactorily or request further work. Throughout the placement, a 'conversation' is maintained across the internet to support student learning. The Profile system provides a flexible platform that can be configured to suit the establishment's requirements and can be applied to other functions such as personal development planning.

Employability and career planning for postgraduates

Peter Fantom

Careers and Appointment Service, University of Aberdeen, AB24 3UX

Employability is often in the limelight, as universities are required to demonstrate how they are enhancing the employability of their graduates & postgraduates. Employability encourages students to reflect on their wider university experiences, as well as their subject knowledge, and helps them identify skills they have and areas which they need to develop.

Peter Fantom the presenter has more than a decade's experience as a Careers Adviser at Aberdeen University. He is also the Editor of a national careers publication, *'What do Graduates Do?'* Peter's workshop aims to draw on experience and resources developed nationally in Scotland during 2004 and 2005. Employability was chosen by the Scottish Higher Education Funding Council (SHEFC), as one of the Enhancement Themes for 2004.

Peter's workshop will give practical advice on how to set realistic and achievable career goals, how to exploit the attractiveness of generic skills to potential employers inside and outside of academia, and find out what other resources and expertise you can access on and off campus to help you achieve the career you want!

Fermentation & Bioprocessing Group / Biochemical Engineering Subject Group of the Institution of Chemical Engineers joint session

Biopharmaceuticals: from conception to production

Biopharmaceuticals: prospects and challenges

Tony Bradshaw

BioProcess UK, London (Email tbradshaw@bioindustry.org)

Moving a biopharmaceutical from early discovery through to the end of clinical trials is a long, fraught and expensive business. Manufacturing even the least sophisticated biological medicine is fundamentally different, and far more complex, than manufacturing small molecule pharmaceuticals. The UK bioprocessing sub sector is significant and economically important, both in its own right, as a high value manufacturing sector, and as a critical component of the biosciences sector overall.

Recognising this the BioIndustry Association (BIA) has been awarded DTI funding totalling £3 million over 4 years to establish a National Knowledge Transfer Network, bioProcessUK, following its bid to the DTI technology programme. The funding follows the recommendation by the BIA/DTI/DH-led Bioscience 2015 report that the UK builds a strong bioprocessing sub-sector (www.bioindustry.org/bigtreport). The first step will be to foster community development, harnessing the expertise in the country to deliver an internationally competitive sector. The outputs of bioProcessUK will be to:

- Develop young scientists and bioprocess leaders in the field of bioprocessing
- Benchmark UK bioprocessing
- Develop a clear and increasing profile for UK bioprocessing
- Support the development of the UK's bioprocessing research & knowledge base
- Develop a consolidated viewpoint on what the UK Government could do to assist bioprocessing

New products: conception and discovery

TBC

The cell as a factory

Ray Field

Director of Cell Sciences, Cambridge Antibody Technology, Milstein Building, Granta Park, Cambridge CB1 6GH

Recent developments in production of recombinant protein biopharmaceuticals from both prokaryotic and eukaryotic cells, have allowed increased yield and scale of operation. Often the complexity of the expressed protein drives the choice of an appropriate scalable cell system. For example, in the case of recombinant human antibody therapeutics, advances in mammalian cell expression and processes have led to increased antibody expression titres, scale of operation, and reduced development timescales. Furthermore, use of chemically defined animal component free culture medium has reduced concerns over potential adventitious contaminants, such as prions. Genetic improvement of the capability of mammalian cells to carry out post-translational modifications has also delivered antibodies with improved potency. Conversely, advances in microbial cell production of antibodies also promises improved post-translational modifications of such complex proteins, coupled with a track record of operation of microbial processes at large scale.

The best route to the effective use of the cell as a factory for protein biopharmaceutical production will depend on specific external and internal technical and commercial drivers for a protein therapeutic. These points will be illustrated with examples from both our own work and current literature.

Product purification

R. Francis

Protherics plc, London

Abstract not received

Millilitre and microlitre scale approaches to speed bioprocess development

Gary J. Lye

Dept of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE (Email g.lye@ucl.ac.uk)

Process development for biologics often distinguishes itself from that of other products by the high frequency of unpredicted and costly events during development and scale-up. This unpredictability derives from the complexity of the product molecules themselves and is likely to increase in the foreseeable future. This presentation will give an overview of ultra scale-down (USD) techniques and how they can be used to identify processing issues early and with the minimum quantity of material. The USD approach involves first generating a fundamental understanding of the nature of the target molecule and the chemical and engineering environment it experiences at all process scales. Experimental data is obtained rapidly in USD mimics of individual unit operations operating at the 10-20 ml scale. This is then used to inform process models to determine operating ranges in which unit performance can satisfy predetermined targets. The presentation will finish with recent work illustrating how data collection is now possible at the microlitre scale (200-1000 μl) and in a parallel manner by the application of laboratory robotics to perform linked sequences of bioprocess operations.

Process analysis

Kym Baker, Imtiaz Alam & Steve Flatman

Lonza Biologics plc, 228 Bath Road, Slough, Berkshire SL1 4DX

The application of the appropriate test methods is imperative to support a product through its lifecycle of process development and validation. The first step is to identify those assays which are required to enable effective process development and process validation studies and those which are critical process assays for manufacturing, including quality control testing. The assay development and assay validation needs will vary for each of these different process requirements and depending on the clinical stage of the product being manufactured. Example case histories will be used to demonstrate the approach for each of these scenarios. These will include in-process test methods aimed at both the fermentation cell culture process and the downstream purification process as well as example final product release tests.

Biopharmaceutical business development

N. Gostick

Nottingham BioCity

Abstract not received

Creating effective research and development facilities

David Sutton

Bovis Lend Lease Technologies, Tanshire House, Shackleford Rd, Elstead, Surrey GU8 6LB (Email david.Sutton@lendlease.co.uk)

Presentation content Organisations undertaking R&D face increasing pressures generated by the rate of change in the scientific and business world. Many existing R&D facilities struggle to adapt to new technologies and processes together with the evolving requirements of management, funding institutions and customers. Organisations are also under pressure to increase R&D productivity with fewer staff and reduced budgets.

This presentation explores how we can rise to this challenge and create R&D facilities that are 'future proof', can boost research productivity and have a reduced whole life cost.

Background *David Sutton* is Director of Design at Bovis Lend Lease. He is an architect and urban designer by training with 20 years experience designing R&D facilities.

Bovis Lend Lease Technologies are a specialist company who provide consultancy, design, construction and regulatory compliance services to the life science, pharmaceutical and healthcare industries. Bovis Lend Lease has offices through out the UK and over 90 offices globally.

Regulatory overview, biopharmaceutical manufacture

Roy Cowell

Quality Control, Cobra Biomanufacturing plc

Regulations, relating to the manufacture of pharmaceutical products, are in place to protect the patient. Adverse drug reactions and deaths, in the EU and US, identified the need to ensure that pharmaceutical manufacturers show due diligence when determining the safety profile of new medicines, and assuring that manufacturing processes are compliant with procedures designed to ensure the safety quality and efficacy of the product. Compliance with Good Manufacturing Practices is not prerequisite for process development but must be applied where facilities may be used for GMP activities. Compliance with GMP, where supporting data may be generated for early regulatory scrutiny, is best practice. Impact on the business arises from the costs associated with maintenance of GMP and Quality Assurance. An effective Quality Assurance function, managing a functional quality system, cannot be undervalued and may only be missed following regulatory inspection. Marketing authorisations for Biopharmaceutical products are issued through the European centralised procedure as required by EU directives and regulations. Applications must demonstrate safety, quality and efficacy for the product and there can be significant challenges presented by the complex upstream and downstream processes associated with the manufacture of biopharmaceutical products.

Clinical trials

J. Makin

Microscience, Wokingham

Abstract not received

Clinical trials production

S. Routledge

Eden Biodesign, Ellesmere Port

Abstract not received

Validation of public/private finance pharmaceutical projects – a case study of the national biomanufacturing centre

A. Jones

SembCorp Simon-Carves Ltd, Cheadle Hulme

The National Biomanufacturing Centre (NBC), which is currently being constructed in Speke, Merseyside, is set to become Europe's leading biopharmaceutical design centre, capable of working with small to medium biotech enterprises to develop and manufacture a wide variety of novel biopharmaceutical medicines for early phase clinical trials.

This project is a significant first in that it is backed by a combination of public funding from the Government and the European Community, and private finance. Consequently the NBC implementation has required the adoption of project structures, methods and interfaces that don't mirror the usual EPCmV / Client construction model.

A vital part of the success of this project is the robust and cost effective delivery of validation to support a multi product / multi client operational facility capable of meeting a broad range of end user needs and attaining regulatory approval.

The presentation will review the validation approach adopted for the NBC project specifically focusing on:

- Validation input into the Basis of Design
- Co-ordination of validation activities between the multi interface project team
- The Design Qualification process
- The use of vendor qualification to support the validation effort
- The role of the Independent Validation Consultant
- Experience that may benefit future public/ private funded projects

The National Biomanufacturing Centre is publicly funded by the North West Development Agency, the European Regional Development Fund and the Department of Trade and Industry.

Production facilities

A. Besso

Jacobs Engineering, Reading

Abstract not received

Production of licensed biopharmaceuticals

Kevin Owen & Mike Harvey

Ipsen Biopharm Ltd, Ash Road, Wrexham Industrial Estate, Wrexham LL13 9UF

Manufacturing's goal is to get the product to the marketplace and keep it there, a complex task in a regulated environment. Many different authorities can audit the plant and process at varying time points as part of pre approval authorisation, and during routine cGMP inspections. The speed of approval for a drug (or for licence variations) into a country can vary dramatically, from instantaneous to many months. Judicious management of inventory can be crucial during this time. It is important to fix the process / site of manufacture / scale of the operation as early as possible to limit post marketing approval changes. However, compliance to process change management can be difficult when dealing with complex biologicals. Maintenance of plant and documentation thereof is essential to maintain a compliant site, as is change control monitoring. Monitor the overall quality of the process and maintain the capacity to achieve it. Trend data to avoid phase shift with time. Be mindful of marketing forecast and try to build in surplus capacity in process. Overall, delay to the marketplace is loss of revenue. Perform risk analysis to establish if spending extra money can be offset against shortening time frame and decreasing risk exposure.

Food and Beverages Group session

Aspects of food spoilage: microbial activity in foods

Can food spoilage be predicted?

Jane Sutherland

Food Microbiology Unit, Dept of Health and Human Sciences, London Metropolitan University, 166-220 Holloway Road, London N7 8DB

Systematic data representing growth of food spoilage micro-organisms were generated using techniques such as viable counting, measurement of enzyme production and activity, accumulation of volatile organic metabolites and changes in absorbance measured using FTIR. Groups of spoilage organisms investigated were *Pseudomonas* and related genera, Enterobacteriaceae, *Brochothrix thermosphacta*, lactic acid bacteria, yeasts and *Bacillus* spp. Each group was inoculated as a cocktail of strains into microbiological media encompassing a range of environmental conditions representative of chilled foods: 2-20ºC; pH 4-7.5 and water activity 0.95-0.99. As microbial growth progressed, estimates were made of viable counts, lipase synthesis, protease activity, volume of CO_2 evolved, accumulation of specific volatile metabolites (measured as peak areas on a chromatogram) and changes in absorbance. The data were modelled and the models verified and validated in relevant foods. Viable count models for pseudomonads, Enterobacteriaceae, *Brochothrix thermosphacta* and yeasts provided reliable and consistent predictions; those for lactic acid bacteria and *Bacillus* spp. were less reliable. Models based on accumulation of volatile metabolites were prepared for *B. thermosphacta* and pseudomonads. The enzymatic data were not straightforward to model and alternative approaches are under investigation. Models based on CO_2 accumulation and absorbance changes could be prepared for growth only at higher temperatures.

Studying microbial activities in the food matrix

Cath Rees & Chris Dodd

Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leics LE12 5RD

While many studies of the physiology and activity of food borne organisms have been undertaken using lab cultures, the data obtained are not always representative of the situation in real foods due to the complexity of the food matrix. Hence microbiologists need to employ methods that will allow investigations to be undertaken without separating the organisms from the food. A range of such techniques have been developed, from sectioning and microscopic analysis, to the use of genetically engineered reporter strains. More recently DNA-based methods for metagenomic studies of populations have been applied to food systems. This talk will review the development of techniques for application to foods and describe current research illustrating how these approaches provide information that cannot be gained by traditional microbiological methods.

Rapid detection method for microbial spoilage using FT-IR Spectroscopy and machine learning

D.I. Ellis, D. Broadhurst, D.B. Kell, J.J. Rowland & R. Goodacre

University of Manchester, M60 1QD

The requirement for real-time monitoring in the modern and highly automated food processing environment has stimulated research into rapid microbiological testing. The conventional microbiological approach to food sampling has changed little over the last half century and it has been estimated that there are currently in excess of 40 methods to measure and detect bacterial spoilage in meats. The ideal method for the on-line microbiological analysis of meat would be rapid, non-invasive, reagentless and relatively inexpensive and these requirements can be met via the application of a spectroscopic approach, in combination with any appropriate data deconvolution strategy based on statistics or machine learning.

Fourier transform infrared (FT-IR) spectroscopy is a rapid, non-invasive technique with considerable potential for application in the food industry. We have shown that this technique can be used directly on the surface of food to produce biochemically interpretable 'fingerprints'. Microbial spoilage in meats is caused by the growth and enzymatic activity of micro-organisms and FT-IR was exploited to measure changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage.

Quantitative interpretation of FT-IR spectra was possible using partial least squares regression and allowed accurate estimates of bacterial loads to be calculated directly from the meat surface in 60 s. Genetic programming was used to derive rules showing that at levels of 10^7 $CFU.cm^2$ the main biochemical indicator of spoilage was the onset of proteolysis. Thus, using Fourier transform infrared spectroscopy (FT-IR) and machine learning we were able to acquire a metabolic snapshot and quantify, non-invasively, the microbial loads of food samples accurately and rapidly in 60 s, directly from the sample surface.

Microbial cell signalling during food spoilage

Lone Gram

Danish Institute for Fisheries Research, Dept for Seafood Research, Søltofts Plads, c/o Technical University of Denmark, Bldg. 221, DK-2800 Kgs. Lyngby, Denmark

It is estimated that between ten and 50% of all foods are lost *post harvest* due to microbial growth and activity. Understanding microbial food spoilage is a multidiciplinary task and is required to provide scientific basis for better preservation methods. In this paper some basic concepts in microbial food spoilage will be defined and discussed and the paper will focus on factors influencing microbial spoilage activity. Microbial spoilage is caused by growth of micro-organisms to high numbers and typically manifests itself as slime (exopolysaccharides or degraded polymers), gas formation or as off-odours and off-flavours. The spoilage of some foods is not just a function of cell biomass but is a complex process where the microbial spoilage processes are regulated by bacterial communication signals such as acylated homoserine lactones (AHLs). These molecules allow the bacterial population to sense its own density and express certain phenotypes only at high cell densities. AHL molecules are found in many spoiling foods and are produced by many food spoilage bacteria. However, only in a few products, does this type of regulation influence the degradation of the product. Such understanding of spoilage processes and their regulation may allow development of more targeted, and often milder, food preservation techniques.

Factors affecting microbial exoenzyme production in foods

P.G. Braun

Leipzig University, Veterinary Faculty, Institute of Food Hygiene, An den Tierkliniken 1, 04103 Leipzig, Germany

In particular with food of animal origin substantial product deteriorations are attributed to the effect of extracellular microbial enzymes. Little is known about their synthesis and activity as affected by intrinsic (e.g. pH, water activity) and extrinsic (temperature of storage) factors in food and it is important in consideration of spoilage to examine interactions between microbial growth and enzyme activity.

The aim of our investigations, funded by Deutsche Forschungsgemeinschaft and the EU-FAIR CT98-4083: Predictive modelling of food spoilage, was to collect and/or model data on

the impact of a three dimensional matrix of temperature, pH and water activity on synthesis and activity of proteases and lipases of selected spoilage organisms.

To generate data on enzymatic activity a modified enzyme assay (agar-diffusion-method) was used with different substrates such as casein, gelatine, tween or tributyrin, adjusted to the defined pH-values and water activities. To measure synthesised lipases, the Reflektoquant Lipasetest (Merck) was used which is based on the conversion of Br,Cl,-indoxylcaprylate by lipases to form a blue dye, the concentration of which is determined reflectometrically.

Data are available as databases and two mathematical models on enzymatic activity (`predictive enzymology`) were developed using the curve of Baranyi which have been verified and validated in milk. Most interesting findings will be introduced and discussed.

Research investigations of microwave-generated UV light as a biocide

Meriel G. Jones[1], Jeffrey D. Cullen[2] & Ahmed I. Al-Shamma'a[2]

[1]School of Biological Sciences, University of Liverpool; [2]Depts of Electrical Engineering and Electronics, University of Liverpool

The food industry is keen to have new techniques that improve the safety and/or shelf life of food products without the use of preservatives. There is considerable interest in developing UV light and ozone (O_3) treatments to enhance shelf life. We have designed a microwave radiation device that is a novel source of germicidal UV and O_3 suitable for the food industry, which may offer speed, cost and energy benefits over existing sources. It differs significantly from conventional UV lamps because there is no warm up time, no deterioration with age, the shapes are adaptable and it offers the possibility of pulsed UV light and therefore of flash sterilisation. One of the drawbacks of using germicidal UV light alone is that it does not work in shadowed areas. However, some products, such as sliced meat, present a flat surface, lending themselves to UV treatment. We have monitored the effectiveness of design and operational parameters on UV output. We have also tested the microbiocidal effectiveness of the device using agar plate colony counts of laboratory strains of *Escherichia coli* and fungi, enumerating the number of viable cells that can be recovered after exposure to UV/O_3.

Determination of factors affecting the photoreactivation of *Listeria monocytogenes* following exposure to pulsed UV-rich light

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

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

Photorepair (photoreactivation) of micro-organisms is an important issue when UV light is used for disinfection purposes. Photoreactivation is a DNA repair process that takes place when cells that have been exposed to, and damaged by, UV light are subsequently exposed to light of a longer wavelength. Although this process has been examined in a number of micro-organisms after their exposure to continuous UV light there is no information available in the literature concerning the effect neither of pulsed UV light nor on the ability of *L. monocytogenes* to exhibit photorepair after UV light damage. In order to address these issues four factors were compared in this study on *L. monocytogenes* these being; (i) the effect of the initial dose of pulsed UV light (4 and 6 pulses); (ii) the effect of post exposure conditions on repair/recovery (light or/and dark repair); (iii) the effect of light intensity during photorepair (high intensity versus low intensity light); and (iv) the influence of growth phase (exponential and stationary phase) prior to treatment. The results demonstrate that *L. monocytogenes* exhibits light repair if the organism is exposed to longer wavelength light following the initial damaging exposure to pulsed high intensity UV-rich light.

Gene activities and metabolism involved in beer spoilage

Rudi F. Vogel & Jürgen Behr

Technische Universität München, Germany (Email rudi.vogel@wzw.tum.de)

The resistance to hop acids is a major prerequisite of beer spoiling lactobacilli to grow anaerobically at low pH in the presence of ethanol and hop acids. Their response to a challenge with hop and low pH was characterized aiming at function based detection systems The cytoplasmic and membrane bound proteomes of the highly hop resistant beer-spoiling isolate *Lactobacillus brevis* TMW 1.465 were compared by 2-D gel electrophoreses at the exponential growth phase at pH 6.0, at pH 4.0 and at pH 4.0 in the presence of 86 µMol/L isohumulone. More than 30 proteins were over expressed more than 1.5fold in the presence of hop. Some of the hop inducible proteins could be sequenced and identified while others were blocked or delivered unkown sequences. Respective genes were identified by reverse genetics and the hop resistance mechanisms were characterised on biochemical level. The so far characterized hop resistance mechanisms include membrane transporters HorA and HitA, enzymes of the arginine deiminase pathway and cell wall modification. The expression analysis of hop resistance genes measured on the cDNA level delineated differences in acid and hop response. While horA and hitA.are little inducible arcB and arcC were extensively overexpressed under hop stress conditions.

Adaptive changes in foodborne micro-organisms exposed to preservative conditions: implications in food processing and safety

Nicholas J. Russell

Dept of Agricultural Sciences, Imperial College London, Wye Campus, Wye, Ashford, Kent TN25 5AH

Foods, particularly those that are highly processed, present a challenge to microbial growth due to the presence of inhibitory compounds or the use of other preservative measures such as modified gas atmospheres. Nonetheless, a very wide range of micro-organisms are able to grow in and spoil food, including bacteria, yeasts and fungi, but only a few are capable of causing illness in the consumer.

This talk will review adaptive biochemical changes in microbial structure and function that facilitate growth in a range of preservative regimes, including the use of solutes to lower water activity, addition of weak acid preservatives to lower pH, mild heat treatment to lower microbial loading and refrigeration to slow growth. The interrelationship between responses and coordination of membrane with intracellular changes will be discussed. This information will then be set in the context of food safety by considering how processing could trigger changes that paradoxically might lead to a foodborne population better able to spoil or even poison food once it reaches retail outlets.

Physiology, Biochemistry and Molecular Genetics Group / Microbial Infection Group / Eukaryotic Microbiology Group joint session

Microbial post-translational modification

Applications of glyco-proteomic and glycan-screening strategies in bacterial glycobiology

Anne Dell, Paul Hitchen, Maria Panico, Stuart Haslam & Howard Morris

Imperial College London, Division of Molecular Biosciences, Faculty of Life Sciences, Wolfson Building, South Kensington Campus, London SW7 2AZ

The information generated from the initial sequencing and secondary interrogation of bacterial genomes is changing the face of prokaryotic glycobiological research. It is now established that protein glycosylation occurs in bacteria and whilst analysis of genomic data is revealing many of the genes in glycosylation pathways, structural knowledge of the ultimate products of genes implicated in glycoprotein biosynthesis is still limited. Our laboratory is addressing this problem by performing detailed mass spectrometric structural characterisation. In particular we are defining the glycans present on individual glycoproteins and sites of glycan occupancy by application of glycoproteomic strategies. Our studies exploit nanospray and on-line nanoLC-ES-MS/MS technology, complementing our MALDI-MS glycan-screening technology. These methodologies have yielded important new information on a variety of novel bacterial glycoproteins and other post-translational modifications (Wacker *et al*., *Science* 2002. Hegge *et al*., *Proc Natl Acad Sci USA* 2004, Feldman *et al*., *Proc Natl Acad Sci USA* 2005) and have proven invaluable in elucidating the roles of gene products involved in these glycosylation pathways. Our strategies will be exemplified by some of our recent collaborative research on glycoproteins from *Campylobacter jejuni* and *Neisseria gonorrhoeae*.

Campylobacter – a tale of two prokaryotic glycosylation systems

Brendan W. Wren

Dept of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT

Campylobacter jejuni is the primary cause of human bacterial gastroenteritis worldwide and can lead to post infection neurological complications due to molecular mimicry of surface structures. This has been the driving force for the genetic characterization of *C. jejuni* strain NCTC11168 over the past few years. Remarkably for a bacterium with a limited genetic repertoire (1.6 Mb), *C. jejuni* has both *O*- and *N*-linked glycosylation systems.

The *O*-linked system involves the addition of pseudaminic acid and/or pseudaminic acid derivatives to the flagellin and is genetically highly heterogeneous between strains. The *N*-linked general glycosylation pathway modifies over 30 *C. jejuni* proteins by the addition of an invariant bacillosamine heptasaccharide glycan. The *C. jejuni* general glycosylation pathway is the first confirmed *N*-linked glycosylation system identified in the bacterial kingdom. Recently, the *N*-linked glycosylation has been transferred into *E. coli* opening up the possibility of producing recombinant glycoproteins and of glycoengineering.

This lecture will review the genetic and structural characterization of the *O*- and *N*-linked glycosylation pathways and will speculate on their role as a pathogen and commensal. Additionally, the glycosylation pathways will be described as models that may facilitate our understanding of *O* and *N*-linked glycosylation systems in eukaryotic organisms.

Towards an anti-adhesive vaccine for *Burkholderia pseudomallei*

Angela E. Essex-Lopresti[1], Collette Johnson[1], Chuk Hai Tsang[1], Lucille Rainbow[2], Kwanjit Duangsonk[2], Craig Winstanley[2], Jim Hill[1] & Richard W. Titball[1]

[1]Dstl Biomedical Sciences, Porton Down, Salisbury, Wiltshire; [2]Dept of Medical Microbiology and Urinary Medicine, Duncan Building, University of Liverpool, PO Box 147, Liverpool L69 3BX

Burkholderia pseudomallei is the causative agent of melioidosis, a severe and fatal spectrum of infectious diseases for which there is currently no licensed vaccine. Bacterial adhesion, an important step in most infectious disease processes, requires the specific interaction of surface proteins (adhesins) with host cell receptors. The genome of *B. pseudomallei* K96243 contains homologues to several adhesins, including genes that could be involved in type IV pili biogenesis. Type IV pili adhesins are widespread amongst pathogenic Gram-negative bacteria, and are being evaluated for use as vaccines against several different organisms, including the closely related *Pseudomonas aeruginosa*. *In vitro* and *in vivo* studies on a type IV pilin subunit homologue, PilA, show it is important for adhesion and virulence in models for melioidosis.

In this study PilA was produced as a Maltose Binding Protein (MBP) fusion and tested as a vaccine in a mouse melioidosis model. MBP-PilA did not protect mice against *B. pseudomallei* challenge. PilA folding and glycosylation status might be important for vaccine efficacy, as might the vaccine delivery method. Current work has focussed on determining the glycosylation state of type IV pilus proteins, refolding recombinant pilA and alternative vaccine delivery systems.

The structure and biosynthesis of trypanosome glycoproteins: targets for drug discovery

Michael A.J. Ferguson

Division of Biological Chemistry & Molecular Microbiology, Wellcome Trust Biocentre, University of Dundee, Dundee DD1 5EH (Email m.a.j.ferguson@dundee.ac.uk)

The African trypanosomes are responsible for human African Sleeping Sickness and Nagana in cattle. These parasites survive and multiply in the bloodstream of individuals infected by the bite of a tsetse fly by virtue of a protective cell surface coat made of tightly-packed glycosylphosphatidylinositol (GPI) anchored variant surface glycoproteins (VSG). The parasite also expresses many other glycoproteins, some of which contain unusually large N-linked oligosaccharides. Recent progress using genetic, chemical and computational approaches to understanding and manipulating protein glycosylation in trypanosomes will be presented.

Flagellar glycosylation systems of bacteria

S.M. Logan

Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada

The flagellin protein is the major structural component of an important surface appendage of prokaryotes, the flagellum. Glycosylation of this protein has recently been described, most notably for a number of

bacterial pathogens. While the biological significance for this process remains ill defined, the biosynthetic pathway offers numerous targets for the development of novel therapeutics. We have identified the novel O-linked glycan structures on flagellin from the bacterial pathogens *Campylobacter jejuni* and *Helicobacter pylori* and have shown that both flagellins are glycosylated with the novel 'sialic acid-like' monosaccharide, pseudaminic acid and related derivatives. We have characterized the enzymes involved in the Pse biosynthetic pathway in both organisms and recently solved the structure of a key biosynthetic enzyme. In addition, we have initiated studies to define the mechanistic basis of the O linked glycosylation process through localization studies as the process appears to be uniquely restricted to the flagellar system suggestive of subcellular compartmentalization. Recently, we have expanded our work on flagellar glycan structural analysis to other bacterial pathogens as well as to the Archaea and can now report on the diversity of glycan structures and linkage types found amongst these species.

Glycosylation of surface layer (S-layer) proteins

Kerstin Steiner, René Novotny, Marc Giry-Laterriere, Sonja Zayni, Andera Scheberl, Christina Schäffer & **Paul Messner**

Universität für Bodenkultur Wien, Zentrum für NanoBiotechnologie, A-1180 Wien, Austria

Cell surface layers (Slayers) have been described for all major phylogenetic groups of bacteria, which may indicate their pivotal role for an organism in its natural habitat. Glycosylation represents the most frequent modification of Slayer proteins. Slayer glycoproteins constitute a class of glycoconjugates first isolated in the mid 1970s, but Slayer glycoprotein research is still in its infancy, possibly because of its 'non-eukaryotic' character. Extensive work during the past 30 years provided evidence of an enormous diversity of Slayer glycoproteins that have been created in nature over three billion years of prokaryotic evolution. These surface-located polysaccharides contribute to an enormous diversification potential of the bacterial cell surface. The S-layer glycoproteins are substantially different from eukaryotic glycoproteins, with regard to both, composition and structure; nevertheless some general structural concepts may be deduced. There is also a difference between archaeal and bacterial S-layer glycoproteins which seems to follow the proposal of a closer phylogenetic distance of archaea to eukaryotes.

These protein modifications add a high application potential to Slayer glycoproteins in the field of modern nanobiotechnology as a base for glycoengineering which recently led to investigations of the Slayer protein glycosylation process at the molecular level. From that work an even more interesting picture of tailored Slayer neoglycoproteins for applications as a nanoscale carrier system in biotechnology and biomedicine is emerging.

Acknowledgements This work is supported by grants from the Austrian Science Fund (projects P15840B10 and P18013B10)

The role of glycoconjugates in the infectious cycle of the protozoan parasite *Leishmania*

Stephen M. Beverley, Althea Capul, Kai Zhang & Salvatore J. Turco

Dept of Molecular Microbiology, Washington University School of Medicine, St Louis, MO 63105, USA (Email beverley@borcim.wustl.edu)

The trypanosomatid protozoan *Leishmania* expresses a diverse array of glycoconjugates on its surface throughout the infectious cycle, many of which have been implicated in critical steps essential for pathogenesis. These include lipophosphoglycan (LPG), a polymer of phosphoglycan (PG) $[Gal\text{-}Man\text{-}P]_n$ repeating units attached to the surface through a heptasaccharide glycan core and glycosylphosphatidylinositol (GPI) anchor, PG-modified and/or GPI-anchored proteins such as proteophosphoglycan (PPG) and GP63 (leishmaniolysin), free GPIs termed glycosylinositol phospholipids (GIPLs), and inositolphosphoceramides (IPCs). LPG expression is restricted to the promastigote stage carried by the sand fly vector, while the remaining molecules are expressed more or less constitutively and in the amastigote stage which resides within mammalian macrophages. Forward and reverse genetic tools were developed for identifying activities implicated in steps essential for the assembly of these glycocoinjugates. These yielded genes affecting nucleotide sugar biosynthesis (UDP-galactofuranose; *GLF*), glycosyltransferases encoded by the *LPG1* and *LPG4* genes, chaperones such as *LPG3*, nucleotide-sugar transporters encoded by the *LPG2* and *LPG5A/B* genes, and ether phospholipid (*ADS1*) and sphingolipid biosynthesis (*SPT1/2*, *SPL*). From these studies we have now developed a clearer picture of how the parasite surface is assembled, and how each of these molecules and/or glycoconjugate domains contributes singly or in association with others to the ability of the parasite to complete its infectious cycle.

Glycosylation of the AIDA-I autotransporter adhesin

Inga Benz, Micha Feld & **M. Alexander Schmidt**

Institut für Infektiologie – Zentrum für Molekularbiologie der Entzündung (ZMBE), Westfälische Wilhelms-Universität/ Universitätsklinikum Münster, Von-Esmarch-Str. 56, D-48149 Münster, Germany (Email infekt@uni-muenster.de)

Protein glycosylation in prokaryotes has become an established fact. Interestingly, especially virulence factors have been identified as glycoproteins in numerous pathogenic bacteria. However, detailed information about glycosylation patterns, the glycosylation machineries involved, and structure-function relationships are not available and, furthermore, the putative functions of glycosylation in pathogenesis are not fully understood.

The AIDA (adhesin-involved-in-diffuse-adherence) autotransporter has been originally described in the clinical *Escherichia coli* diarrhoea isolate 2787 (O126:H27). The 'passenger' AIDA-I adhesin is the first protein to be post-translationally modified by heptose residues. AIDA-I is covalently modified with – on average – 19 heptose residues which appear to be essential for stability and/or function. This unique protein modification is mediated by a novel glycosyltrans-ferase termed autotransporter adhesin heptosyltransferase (AAH). AAH can be functionally replaced by the TibC protein of enterotoxigenic *E. coli*. The AAH enzyme is encoded immediately upstream of the *aid*A gene coding for the AIDA autotransporter and is always associated with functional autotransporter adhesins of the AIDA family. Previously, all heptosyltrans-ferases (HepI-III) described are involved in the coordinated and highly specific assembly of heptose residues in the inner core of LPS. Interestingly, the AAH protein shows only limited sequence similarities to the homologous HepI-III but appears to be structurally related.

Glycosylation of the Arg gingipains of *Porphyromonas gingivalis*

Mike Curtis

Institute of Cell and Molecular Science, Barts and the London Queen Mary's School of Medicine and Dentistry

The Arg-gingipains (Rgps) of *Porphyromonas gingivalis* are a family of extracellular cysteine proteases with specificity for arginyl peptide bonds and are important virulence determinants of this periodontal bacterium. The maturation pathway of these enzymes leads to the generation of a range of isoforms including a heterodimer and several distinct monomeric species comprising the catalytic chain variably glycosylated with 15-30% (w/w) carbohydrate. Glycosylation of these enzymes influences both their stability and their recognition by the immune system of patients colonized by this organism and hence may play a role in the virulence of the bacterium in periodontal disease. The glycans attached to the Rgps are predominantly O-linked but are highly heterogeneous in terms of their length, monosaccharide composition and linking sugar to the protein chain. However one of the glycans attached to some of the Rgp isoforms is immunologically related to a cell surface repeating unit polymer. Recent purification and characterization of this polymer has revealed an anionic polysaccharide (APS) distinct from LPS and capsule: a branched phosphomannan. Disruption of APS synthesis also influences production of the Arg-gingipains indicating that the maturation

pathway of these proteases is linked to the biosynthesis of this novel carbohydrate.

Glycosylation of cell wall proteins in *Candida albicans*

N.A.R. Gow, J.M. Bain, S. Bates, G. Bertram, R.P. Hobson, H.B. Hughes, C.A. Munro, D. MacCallum, F.C. Odds & A.J.P. Brown

Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD (Email n.gow@abdn.ac.uk)

The outer layer of the cell wall of *Candida albicans* is heavily enriched in glycosylated proteins that play critical roles in cell adherence, and act as major antigens and in the immunoregulation of the host. A null mutant in the Golgi manganese transporter gene *PMR1* was viable in vivo, had greatly reduced N- and phosphomannan and was attenuated in virulence. Therefore protein mannosylation is required for pathogenesis. The *C. albicans O*-linked mannan consists of a tetrasaccharide in which Mnt1p and Mnt2p participate as partially functionally redundant enzymes in the assembly of the terminal two α-1,2-mannose residues. Deletion of either *MNT1*, *MNT2* or both *MNT1* and *MNT2* resulted in strains with reduced adherence to epithelia and attenuation of virulence. This suggests that *O*-mannan functions as a ligand in interactions with host surfaces. Mutants with deletions in the *MNN4* gene are almost devoid in phosphomannan, which has been implicated in recognition of *C. albicans* by macrophages, but were phagocytosed normally. Deletion of the *OCH1* resulted in elimination of the outer *N*-mannan chains, induction of the cell wall salvage pathway and loss of virulence. Analysis of glycosylation mutants demonstrates that the carbohydrate epitopes of mannoproteins play key roles in pathogenesis of *C. albicans*.

Lipoprotein maturation in mycobacteria

Peter Sander, Mandana Rezwan, Silvana K. Rampini, Thomas Grau, Andreas Tschumi, Max Dolder, Sonja Kuhn, Dimitri Cloetta & Erik C. Böttger

Institute of Medical Microbiology, University of Zurich, Switzerland

Lipoproteins (Lpp) represent a class of membrane-anchored proteins. Lpp maturate by post-translational enzymatic modifications mediated by prolipoprotein diacylglyceryl transferase (Lgt), lipoprotein signal peptidase (LspA) and eventually lipoprotein N-acyl transferase (Lnt). We have previously established that LspA is a major determinant in the pathogenesis of *M. tuberculosis* (*Mol Microbiol* 2004, 52, 1543). To address the *in vivo* attenuation conferred by inactivating LspA at a mechanistic level we performed infection experiments in mouse macrophages. Intracellular growth of the mutant was significantly decreased as compared to the parental wild-type strain. However, both mutant and wild-type strains resided in a compartment with early endosomal characteristics (mildly acidified (pH6.3) and absence of Lamp1), indicating that the mutants attenuation is not due to enhanced phagosome maturation. These findings point to an important role for LspA in ensuring multiplication in early endosomal compartments. Allelic replacement techniques, heterologous expression of recombinant Lpp, Triton X114 partitioning and cell fractionation were used to generate and characterize a *M. smegmatis* Lgt mutant. Recombinant Lpp expressed in Lgt mutant showed altered solubility (aqueous vs. detergent phase) and altered subcellular localization (cytoplasmic membrane vs. cell wall), indicating absence of diacyl glycerol modification and the importance of lipid modification for correct transport and localization of lipoproteins in the mycobacterial cell envelope.

Streptococcal lipoproteins

Dean Harrington[1], Andrea Hamilton[2], Carl Robinson[3], Andrew Waller[3], Josh Slater[4] & Iain Sutcliffe[5]

[1]Dept of Biomedical Sciences, University of Bradford; [2]School of Life Sciences, University of Sunderland, UK; [3]Animal Health Trust, Newmarket; [4]Royal Veterinary College, Hatfield; [5]School of Applied Sciences, Northumbria University

N-terminal lipidation is a major mechanism by which bacteria tether proteins to their membranes. Bioinformatic analysis of streptococcal genomes suggests that lipoproteins represent approximately 2% of their predicted proteomes. A number of putative lipoprotein functions are likely to be important in bacterial interactions with the host particularly since some lipoproteins are likely to be surface-exposed. This has led to interest in lipoproteins as potential vaccine candidates. Lgt is an enzyme essential for the lipidation of lipoproteins. To investigate the role of lipoproteins in streptococcal virulence we constructed a mutant strain of *Streptococcus equi* 4047 (the causative agent of the equine disease strangles) that harboured an in-frame deletion in *lgt* (ΔLgt). Palmitate radiolabelling confirmed the lack of lipoprotein lipidation in the ΔLgt mutant, as did Western blotting of individual lipoproteins. The ΔLgt mutant was significantly less virulent than the parent strain in a mouse model of strangles. Moreover, the mutant strain was less able to colonise horse epithelial tissues in an air-interface model of colonisation. However, the virulence of the ΔLgt mutant was not significantly attenuated in a horse infection study. These results suggest that loss of lipoprotein lipidation does not necessarily result in the loss of lipoprotein function.

Construction and characterization of a lipoprotein-deficient mutant of *Streptococcus agalactiae*

Beverley Bray[1], Iain Sutcliffe[2] & Dean Harrington[1]

[1]Dept of Biomedical Sciences, University of Bradford; [2]School of Applied Sciences, Northumbria University

Streptococcus agalactiae (group B streptococcus; GBS) is the major bacterial pathogen of neonates affecting approximately 1 in 1000 births (case fatality rate; 4-6%). However, little is known about the molecular basis of GBS virulence. N-terminal lipidation is a major mechanism by which bacteria tether proteins to membranes. Bioinformatic analysis of GBS genomes suggests that lipoproteins represent approximately 2% of the predicted proteome. Lgt is an enzyme essential for the lipidation of lipoproteins. To investigate the role of lipoproteins in GBS physiology we used allelic replacement to construct a mutant strain of *S. agalactiae* A909 with an in-frame deletion in *lgt*. Growth of the ΔLgt mutant was comparable to the parent strain in both Todd-Hewitt broth (THB) and Chelex-treated THB. There was no difference in the haemolytic activity or pigmentation of the mutant compared to the parent strain. Despite the identification of a putative calcium-binding lipoprotein, within a region of the genome responsible for the synthesis of the rhamnose-containing group B wall carbohydrate antigen, the mutant strain agglutinated normally with the Streptex group B reagent. However, Western blotting of cell wall fractions revealed that a putative manganese-binding lipoprotein was inefficiently retained in the membrane emphasising the importance of lipidation in tethering.

Application of immunoproteomics to analysis of post-translational processing of surface and secreted proteins of *Streptococcus pyogenes*

Michael D. Boyle

Dept of Biology, Juniata College, 1700 Moore Street, Huntingdon, Pennsylvania 16652, USA

Post-translational modification of the anti-phagocytic M1 protein of *Streptococcus pyogenes* by secreted bacterial enzymes can influence its invasive potential and its non-immune binding properties for human immunoglobulin G subclasses. Current methods of monitoring this modification event involve N-terminal sequencing and are cumbersome, slow and not amenable to routine analysis. In order to study these interactions my laboratory has developed a rapid, sensitive, immunoproteomic methodology using surface enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF). This approach can be used to monitor modification of the M1 protein by the secreted bacterial cysteine protease, SpeB, as well as production and activation of the cysteine protease itself. The application of immunoproteomics to the study of post-translational modification and bacterial pathogenesis will be discussed.

Protein stability as a regulatory component of proteome expression

Robert J. Beynon

Protein Function Group, Faculty of Veterinary Science, University of Liverpool, Crown Street, Liverpool L69 7ZJ

As there are an increasing number of studies that measure the abundance of individual proteins in a proteome as well as the cognate mRNAs in the transcriptome, a frequent observation is the absence of a perfect correlation between the two. Most proteins in a cell are in a state of flux, such that new molecules are continually synthesised in a zero order process, balanced by the stochastic removal of existing protein molecules in a first order, degradative process. It follows that if we are to understand the relationship between proteome and transcriptome, we must be define the rates of synthesis and of degradation for each protein within that proteome.

We have developed stable isotope methods for the measurement of protein turnover on a protein-by-protein basis, on a proteome wide scale. In *Saccharomyces cerevisiae*, proteins are prelabelled with the stable isotope labelled amino acid [$^2H_{10}$]-leu and the subsequent loss of label from individual proteins (resolved by 2-D GE and assessed by MALDI-ToF MS) then reports on the rate of degradation.

This work is supported by BBSRC

Significance of hydroxylation and glycosylation of a ubiquitylation-associated protein in *Dictyostelium*

Christopher M. West[1], Hanke van der Wel[1], Altan Ercan[1] & Slim Sassi[2]

[1]Dept of Biochemistry & Molecular Biology, Oklahoma Center for Medical Glycobiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104; [2]Dept of Anatomy & Cell Biology, University of Florida College of Medicine, Gainesville, FL 32610, USA

Dictyostelium is a mycetezoan that likely branched from the tree of life between the divergence of plant and fungal lineages. Genetic screens have revealed that E3(SCF)-Ubiquitin ligases figure importantly in regulation of terminal cell differentiation in the *Dictyostelium* fruiting body. Skp1, a subunit of these ligases, is subject to oxygen-dependent prolyl 4-hydroxylation and subsequent addition of a pentasaccharide chain by a novel cytoplasmic hydroxylation/glycosylation pathway. Most of the enzymes have evolutionary origins traceable back to bacteria and are distantly related to enzymes in the Golgi of animals, plants and other microbes. Disruption of the gene that encodes the Skp1 prolyl-4-hydroxylase interferes with an oxygen-dependent step in fruiting body formation, whereas disruption of this or one of the glycosyltransferase genes affects cell type differentiation. Biochemical studies suggest that Skp1 is the sole target of this modification pathway, and the similarity of these phenotypes to those of other SCF-pathway mutants implicates a role for hydroxylation/glycosylation in the degradation of developmental regulatory proteins. Bioinformatics studies suggest the occurrence of these modifications in other unicellular eukaryotes including agents of plant and animal diseases. These findings emphasize that complex *O*-glycosylation can occur on cytoplasmic and nuclear as well as extracellular proteins, but the structures and functions are distinct.

Multiple roles of ubiquitin in response of yeast cells to extracellular amino acids

B. André

Université Libre de Bruxelles, IBMM, Molecular Cell Physiology, Gosselies, Belgium

Yeast cells possess a plasma-membrane sensor for detection of amino acids in the external medium. A key component of this sensor is Ssy1, a homologue of amino-acid permeases devoid of transport activity and likely involved in detection of external amino acids. Ssy1 acts in tight conjunction with Ptr3, a protein with a C-terminal WD40-repeat domain. Upon detection of amino acids, this Ssy1-Ptr3 sensor activates the serine-protease-like Ssy5 factor that mediates endoproteolytic cleavage of the inactive precursor form of Stp1 transcription factor. The processed Stp1 is thus translocated into the nucleus where it activates transcription of amino acid permease genes, e.g. *AGP1* encoding a broad-specificity amino-acid permease. Data will be presented showing that ubiquitin intervenes at multiples levels in this signalling pathway.

Proteomic analysis of nitrogen signalling in *Aspergillus nidulans*

Igor Morozov, Meriel G. Jones, Huw H. Rees & Mark X. Caddick

School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB

Nitrogen metabolite signalling in *Aspergillus nidulans* is subject to regulation by the GATA transcription factor AREA, the activity of which is determined by the quality and quantity of the available nitrogen sources. Under conditions of nitrogen limitation, AreA activity increases and this results in the expression of a large, though as yet undefined, number of genes. Here we report that proteomic based analysis of nitrogen signalling revealed differential expression of three genes. The expression of AN3674.2 increased significantly within 2 minutes of adding Gln. The predicted AN3674.2 protein shows homology to domain of Pleckstrin which binds phosphoinositidies and involved in targeting proteins to the plasma membrane. A second gene, AN6921.2, appeared in two forms with different molecular weight. Under conditions of nitrogen limitation the lower molecular weight form predominates while the opposite occurs under derepressed conditions. The predicted protein displays homology to the yeast SGT protein which is involved in assembling of the SCF ubiquitin ligase. The third gene is only expressed in the presence of Gln is AN0084.2 and shows homology to the Ran-binding domain (RdnBP1). This regulates receptor-mediated transport of RNA and proteins between nucleus and cytoplasm. The implication of these proteins in nitrogen metabolite signalling pathway will be discussed.

Regulated proteolysis in *Bacillus subtilis*

K. Turgay

Institut für Biologie, FB Biologie, Chemie, Pharmazie, Freie Universität Berlin, Königin-Luise-Str. 12-16, 14195 Berlin, Germany

The AAA+ protein ClpC, which is part of the ClpCP protease, is not only involved in the removal of unwanted misfolded and aggregated proteins but also controls, through regulated proteolysis, key steps of several developmental processes in the Gram-positive bacterium *Bacillus subtilis*. In contrast to other AAA+ proteins, ClpC is unable to mediate these processes without an adaptor protein like MecA.

To elucidate the complex role of ClpC and its adaptor proteins in the regulatory network and the general protein quality control system of *B. subtilis*, we studied the activation of ClpC by MecA in more detail. Our results demonstrate that the general activation of ClpC is based upon the ability of MecA to participate in the assembly of an active and substrate-recognizing higher oligomer consisting of ClpC and the adaptor protein, which is a prerequisite for all activities of this AAA+ protein. Using hybrid proteins of ClpA and ClpC, we identified the N-terminal and the Linker domain of the first AAA+ domain of ClpC as the essential MecA interaction sites. This new adaptor-mediated mechanism adds another layer of control to the regulation of the biological activity of AAA+ proteins.

References Turgay, K., Hahn, J., Burghoorn, J. & Dubnau, D. (1998) Competence in *Bacillus subtilis* is controlled by regulated proteolysis of a transcription factor. *EMBO J* 17, 6730-38; Schlothauer, T., Mogk, A., Dougan, D.A., Bukau, B. & Turgay, K. (2003) MecA, an adaptor protein necessary for ClpC chaperone activity. *Proc Natl Acad Sci USA* 100, 2306-11; Kirstein, J., Schlothauer, T., Dougan, D.A., Tischendorf, G., Mogk, A., Bukau, B. & Turgay, K. (2005) Adaptor protein controlled oligomerization activates the AAA+ protein ClpC. Submitted for publication.

Transferring substrates to the 26S proteasome in the fission yeast *Schizosaccharomyces pombe*

Colin Gordon

MRC Human Genetics Unit, Western General Hospital, Edinburgh

The ubiquitin pathway is found in all eukaryotes. In this pathway, target proteins are covalently modified by the addition of ubiquitin, a 76 amino acid protein, to specific lysine residues. The ability of multi-ubiquitin chains to function as a signal to target proteins for degradation by the 26S proteasome is well documented. A key question is how is the multi-ubiquitin chain is recognised as a signal? Fission yeast Rhp23/Rad23 and Pus1/Rpn10 represent two families of multi-ubiquitin chain binding proteins that can associate with the proteasome as well as some E3 ubiquitin ligases. They seem to provide a link to shuttle ubiquitinated substrates from the E3 ubiquitin ligases to the 26s proteasome. A detailed characterisation of their proteasome binding will be presented along with their potential role in ubiquitin conjugate dynamics. Finally data will be presented indicating that an additional substrate presentation pathway exists in fission yeast which is also conserved in higher eukaryotes.

Drug and target discovery in the malarial proteome

T. Haystead

Duke University Medical Centre, Durham, USA

Abstract not received

Chemotaxis in *Escherichia coli*

J.B. Stock

Princeton University, New Jersey, USA

Abstract not received

Two-component signalling in the myxobacteria

P.J.A. Cock, P.F. Hawkins, L. Evans, D.A. Hodgson & **D.E. Whitworth**

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

Two-component signal transduction systems (TCSs) consist of partner sensor kinase and response regulator proteins, containing conserved transmitter and receiver domains respectively. *Myxococcus xanthus* possesses a huge number of TCSs (190 receiver and 135 transmitter domains). A bioinformatic survey of all TCSs in 229 genomes suggests a particularly significant communication between the TCS and cyclic nucleotide-mediated signalling networks of *M. xanthus*. The increased regulatory capacity of *M. xanthus* allows a complex life-cycle involving vegetative social predation of other micro-organisms, and starvation-induced multicellular development/sporulation.

We have performed a large-scale but directed yeast two-hybrid screen for protein-protein interactions between transmitter and receiver domains of *M. xanthus,* complemented by *in vitro* phosphotransfer assays. False-positives were reduced by cross-validation against microarray data, and several suggested interactions were confirmed by phenotypic analysis. We found that in addition to identifying specific partnerships, comparisons of yeast two-hybrid interaction profiles allowed clustering of TCS proteins into functionally related groups.

M. xanthus appears to integrate the activities of multiple TCSs into complicated regulatory circuits, through their serial or parallel combination. We have also identified a novel form of 'phosphorelay', and a phosphate-acquisition regulon that is far more complicated than other known examples.

Chemotaxis in *Dictyostelium*

Rob Kay

MRC Laboratory of Molecular Biology, Cambridge

Chemotaxis plays a crucial role in development, wound healing and the function of the immune system. *Dictyostelium* cells chemotax to cyclic-AMP as part of their aggregation mechanism. Polarization of cells in a cyclic-AMP gradient involves the rapid recruitment of PI3kinase and PH-domain proteins to the membrane, followed by actin polymerization, driving pseudopod expansion. This expansion is only one of the many ways in which a moving cell changes in shape, raising the question of whether the surface area also changes during movement and, if so, how this is accommodated by the plasma membrane. Accommodation cannot be assumed to be a passive process, since the membrane, though flexible is basically inextensible. I shall describe experiments suggesting that plasma membrane dynamics are in fact an essential part of cell movement. We devised a method for measuring the surface area of individual cells as they move, and find that randomly moving and chemotacting cells constantly change in surface area. Cells in which exocytosis of membrane is genetically blocked are unable to move, or significantly extend pseudopodia, although they can efficiently polarize in a cyclic-AMP gradient.

Converging physics and biology to understand basic mechanisms in the phosphorelay

James A. Hoch

The Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla CA 92037, USA

Two-component systems consisting of a sensor histidine kinase that phosphorylates and activates a response regulator/transcription factor are widespread in bacteria and regulate a variety of genes to cope with environmental responses as well as cell cycle progression and other processes. Phosphorelays are a more complex version with two intermediate phosphoryl transfer proteins separating the two-components that serve to amplify the number of sites for regulatory proteins to interdict the flow of phosphoryl groups to the response regulator/transcription factor. The best example of this type of regulation is the Bacillus sporulation phosphorelay that has a multitude of phosphatases regulating phosphoryl flow in response to a variety of cellular signals. Two-component systems were amplified in the genome by sequential rounds of gene duplication followed by adaptation to sense unique signals and control unique gene sets with the overall structures of the interacting domains remaining unchanged. In order to understand evolution of molecular recognition in greater detail a study of two-component pairs from genomic data using a new mathematical system to identify covariance of amino acids in the two domains, has been developed. The system revealed specific residues in a region of the sensor histidine kinase that are believed to be critical for transmitting the signal and the location and nature of these residues has given rise to a theory of how the signal ligand bound sensing domain communicates with its phosphoryl transfer domain.

From signal transduction to cell biology – phosphoproteomics for plant–microbe interactions

T. Nuhse

John Innes Centre, Norwich

The basis of innate immunity in plants is a combination of constitutive structural barriers and the perception of characteristic molecules released by microbes. These elicitors or 'pathogen-associated molecular patterns' (PAMPs) trigger defence responses that are sufficient to contain non-host pathogens but can be suppressed by successful pathogens that express effector proteins. PAMP-dependent signalling is genetically not well characterised; many of the components may be essential genes or have redundant paralogs and are therefore not accessible to forward genetics.

We have developed techniques to study changes in protein phosphorylation in elicitor-treated Arabidopsis cells. Many rapidly phosphorylated proteins have been identified with an *in vivo* labelling/2D-PAGE approach, and several have proven to be essential components of antimicrobial defence.

Many of the earliest responses to elicitors are changes in plasma membrane (PM) protein activity (such as the NADPH oxidase RbohD, or ion channels), and the PM also plays a key role in guiding the

cellular polarisation towards the site of attempted cell penetration by fungi. To study the dynamics of PM protein phosphorylation, we developed an approach based on phosphopeptide affinity chromatography and quantitative mass spectrometry. The identified rapidly regulated proteins point to diverse cell biological responses and unanticipated processes.

Redox regulation of transcription factor activity

Matthew J. Wood

National Institutes of Health, Cell Biology and Metabolism Branch, 18 Library Dr., Bethesda, MD 20892-5430, USA

The budding yeast *Saccharomyces cerevisiae* has provided a valuable model for understanding how eukaryotic organisms respond to and defend against oxidative stress. It has a complex adaptive response to oxidative stress that involves the transcription factor Yap1, which is post-translationally regulated by a reversible disulfide bond-relay cascade and controls the expression of ~70 genes. Yap1 normally exists in a reduced form and is distributed throughout the cytoplasm and nucleus because it contains both a nuclear import signal and nuclear export signal (NES). In response to oxidative stress, Yap1 rapidly oxidizes and accumulates in the nucleus, which results in the induction of antioxidant defense genes. We have identified the minimal redox-regulated domain of Yap1 and determined its high resolution structure. In the active oxidized form, an NES in the C-terminal cysteine rich domain is masked by disulfide bond-mediated interactions with a conserved N-terminal alpha-helix. Upon reduction of the disulfide bonds, Yap1 undergoes a change to an unstructured conformation that exposes the NES and allows redistribution to the cytoplasm. We suggest that redox-controlled masking of a signal sequence may represent a general stress sensitive mechanism for controlling accessibility of protein localization signals.

Post-translational modifications and the regulation of nitrogen metabolism

Ray Dixon

Dept of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH

PII signal transduction proteins are small homotrimeric proteins that are widely distributed in bacteria, archaea and plants and play a pervasive role in the regulation of microbial nitrogen metabolism. The nitrogen status is relayed to these proteins via covalent modification and the signal is transduced by interaction of PII proteins with a variety of different targets including metabolic enzymes and signal transduction components. The mode of covalent modification varies in different bacteria and involves uridylylation in proteobacteria, adenylylation in actinomycetes and phosphorylation in cyanobacteria. PII proteins also bind 2-oxoglutarate and ATP, enabling integration of carbon and energy signals with the nitrogen status signal conveyed by covalent modification. In this talk I will review the mechanisms employed by PII proteins to communicate with their target receptors and will focus in particular on our work on the interaction of PII-like proteins with transcription factors that regulate nitrogen fixation.

Role of the PII and AmtB proteins in the post-translational modification of nitrogenase in *Azospirillum brasilense*

Luciano F. Huergo[1,2], Leda S. Chubatsu[1], Fabio O. Pedrosa[1], Emanuel M. Souza[1] & Mike Merrick[2]

[1]Dept of Biochemistry and Molecular Microbiology, Universidade Federal do Parana, Curitiba, PR, Brazil; [2]Dept of Molecular Microbiology, John Innes Centre, Norwich

In most bacteria studied so far the regulation of nitrogen metabolism relies on post-translational modification of members of the PII family of signal transduction proteins. These proteins are modified according to cellular nitrogen levels and in turn transduce the prevailing nitrogen level through protein-protein interactions that ultimately lead to post-translational modification, and thus regulation, of key enzymes and transcriptional activators. Nitrogen fixation in *Azospirillum brasilense* is regulated by mono-ADP-ribosylation of dinitrogenase reductase (NifH) that occurs in response to addition of ammonium to the extracellular medium. This process is mediated by dinitrogenase reductase ADP-ribosyltransferase (DraT) and reversed by dinitrogenase reductase glycohydrolase (DraG), but the molecules responsible for regulating the activities of these enzymes are unknown. Using NifH western blot analysis we have investigated the role of the PII proteins (GlnB and GlnZ) and the ammonium transporter AmtB in the NifH-modification process. Post-translational modification of NifH is defective in an *amtB* mutant suggesting that AmtB acts as an extracellular ammonium sensor. We have also shown that GlnB and GlnZ bind to the membrane in an AmtB-dependent manner after an ammonium shock. The significance of the cellular localization of these proteins in facilitating the ammonium-dependent post-translational modification of NifH will be discussed.

Post-translational modifications of Rab GTPases in *Saccharomyces cerevisiae* and the role of the YIP1 family

Catherine Z. Chen[1,2] & **Ruth N. Collins**[2]

[1]Graduate Program in Pharmacology, Cornell University, Ithaca, NY 14853; [2]Dept of Molecular Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Tel +1 607 253 4123; Email rnc8@cornell.edu)

The Rab family is unique amongst Ras superfamily members in their post-translational acquisition of two geranylgeranyl moieties at their COOH-terminus. The YIP1 family comprises an evolutionarily conserved group of membrane proteins that share the ability to bind di-prenylated Rab proteins. The biochemical interactions of YIP1 family proteins suggest a possible participation in the process by which Rab proteins are recruited onto membranes. *YIP1* is essential for viability in yeast and a deletion of *YIP1* can be rescued with the human ortholog YIP1A. We have made use of this evolutionary conservation of function to generate a series of mutant alleles of *YIP1* to investigate the biological function(s) of Yip1p. Our findings indicate evidence for the participation of Yip1p in both Rab action and COPII vesicle biogenesis function; at present we are extending these observations to other YIP1 paralogs to obtain a general view of YIP1 family function.

Young Microbiologist of the Year Competition

Molecular characterization of the *Salmonella*-specific protein PagN

M.A. Lambert & S.G. Smith

Trinity College Dublin, Dublin, Ireland

Salmonellae are enteric pathogens that cause a wide range of host-specific illnesses, including gastroenteritis and enteric fever. During infection *Salmonella* adhere to and invade intestinal M-cells and epithelial cells. Once internalised, they are capable of multiplication and subsequent dissemination.

The PhoP-activated gene *pagN* is broadly distributed within and confined to the *Salmonella* genus. This gene encodes a protein predicted to have a beta-barrel structure showing extensive homology over its entire length to the *Escherichia coli* adhesins Tia and Hek. The gene-product PagN possesses a typical signal peptide at its amino terminus indicating that it is translocated across the cytoplasmic membrane via the Sec apparatus.

We cloned the *pagN* gene into the multiple cloning site of pTrc99a creating the expression vector pML1. Analysis of outer membrane fractions from recombinant *E. coli* XL-1 Blue carrying pML1 reveals that *pagN* directs the production of a 25.7 kDa protein that is localised to the outer membrane. Synthesis of this protein was shown to be up regulated in MOPS minimal medium representing intra-macrophage conditions.

Expression of PagN was directly correlated with the ability of recombinant *E. coli* to adhere to erythrocytes bringing about mannose-resistant haemagglutination. Recombinant *Salmonella* LT-2 over expressing PagN failed to agglutinate erythrocytes. However, recombinant *Salmonella* CH133, a *galE* mutant, that expresses rough LPS, supported haemagglutination indicating a possible role for LPS in masking PagN.

Induction of PagN expression in recombinant *E. coli* DH5α brings about adhesion to and invasion of CHO cells. Adhesion and invasion was shown to be inhibited by the addition of both heparin and heparan-sulfate. CHO cells grown in the presence of xylopyranoside, which prevents proper glycosylation of proteoglycans, showed reduced PagN-promoted cell adhesion, indicating that PagN promotes adhesion to CHO cells via the heparin moieties of proteoglycan glycosaminoglycans.

Intracellular localization, modification and toxicity of the pseudomonal type III secreted toxin ExoU

F.R. Stirling & T.J. Evans

Division of Immunology, Infection and Inflammation, Faculty of Medicine, University of Glasgow

ExoU is a toxin secreted by the type III secretion system of *Pseudomonas aeruginosa* into eukaryotic cells. ExoU acts as a potent phospholipase that contributes to increased bacterial virulence. In common with other type III secreted Pseudomonal toxins, ExoU requires an unknown eukaryotic cofactor for activity. The aim of this study was to characterise the intracellular target of ExoU, and determine how the eukaryotic cell activates the toxin. Immunofluorescence and western blot analysis of fractionated cells revealed that ExoU is localised at the plasma membrane after transfection and infection. The region required for this localisation is within the extreme C-terminal residues. We found that ExoU is modified to a higher molecular weight form following transfection and infection, which was exclusively found in membrane fractions and required the same extreme C-terminal residues needed for membrane localization. Full toxicity of ExoU, as assessed by luciferase and LDH assays, also required a similar region of the molecule. We conclude that the C-terminus of ExoU is required for membrane localization and modification to a higher molecular weight modified form, which seems to be necessary for toxicity.

A DEAD-box RNA-helicase is required for normal zoospore development in the potato late blight pathogen *Phytophthora infestans*

Claire Walker[1], Maico Köppe[1], Anna Avrova[2], Laura Grenville-Briggs[1], Paul Birch[2], Stephen Whisson[2] & Pieter van West[1]

[1]University of Aberdeen, Aberdeen Oomycete Group, College of Life Science and Medicine, Aberdeen; [2]Scottish Crop Research Institute, Plant Pathogen Interactions Programme, Invergowrie

Zoospores of *Phytophthora infestans*, the potato late blight pathogen, are released from sporangia and play a key role in pathogenicity. Zoospore formation occurs within minutes after a cold-shock and it is considered one of the fastest developmental processes in a biological system. Therefore, we predict that there is a stored pool of 'masked mRNAs', which can be rapidly translated during zoosporogenesis. Recently, we isolated a putative DEAD-box RNA-helicase, *rnh1*, from *P. infestans*. RNA-helicases are involved in mRNA processing and translation initiation. Expression of *Rnh-1* increased 20-fold in young zoospores compared to mycelium. We used RNA-interference to functionally characterize *rnh-1*. Silenced *rnh1* strains produced large aberrant zoospores. These had undergone partial cleavage and often had multiple flagella on their surface. The *rnh1*-silenced zoospores were also sensitive to osmotic pressure and they often burst upon release from sporangia. We speculate that *rnh-1* may be involved in activation of stored or 'masked mRNAs'.

Effect of light on the interaction between cyanophage S-PM2 and *Synechococcus* sp. WH7803

Ying Jia & Nicholas H. Mann

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

S-PM2 is a bacteriophage, with a double-stranded, circular genome, which infects the ecologically important marine cyanobacterium *Synechococcus* sp. WH7803. We are currently investigating fundamental features of phage-host interactions. An initial investigation of the role of light on phage attachment has indicated striking light-dependence. In the darkness, phages were not capable of absorbing to cells, but absorption resumed as soon as the light was switched on. A collection of marine cyanophages is being screened in order to see if this is a common phenomenon. Attempts to clarify the mechanism of phage absorption showed that the photosynthetic inhibitor and uncoupler, DCMU and CCCP could not prevent phage absorption in light. This finding indicates that there must be other factors controlling phage absorption rather than photosynthesis-related processes. Because light/dark cycles are environmental stimuli that synchronize the circadian clock, the potential role of cyanobacterial circadian rhythm in the cyanophage absorption is under investigation. Additionally, investigation of the possible presence of a light receptor either located in the phage or in *Synechococcus* will be carried out. All these studies will offer significant insights into the factors affecting cyanophage-cyanobacterium dynamics under natural environmental conditions.

Biodegradation of the high explosive RDX by *Rhodococcus* sp.

James Edwards[1], Rosamond G. Jackson[1], Helena M. B. Seth-Smith[2], Deborah A. Rathbone[1] & Neil C. Bruce[1]

[1]CNAP, Dept of Biology, University of York, York YO10 5YW; [2]The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA

Royal Demolition Explosive (RDX) is a widely manufactured high explosive and today is a recognized environmental hazard owing to its toxicity and recalcitrance. Concern is growing regarding the large areas of land and ground water polluted worldwide as a result of the continued manufacture, use and disposal of this compound. We have isolated 19 strains of bacteria belonging to the genus *Rhodococcus* that are capable of utilizing RDX as a nitrogen source. The gene responsible for RDX biodegradation (*xplA*) has been cloned from *R. rhodochrous* strain 11Y, and shown to be a unique cytochrome P450. Investigations of the remaining strains using a P450 inhibitor (metyrapone) showed greatly reduced RDX degradation suggesting a P450 involvement in these strains. We now have evidence to show that the *xplA* gene is present both in these 19 RDX degrading strains, and in other strains isolated from different geographical sites from within the UK and Australia. This gene appears to be specific only to strains of bacteria isolated from RDX contaminated sites. Interestingly, the homologues cloned thus far have a remarkable degree of similarity to one another (>99% amino acid identity). Further, the recombinant expression of XplA has recently been achieved in *E. coli* which will permit future characterization of this novel enzyme.

Special Lectures

Fred Griffith Review Lecture

Bioengineering beneficial Bacillus toxins

David J. Ellar

University of Cambridge

After gaining his PhD from Syracuse University, New York studying bacillus sporulation, Professor Ellar went to New York University to work on bacterial membranes with Professor Milton Salton. Upon appointment to the Biochemistry Department at Cambridge he began to compare the biochemistry of developing, dormant and germinating bacillus spore membranes and showed that activation of a cortex-lytic enzyme by the germinant is a triggering mechanism for spore germination. In 1980 he began investigating two groups of insecticidal proteins (Cry and cyt delta-endotoxins) synthesised by *Bacillus thuringiensis* during sporulation. These 'biopesticides' which destroy gut epithelial cells are alternatives to chemical pesticides either as sprays or in transgenic plants. At the outset, relatively little was known of the structure, genetics or mode of action of these toxins. In the dramatic improvement in this picture in the succeeding 25 years, his laboratory has played a major part as a leading international centre for research on these toxins. By combining the 'killing' domains of the Cry toxins with a diverse library of human antibody recognition domains, the group is constructing novel immunotoxins –'Crybodies'- that can target selected cells for destruction.

Peter Wildy Prize for Microbiology Education

Yes, but is it microbiology...?! (Using links between microbiology and art in undergraduate programmes)

Joanna Verran

Professor of Microbiology, Dept Biological Sciences, Manchester Metropolitan University, Chester St., Manchester M1 5GD

In order to deliver key/transferable skills to undergraduates (first and second year), I initiated a range of novel exercises which involve the use of microbiological knowledge in a 'different' way. Essentially, students worked in groups to produce information for the public in various formats.

These design-based activities progressed into interdisciplinary work on history and literature, and art. Exploration of the links between microbiology and art revealed many associations beyond the obvious use of art to represent disease and disaster, posters and cartoons to inform and provoke, beautiful images and models of micro-organisms – and deterioration and restoration of art. More specific science-art collaborations have found particular value in assisting in the communication of scientific ideas, principles and issues to the public.

A number of collaborations between staff and students in the Art and Design and Science and Engineering faculties have been established. The exercises have challenged the thinking of students from the different disciplines, and the outcomes have often (but not always!) been positive. Thinking across subject boundaries, communicating ideas, time management and product delivery are important skills – but the underlying theme is microbiology.

The lecture will outline the diversity of links between microbiology and art, and review (with honesty) some of the experiences encountered in this unusual arena.

Posters

Cells & Cell Surfaces Group

CCS 01 The ABC carbohydrate uptake transporters of *Streptococcus mutans*

Alexander J. Webb & Arthur H.F. Hosie

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

Dental diseases, such as dental caries (tooth decay), are possibly the most frequent bacterial associated diseases in humans. Although non-life threatening, they constitute a considerable economic burden. *Streptococcus mutans* is the bacterium most commonly associated with dental caries. Carbohydrate metabolism by this organism results in acid production, which contributes to caries. Members of two subfamilies (CUT1 and CUT2) of the ubiquitous ATP-binding cassette (ABC) transporter superfamily are involved in carbohydrate uptake. We have identified by bioinformatic analysis two CUT1 and one CUT2 ABC transporters in *S. mutans* UA159. One of the CUT1 and the CUT2 transporters have not been previously characterised. Investigation of their function will yield important information on carbohydrate transport and metabolism by this species. We have constructed mutants of these CUT1 and CUT2 ABC transporters and present here the phenotypic analysis of these mutants.

CCS 02 The flagellar specific sigma factor, σ^{28}, acts as a Type III secretion substrate for its anti-sigma factor FlgM in *Salmonella enterica* serovar Typhimurium

Phillip Aldridge[1,2], Joyce Karlinsey[2], Christopher Birchall[1], Jin Yagaskai[2] & Kelly Hughes[2]

[1]Institute for Cell and Molecular Biosciences, University of Newcastle, Framlington Place, Newcastle upon Tyne NE2 4HH; [2]Dept of Microbiology, Box 357242, University of Washington, Seattle, WA 98195, USA

Flagellar associated Type III secretion chaperones (T3SC) are bi-functional proteins: acting as secretion chaperones during one stage of flagellar assembly and regulators of gene expression at later assembly stages. In *Salmonella enterica* serovar Typhimurium a major checkpoint during flagellar assembly is completion of the hook-basal body (HBB), coinciding with FlgM secretion. Cytoplasmic FlgM acts as an anti-sigma factor of the flagellar specific sigma factor σ^{28}. T3SC's facilitate substrate recognition and secretion only after HBB completion. However, the only protein secreted after HBB completion that does not have a defined T3SC is FlgM. We will present secretion data that shows σ^{28} itself acts as the FlgM T3SC. We have isolated σ^{28} mutants defective in chaperone activity to determine if σ^{28} acts in a similar to that of known T3SC's during substrate delivery. Analysis of isolated mutants suggests that the majority are defective in FlgM binding whereas mutants defective in substrate delivery represent a minor class of mutants. This is the first time that a known transcriptional regulator has been shown to act as a T3SC. We propose a model in which the chaperone activity of T3SC's is a mechanism for sensing the assembly status of the growing type III secretion apparatus.

CCS 03 A comparison of flagellar assembly between *Caulobacter crescentus* and *Salmonella enterica* serovar Typhimurium identifies significant divergence from the accepted paradigm, dictated by enteric bacteria

Alex Faulds-Pain & Phillip Aldridge

Institute of Cellular & Molecular Biosciences, Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH

The flagella system of *Salmonella enterica serovar* Typhimurium dictates the paradigm for flagella assembly in bacteria. Assembly requires an associated Type III secretion apparatus (T3SS) to secrete flagellar subunits through the base of the growing structure. All T3SS's identified, either experimentally or through genome projects, possess Type III secretion chaperones (T3SC's) suggesting that they may be an essential component of T3SS's in all bacteria.

The alpha proteobacteria *Caulobacter crescentus* possesses a single polar flagellum compared to the six to eight lateral flagella of *S. enterica*. We will present a comparison of flagellar genes between *C. crescentus* and *S. enterica* that identifies a significant divergence from the accepted paradigm. Most strikingly, none of the known T3SC's from *S. enterica* was found in the *C. crescentus* genome. Considering that all other T3SS's possess T3SC's, we have performed a subsequent analysis of the *C. crescentus* genome to identify putative T3SC's amongst hypothetical open reading frames and known flagellar genes that possess T3SC characteristics and are encoded in the same loci as other flagellar genes. We will present genetic and biochemical data used to determine whether the identified hypothetical ORF's are required for flagellar assembly and any identified gene possesses chaperone activity.

CCS 04 Role of the BacA protein in *Sinorhizobium* bacteroid development

Victoria L. Marlow[1], An Jansen[2], Anup Datta[3], Russ W. Carlson[4], Graham C. Walker[2] & Gail P. Ferguson[1]

[1]Institute of Structural and Molecular Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JR; [2]Dept of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA; [3]Glycobiology Research and Training Centre, Dept of Molecular and Cellular Medicine, University of California, San Diego, La Jolla, CA 92093-0687, USA; [4]Complex Carbohydrate Research Centre, University of Georgia, Athens, GA 30602, USA

The α-proteobacterium *Sinorhizobium meliloti* can exist free-living in the rhizosphere or in a symbiotic relationship with leguminous plants. During the symbiosis, *S. meliloti* are released into membrane-bound compartments, where they differentiate into morphologically distinct, Y-shaped bacteroids that fix N_2. The precise signals and changes involved in bacteroid differentiation are still poorly understood; however, the inner membrane BacA protein is essential for this process. Although the precise function of BacA is unknown, *S. meliloti bacA* null mutants display an array of phenotypes including a low-level resistance to the glycopeptide, bleomycin and an increased sensitivity to detergents. The latter phenotype led us to discover that BacA affects an unusual fatty acid modification of *S. meliloti* lipid A. Subsequent analysis revealed that the unusually modified lipid A observed in free-living *S. meliloti* is important, but not crucial for bacteroid differentiation, suggesting additional host-induced lipid A changes may be occurring. We recently discovered that the bleomycin resistance phenotype of the *bacA* mutant is independent of the unusual lipid A. Thus we are currently investing the molecular basis of the bleomycin resistance phenotype of the *bacA* mutant. This research will greatly improve our understanding of bacteroid development during the *S. meliloti* legume symbiosis.

CCS 05 A new method for the rapid purification of FanC, the major subunit of K99

Mehdi Golchin[1] & Robert Aitken[2]

[1]Faculty of Veterinary Medicine, Shahid Bahonar University, Iran; [2]Division of Infection & Immunity, IBLS, University of Glasgow

Several strategies and methods have been attempted to purify K99 fimbriae from enterotoxigenic *Escherichia coli* strains. Most of these

methods are technically complex and time consuming. We have developed a single step ion-exchange chromatography method that takes just a few hours for purification of this important virulence factor.

K99 fimbriae were stripped from *E. coli* B41 by heat treatment and phosphate urea buffer. The crude extracts were then equilibrated with tris buffer and were loaded to a HiTrap SP-XL column. By step-wise elevation of sodium chloride concentration, the FanC was eluted as a single species. The purified protein agglutinated sheep and horse red blood cells and had high activity with anti-K99 monoclonal antibody in ELISA. Also, pre-incubation of red blood cells with the purified protein blocked adhesion of B41 bacteria. LPS contamination was undetectable using a silver staining method.

In summary, this simple, inexpensive method yields purified FanC that has good purity, stability and biological activity, making it suitable for many purposes including vaccination.

CCS 06 The role of the DAD motif in the membrane association of KfiB protein and incorporation of GlcNAc into membrane

Meraj Pourhossein[1], Nigel Hodson[2], Brendan Barrett[2] & Ian Roberts[2]

[1]Dept of Molecular Biology, School of Medicine, Isfahan Medical Sciences University, Isfahan, Iran; [2]Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT

Site-directed mutagenesis is a very effective technique to make point mutations in order to switch amino acids to another desired amino acids.

It has been revealed that the DDD motif is conserved within the C family of glycosyltransferases DDD motif is catalytically important in the transfer of GlcNAc and synthesis of the glycosidic bond (Breton *et al.,* 1998; Hodson *et al.,* 2000). To clarify the importance of the DAD motif in KfiB function, Quick Change site directed mutagenesis (Stratagene) was performed to change the Aspartic acid residue to Glutamic acid and Alanin (D512E, D512A, D514E, and D514A).

The effect of the site-directed mutations on the function of the KfiB protein in polysaccharide export was determined by complementation assay. The complementation analysis showed that all of the mutations could complement the *kfiB* mutation in pPC6::23 and restore capsule formation. However, plaque titer and morphology was altered (less in number with smaller and irregular shape) in strain XL1-Blue[pPC6::23]pMA1(D514A).

The *in vitro* transferase activity of membranes containing these proteins was reduced in D514A compared to the activity of membranes with the parental KfiB construct (69.3%), which was statistically significant (P=0.01). It is apparent this amino acid is necessary for correct functioning of KfiB.

CCS 07 Induction of *Staphylococcus epidermidis* biofilm formation via proteolytic processing of the accumulation associated protein by staphylococcal and host proteases

H. Rohde[2], C. Burdelski[2], M. Hussain[3], F. Buck[5], M.A. Horstkotte[2], J.K.-M. Knobloch[2], C. Heilmann[3], M. Herrmann[4] & **D. Mack**[1,2]

[1]Medical Microbiology and Infectious Diseases, The Clinical School, University of Wales Swansea, Swansea; [2]Institut für Infektionsmedizin, Universitätsklinikum Hamburg-Eppendorf; [3]Institut für Medizinische Mikrobiologie, Universitätsklinikum Münster, Münster, Germany; [4]Abteilung Bakteriologie und Hygiene, Institut für Medizinische Mikrobiologie und Hygiene, Universitätskliniken des Saarlandes, Homburg/Saar, Germany; [5]Institut für Zellbiochemie und klinische Neurobiologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

Due to its biofilm forming potential *Staphylococcus epidermidis* has evolved as a leading cause of device related infections. The polysaccharide intercellular adhesin (PIA) is significantly involved in biofilm accumulation. However, infections due to PIA-negative strains are not uncommon, suggesting the existence of PIA-independent biofilm accumulation mechanisms. Here we found that biofilm-formation in the clinically significant *S. epidermidis* 5179 depended on the expression of a truncated 140 kDa isoform of the 220 kDa accumulation associated protein Aap. As expression of the truncated Aap isoform lead to biofilm formation in *aap*-negative *S. epidermidis* 1585, this domain mediates intercellular adhesion in a polysaccharide independent manner. In contrast, expression of full length Aap did not lead to a biofilm-positive phenotype. Obviously, to gain adhesive function, full length Aap has to be proteolytically processed through staphylococcal proteases as demonstrated by inhibition of biofilm formation by α_2-macroglobulin. Importantly, also exogenously added granulocyte proteases activated Aap, thereby inducing biofilm formation in *S. epidermidis* 5179 and 4 additional, independent clinical *S. epidermidis* strains. It is therefore reasonable to assume that *in vivo* effector mechanisms of the innate immunity can directly induce protein dependent *S. epidermidis* cell aggregation and biofilm formation, thereby enabling the pathogen to evade clearance by phagocytes.

Posters

Education & Training Group

ET 01 Embedding employability in the curriculum

Joy Perkins[1] & Peter Fantom[2]

[1]School of Medical Sciences, Foresterhill, Aberdeen University, Aberdeen AB25 2ZD; [2]Careers & Appointments Service, 48 College Bounds, Aberdeen University, Aberdeen AB24 3UX

'Working Out? Placement & Career Skills' is a new module for the academic year 2004-5. The module aims to provide increased support and guidance in order to help Level III Molecular & Cell Biology students secure an industrial placement. As well as providing undergraduate students in their penultimate year with essential careers information, the module covers the necessary skills to complete placement applications by exploring CV preparation, writing covering letters, completing application forms, skill identification and good interview techniques. The aim is to help students get the most from a placement and also to aid their career planning. Students are not guaranteed a placement by completing the module but material covered during the module will assist undergraduates to submit high quality placement applications and perform well during an interview to maximise their chances of success. This twelve-week module taught jointly by the School of Medical Sciences and the Careers & Appointments Service is a pre-requisite for all students wishing to undertake an industrial placement year and is strongly recommended as part of career development for all other Molecular and Cell Biology students. The module provides an example of how an existing curriculum may be 'tuned' in order to enhance students' employability.

ET 02 Working in the wilderness

Kyle Whyte[1] & **Joy Perkins**[2]

[1]Integrin Advanced Biosystems Ltd, Marine Resource Centre, Barcaldine, Oban, Argyll; [2]School of Medical Sciences, Foresterhill, Aberdeen University, Aberdeen AB25 2ZD

Bioscience undergraduates often have the opportunity to apply for industrial placements with large biology organisations in major cities. However, a small minority of students opt for placements in more remote locations, such as Integrin Advanced Biosystems, Oban; a marine biotechnology company based on the shore of Loch Creran in Argyll.

Integrin currently has two main areas of operations. Firstly, as a seafood safety company, Integrin tests for toxins hazardous to human health in shellfish for both the Food Standards Agency and private commercial companies. Secondly, the company performs biodiscovery and marine natural products research. Placement students are offered the unique experience of being involved in both aspects of the organisation and this poses both great rewards and challenges not available to placement students in multinational companies. Additionally, the family atmosphere and support available to placement students from staff at all levels makes work-based learning in a remote location more of a molehill than a mountain to climb! This presentation highlights the important role of small to medium sized enterprises (SMEs), in providing undergraduate work experience.

ET 03 Avenue Middle School Science Group: a happy relationship between a bacterium and a pea plant

Kay Yeoman[1], Jane Dye[2], Lucy Cousins[1], Liz Battle[1], Rebecca Noakes[1], Judy Geeson[1] & Juri Zarins[2]

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

A Royal Society partnership grant was awarded to Avenue Middle School and the School of Biological Sciences, University of East Anglia. Twenty five year seven pupils were given the opportunity to take part in an after school science club. The theme behind the club was the 'happy relationship between a bacterium and a pea plant'. Firstly, this involved the pupils knowing about micro-organisms and how to isolate and handle them. The pupils leant how to distinguish between Gram positive and negative bacteria using the Gram stain and microscopes. They leant how to use micropipettes and inoculated pea plants with *Rhizobium leguminosarum* to induce the formation of root nodules. The bacteria were then re-isolated from surface sterilised nodules induced on the pea roots and also from wild clover plants using streak plating. They extracted DNA from pea seeds and *R. leguminosarum* and analysed it using DNA gel electrophoresis. They learnt that DNA analysed in this way looks identical, despite coding for two very different organisms.

Posters

Fermentation & Bioprocessing Group

FB 01 Optimized expression of recombinant proteins in *Escherichia coli* using a variety of media

Paul Homes, Praveen K. Singh, Jo J. Jones, Adam N. Wong & Richard M. Hall

Gene Expression and Protein Biochemistry, GlaxoSmithKline, New Frontiers Science Park, Coldharbour Road, Harlow, Essex CM19 5AD (Email Paul.2.Homes@gsk.com)

In pursuit of over-expressed soluble recombinant proteins in *E. coli*, it is often essential to optimise growth conditions of the construct. One important method of producing soluble protein rather than as insoluble aggregates is the choice of media. Historically, Luria Bertani broth (LB) has been the medium of choice; however it is not always the medium that yields the highest biomass and/or soluble biologically active protein. Other media such as Modified terrific broth (MTB), as well as those available from external vendors has been evaluated in terms of solubility enhancement in comparison to LB.

In instances where insoluble aggregates are the only end product and refolding of inclusion bodies is successful (in that the correctly folded protein is biologically active), the production of a large amount of insoluble material is essential. This is due to the fact that refolding of proteins is expensive and inefficient.

A variety of proteins have been studied including human and microbial derived proteins, from a range of target classes including kinases and other enzymes. Different media have been evaluated in the production of over-expressed protein in both the soluble and insoluble fraction. We have shown that media plays a vital role in the optimisation of expressed protein in both shake flasks and bioreactors.

FB 02 A comparison of methods used to enhance the solubility of recombinant proteins expressed in *Escherichia coli*

Praveen K. Singh, Paul Homes, Jo J. Jones, Adam N. Wong & Richard M. Hall

Gene Expression and Protein Biochemistry, GlaxoSmithKline, New Frontiers Science Park, Coldharbour Road, Harlow, Essex CM19 5AD (Email Praveen.K.Singh@gsk.com)

Recombinant proteins are expressed in *E. coli* either in inclusion bodies or as soluble protein. In the soluble form, the recombinant protein is normally in the correct conformation and often biologically active, thus simplifying downstream processing. When expressing the protein, physical factors such as temperature, aeration and pH greatly influence expression. At the molecular level codon optimisation of the host strain and silent mutations in the gene sequence have been routinely employed. Co-expression of the recombinant protein with a fusion partner such as GST or the addition of a tag to facilitate solubility and purification has also been successfully applied. Supplementing the media with various additives like ethanol, osmolytes, amino acids and antibiotics can also greatly influence the solubility of recombinant proteins expressed in *E. coli*. We explore the potential of using certain additives on poorly expressed soluble proteins to enhance their expression. The effects of these additives on the solubility of various proteins are presented here.

FB 03 Continuous aseptic production of biosurfactants

Simon C. Baker[2], Chien-Yen Chen[1] & Richard C. Darton[1]

[1]Dept of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PJ; [2]School of Biological and Chemical Sciences, Birkbeck College, Malet Street, London WC1E 7HX

Biosurfactants are commercially important compounds with potential for use in environmental protection, petroleum, food, pharmaceutical and other industries. However, the high cost of recovery has limited their application even as fine chemicals. To address this problem, a reactor with integrated foam fractionation was designed and implemented for *in situ* product removal of a model biosurfactant, surfactin of *B. subtilis*. The foam in the reactor was routed through a foam fractionation column to a mechanical foam breaker and an approximately a 50-fold biosurfactant-enriched foamate was then collected. Surfactin A could then be precipitated after acidification, and was of sufficient purity for direct characterisation by mass spectroscopy. Continuous cultivation in a glucose-limited chemostat was then used to determine the growth parameters of *B. subtilis* BBK006 for biosurfactant production. The continuous cultivation exhibited low maintenance metabolism (m=0.39 $mmol_{glucose}\ g_{bacteria}^{-1}\ h^{-1}$) and high growth yield (=30.8 $g_{bacteria}\ mol_{glucose}^{-1}$). A high steady state concentration of surfactin (18 mg l^{-1}) was maintained at a dilution rate of 0.2 h^{-1} when glucose concentration of the feed was 0.25 g l^{-1}. This demonstration of continuous aseptic surfactin production illustrates a principle that could be applied to other surfactants which may be produced at low concentrations.

Posters

Food and Beverages Group

FdBev 01 Sites of injury in Salmonella typhimurium cells treated at different temperatures and water activity levels

Khalid M. Aljarallah & Martin R. Adams

Food Safety Research Group, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey GU2 7XH (Email k.aljarallah@surrey.ac.uk; m.adams@surrey.ac.uk)

It is generally thought that the heat resistance of bacterial cells increases as the water activity (a_w) of the medium decreases. However, evidence exists which suggests this is not the complete picture, as lowering a_w may increase or decrease heat resistance depending on factors such as heating temperature, level of a_w, matrix or humectant used and microbial strain.

Previous investigation (in our lab) found that at temperatures > 51°C, *Salmonella* Typhimurium cells at low a_w (0.94) were more heat resistant than those treated in high a_w (0.99) media. But, at < 51°C the reverse was true. This difference in behaviour could reflect different mechanisms of heat inactivation at the two temperatures

At 50°C the Decimal Reduction Times (D values) based on counts on non selective nutrient agar (NA) (detecting damaged and undamaged cells) and on NA+4% NaCl (detecting cells with undamaged cytoplasmic membrane) are very similar for the low aw heating medium. This indicates that the cells surviving this heat treatment at low aw do not carry cytoplasmic membrane injury. When the heating medium is nutrient broth (high a_w) the big difference in the D50 values on NA+4% NaCl and NA shows that a lot of the survivors do have cytoplasmic membrane injury. This suggests that during heating at low a_w at 50°C injury to the cytoplasmic membrane results in death where as in high aw media these injured cells survive.

Outer membrane injury (detecting using XLD agar) occurred under both high and low aw heating conditions to a comparable extent.

At 60°C the D values are lower on NA + 4% NaCl in both high and low a_w heating media suggesting that cytoplasmic membrane damage is not the principle cause of death at this temperature.

At 60°C, 16S and 23S RNA subunits showed more rapid degradation than at 50°C. However, while the stability of rRNA was similar in both high and low a_w media at 50°C, degradation was much reduced by low a_w at 60°C.

Acknowledgments Grateful gratitude to the Saudi Arabian Standards Organisation (SASO) for sponsoring this study

FdBev 02 Effect of pulsed UV light treatment on waterborne bacterial pathogens

A. Andoh, J.G. Anderson & S.J. MacGregor

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

Over many years, various methods have been developed for water treatment and disinfection. Recent advances have indicated that pulsed UV-rich light technology represents a novel treatment that can be exploited to inactivate waterborne pathogens.

In this study, four Gram negative organisms of concern in water microbiology were compared for their susceptibilities to pulsed UV light. The organisms used were *Escherichia coli* NCTC 9001, *Aeromonas hydrophila* NCTC 8049, *Pseudomonas aeruginosa* LMG 9009 and *Vibrio cholerae* NCTC 11348. These organisms were cultured in nutrient broth in shake flasks for 18 hours at 37 °C for *E. coli* and at 30 °C for *P. aeruginosa, V. cholerae,* and *A. hydrophila*. The organisms were centrifuged, re-suspended in phosphate buffered saline and 20ml added to open top Petri dishes which were then subjected to 5, 10, 20, 40, and 80 pulses of UV-rich light and surviving populations enumerated using the spiral plate method. The results showed that pulsed UV-rich light was highly effective for inactivation of all four species of bacteria. Under the test conditions used *Vibrio cholerae* was the most resistant of the four organisms although further tests are being carried out to confirm this finding under a range of exposure conditions.

FdBev 03 Development of specific oligonucleotide probes to detect different *Vibrio* species

Umberto Molini[1,2], Narut Thanantong[1], Annunziata Giangaspero[3] & **Olivier Sparagano**[1]

[1]School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne NE1 7RU; [2]Dept of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, Università degli Studi di Teramo, Teramo, Italy; [3]Dept PR.I.M.E., Università degli Studi di Foggia, Foggia, Italy

Vibriosis is an economically important disease of fish, marine invertebrates (particularly Penaeid shrimps) and large marine mammals responsible for high mortality rate in aquaculture in diverse farming locations throughout the world. Some Vibrio species are also responsible for zoonoses, whereas, others are relatively non-pathogenic. Two Vibrio species; *V. cholerae* and *V. vulnificus* are known to be extremely debilitating when they infect humans; contamination being either made by direct contact or through water and food consumption. Furthermore some Vibrio species can become more pathogenic when their hosts go through higher stress conditions.

The aim of this study was to obtain, from a phylogenetic analysis of the 23S ribosomal RNA (rRNA) gene, sequences of different *Vibrio* species coming from marine and human organisms.

A 474 bp fragment of the 23S rRNA was amplified by PCR using specific *Vibrio* primers. Amplified fragments were sequenced and a Blast analysis allowed the identification species-specific DNA zones to be used as potential probes. Species-specific probes were identified for *V. parahaemolyticus*, *V. fortis*, *V. lentus* and for two clusters of taxonomically related species: *V. harveyi/campbelli* and *V. splendidus/aesturianus*.

A reverse line blot assay showed that the designed probes can specifically detect the different *Vibrio* species proving that these probes can be used as a fast, accurate and economical technique to value the presence of pathogenic/non-pathogenic *Vibrio* species in the sea and from marine organisms to investigate environmental risks.

FdBev 04 Identification and antimicrobial activity of Bacillus spp. isolated from Bikalga, an alkaline fermented product

A.H. Varnam & I. Ouoba

Food Microbiology Unit, Dept of Health & Human Sciences, London Metropolitan University, North Campus, 166-220 Holloway Road, London N7 8DB

Bikalga is an alkaline fermented food prepared from the seeds of *Hibiscus sabdariffa*. Bikalga is used as a condiment in Burkina Faso and is traditionally associated with health benefits. This work was undertaken to identify the species of *Bacillus* that mediate fermentation and to make a preliminary assessment of their antimicrobial properties.

Bacillus spp. were isolated from Bikalga produced in different regions of Burkina Faso. Twenty isolates were chosen at random for identification by sequencing the chromosomal 16S rRNA gene. The antimicrobial activity of the same isolates against foodborne pathogens was investigated using agar plate inhibition tests.

Seven isolates were identified as *B. subtilis*, six as *B. licheniformis* and three as *B. sphaericus*. Isolates were also identified as *B. cereus*, *B. badius*, *B. pumilus* and *Brevibacillus bortelensis*. Antimicrobial activity of the *Bacillus* isolates was variable according to the *Bacillus* isolate and the pathogen. Two isolates of *B. subtilis* were able to inhibit all the pathogens while two isolates of *B. licheniformis* and *Brevibacillus bortelensis* had no inhibitory activity.

B. subtilis and *B. licheniformis* are of greatest importance in production of Bikalga, irrespective of geographical origin and methods of production. Antimicrobial activity is variable, but may be beneficial with some strains.

FdBev 05 An investigation of the probiotic potential of bacteria isolated during fermentation of a sorghum drink

T. Mashababe & A.H. Varnam

Food Microbiology Unit, Dept of Health & Human Sciences, London Metropolitan University, North Campus, 166-220 Holloway Road, London N7 8DB

Fermented sorghum products are popular weaning foods in sub-Saharan Africa and may have a protective effect against diarrhoeal disease. This work was undertaken to investigate the probiotic potential of lactic acid bacteria (LAB) isolated from the fermentation and the possibility of developing a sorghum-based probiotic drink.

Sorghum-based drink was made using (i) a probiotic strain *L. acidophilus* 701748-FIMBC (ii) a natural fermentation (no starter). Fermentation was at 37° for 48 h and drinks were then stored for 21 days at 5° and 25°C. Numbers of LAB were determined at regular intervals. Selected isolates were identified and tested for tolerance to bile and gastric acidity and inhibitory activity against common foodborne pathogens.

Numbers of LAB were in the order of 10^8 at the end of fermentations and showed only limited fall over 21 days. Isolates were identified as *Lactobacillus acidophilus, Enterococcus hirae* and *Pediococcus pentosaceus*. All isolates from natural fermentations were resistant to bile and gastric acidity. Inhibitory activity was demonstrated against all pathogens tested except *B. cereus*.

Fermented sorghum drink provides a stable vehicle for probiotic bacteria. Isolates of LAB from natural fermentations had *in-vitro* properties similar to those of the control strain and may provide the basis for the wider application of probiotics in Africa.

FdBev 06 Effects of Lactobacilli against *Helicobacter pylori* in a gel stabilized glucose-gradient system

H. Abdollahi & E. Reza-Zadeh

Microbiology Dept, Kerman Medical School, Kerman, Iran

Background *H. pylori* resides in human gastric mucosa and may cause gastritis, peptic and duodenal ulcers. *Lactobacilli* resist acidic condition of stomach and are regarded as important probiotic. Their effects against a locally isolated *H. pylori* were tested in a novel laboratory model.

Methods The system consisted of a solid layer (PYS medium plus glucose) at the bottom and a semi-solid layer (same medium without glucose but with known number of pure or mixed bacterial cells) at the top, set in large tubes and incubated at various conditions. Viable counts, pH, glucose, and optical density of segments from sample cores were determined.

Results 1) *L. plantarum* was able to eliminate *H. pylori*. 2) The pattern of band formation in mixed cultures was similar to pure *Lactobacillus*. 3) *H. pylori* was able to use glucose and lower pH. 4) The number of bands (condensed bacterial growth) increased with incubation time.

Conclusion Opposing gradients of glucose and oxygen allowed bacteria to select the preferred positions to form specific patterns of bands. We found *L. plantarum* eliminating *H. pylori* mainly by lactic acid and bacteriocin production.

Posters

Microbial Infection Group

MI 01 Identification and characterization of the O-antigen gene cluster of *Francisella tularensis* subsp. *novicida*

Rebecca M. Thomas, Joann L. Prior, Stephen L. Michell & Richard W. Titball

Dstl, Porton Down, Salisbury SP4 0JQ

Francisella tularensis is the causative agent of tularemia, a disease affecting many mammalian species. The infectious dose of *F. tularensis* in humans by the aerosol route is as low as 10 cells and for this reason the organism is considered as having the potential for misuse as an agent for biological terrorism. *F. tularensis* has been divided into four subspecies: *F. tularensis* subsp. *tularensis* is the most virulent. *F. tularensis* subsp. *holarctica* is less virulent and *F. tularensis* subsp. *mediaasiatica* is considered to be of relatively low virulence. *F. tularensis* subsp. *novicida* is considered to be pathogenic only in immunocompromised humans.

The structures of the O antigens of the LPS of *F. tularensis* subsp. *novicida* and *tularensis* are known, however the genetic information for subsp. *novicida* is unknown. Based upon sequence similarities to strain Schu S4 a gene cluster has been identified in *F. tularensis* subsp. *novicida*. Putative functions were assigned and they indicate that the gene cluster is involved in LPS O antigen biosynthesis. The *F. novicida* gene cluster was functionally disrupted by the mutation of a single gene and the mutant strain was characterised.

MI 02 The development of assays to measure DAM activity

R. Wood[1], R. Griffiths[2], **V. Taylor**[2], R. Titball[2] & P. Roach[1]

[1]School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ; [2]Dstl, Porton Down, Salisbury SP4 0JQ

DNA adenine methylation (DAM) plays important roles in many bacteria and has been shown to have a role in virulence. Inactivation of the *dam* gene in *Salmonella enterica* serovar Typhimurium, *Yersinia pestis* and *Yersinia pseudotuberculosis* has resulted in attenuation. Therefore, inhibitors specific in blocking adenine methylation may be suitable as broad-spectrum antibiotics. DAM inhibitors must be able to cross the cell membrane in order to be developed as antibacterials. We have developed assays that measure the activity of DAM in the presence of potential inhibitors and the ability of those compounds to cross the cell membrane. In the longer term this might allow a new class of antimicrobial compounds to be developed.

MI 03 Dam inhibitors: the construction of the bioassay sensor

R. Griffiths, V.L. Taylor, P.C.F. Oyston & R.W. Titball

Dstl, Porton Down, Salisbury SP4 0JQ

DNA adenine methylation (DAM) plays a role in several main cell functions. One of these functions is in the post-replicative repair process of the bacterial genome. In the absence of DAM methylation, increased rates of spontaneous and inducted mutations are observed. DAM has been shown in to play a role in virulence and inhibitors of DAM have been shown to be feasible. There is an urgent need for generic antibacterials as alternatives to antibiotics due to the increase of bacterial resistance. DAM inhibitors would be effective countermeasures, and difficult to defeat since the target for DAM is DNA. The construction of a bioassay sensor forms part of the project. The construction of a bioassay sensor would allow candidate compounds to be screened in an in vivo assay to test their ability to reduce the growth of an *Escherichia coli dam* mutant. The minimum inhibitory concentrations of the compounds could then be determined. The screening work would also demonstrate the ability of the compounds to cross the bacterial cell wall. The *dam* gene from *E. coli* was inactivated and replaced with a functional copy from *Yersinia pestis* to produce the *E. coli dam* mutant.

MI 04 Does *Francisella tularensis* subspecies *tularensis* express a *Francisella tularensis* subspecies *novicida*-like polysaccharide?

Joann L. Prior, Gill M. Hartley, Rebecca M. Thomas & Richard W. Titball1,2

Dstl, Porton Down, Salisbury SP4 0JQ

Francisella tularensis subspecies *holarctica* produces a lipopolysaccharide (LPS) containing an O-antigen (O-ag), the structure of which has been determined. When individuals are immunised with the live vaccine strain (LVS) of *F. tularensis* (subsp. *holarctica*) their sera has been shown to contain antibodies which react with the LPS O-ag of this bacterium. When this sera is screened with LPS from *Francisella tularensis* subspecies *novicida* there are no antibodies present which recognise this LPS. However, sera taken from individuals who have had a Tularemia infection (convalescent sera) contain antibodies which recognise both *Francisella tularensis* subspecies *holarctica* and *Francisella tularensis* subspecies *novicida* LPS. Possible explanations for this phenomenon include the possibility that *Francisella tularensis* has the ability to express both types of LPS O-ag or that at different stages in infection different epitopes of the LPS O-ag are revealed so that different antibodies are generated in the immune response.

MI 05 A *capB* mutant of *Francisella tularensis* subspecies *tularenisis* is attenuated in a mouse model of infection

Stephen L. Michell, Petra C.F. Oyston & Rick W. Titball

Biomedical Sciences, Dstl Porton Down, Salisbury SP4 0JQ

Francisella tularensis is a Gram negative bacteria that has been described as the most infectious pathogen known to man. The recent completion of the genome sequence of the virulent stain *tularensis* subspecies *tularensis* SchuS4 revealed a genome of 1.8Mb encoding approximately 1800 genes. Analysis of the genome failed to reveal the presence of such classical virulence factors such as toxins or type III secretion systems that predominate in many other pathogens. Interestingly the genome analysis revealed a region encoding an operon of genes with similarity to the cap operon of *Bacillus anthracis*. This region in *B. anthracis* winch includes the genes *capBCAD* encodes for the capsule poly-D-glutamic acid, which is an essential virulence factor of anthrax. Historically it has been observed that a thickening of a capsule of *F. tularensis* has correlated with an increase in its virulence. Whether the *cap* cluster of genes in *Francisella* encodes a capsule of similar structure to that of *B. anthracis* remains to be determined. The effect of inactivation of *capB* in *F. tularensis* on virulence and antigenicity will be presented.

MI 06 Detection and suppression of *Verticillium albo-atrum* aggressive on Ve-resistant tomato cultivars

Vinodh Krishnamurthy[1], Matt Dickinson[1], Tim O'Neill[2] & Steve Rossall[1]

[1]Division of Plant Science, University of Nottingham, LE12 5RD; [2]ADAS Arthur Rickwood, Mepal, Ely CB6 2BA

Commercial tomato varieties carrying a single resistant gene (Ve) has previously provided good control for wilt pathogen, *Verticillium*

albo-atrum. However, since 1995 strains of *V. albo-atrum* have infected and caused disease in Ve-resistant tomato varieties in the UK and Netherlands. Traditionally *V. albo-atrum* is a soil-borne pathogen and the classic mode of entry into the host is through the root. However, current outbreaks in tomato have rockwool substrates which have no direct contact with the soil. These 'new strains' of *V. albo-atrum* threaten the profitability of UK glasshouse tomato production. Our study investigates the epidemiology and control of *V. albo-atrum* within glasshouses.

A PCR assay using *V. albo-atrum* specific primers derived from random genomic probes was developed to detect *V. albo-atrum*. This molecular method is been used to examine potential inoculums sources in glasshouses including water, air-borne spores, insects, seeds, plant debris and dripper pegs. Our experiments have identified that these 'new strains' of *V. albo-atrum* spores were able to infect rockwool grown tomatoes through root drenching and fresh de-leaf scars. A range of bacteria and fungi isolated from roots and stems of tomatoes are being examined as possible components of an integrated control strategy that could be used alone, or in conjunction with disinfection, to control Verticillium wilt outbreak in glasshouses.

MI 07 Characterization of a prolipoprotein diacyl glyceryl transferase (*lgt*) mutant of *Staphylococcus aureus*

Alan Cockayne[1], Julie Morrissey[2] & Paul Williams[1]

[1]Centre for Biomolecular Sciences, University Park, Nottingham; [2]Dept of Genetics, University of Leicester

Lipoproteins are post-translationally modified, secreted proteins with important functions in several key areas of bacterial physiology. These include roles in solute transport, cell wall biosynthesis and protein secretion. In order to investigate the importance of protein lipidation in these processes we have constructed an *lgt* mutant in the Gram positive pathogen *Staphylococcus aureus*.

In addition to the predicted effects of *lgt* mutation on lipoprotein anchorage to the staphylococcal cytoplasmic membrane, the *lgt* mutant showed an altered cell wall protein profile, reduced iron content and increased siderophore production consistent with a defect in iron transport or storage. The *lgt* mutant also showed an altered cell morphology suggestive of alteration in autolysin production or regulation. Enhanced autolytic activity was also suggested by increased sensitivity of the mutant to Triton X-100-induced cell lysis and alteration in autolysin profiles.

These observations show that inhibition of protein lipidation and its effect on targetting of lipoproteins to the cytoplasmic membrane has a significant, pleiotropic effect on several important aspects of cell physiology in *S. aureus*. It seems likely that *lgt* mutation will also reduce the virulence of *S. aureus* and protein lipidation may therefore represent an interesting alternative target for development of new anti-staphylococcal agents.

MI 08 Characterization of an atypical EPEC strain associated with food-borne infection in adults

Amy L. Wedley, Peter A. Chapman & Jonathan N. Fletcher

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

Enteropathogenic *E. coli* (EPEC) strains are commonly associated with infantile diarrhoea in developing countries, and with decreasing frequency outbreaks and sporadic cases in infants in developed countries. Cases of disease in adults, though not unknown, are rare, and the proven acquisition via contaminated food is highly unusual. In the latter part of 1997 59 people from two separate coach parties to Northern France suffered gastrointestinal illness, which was epidemiologically linked to the consumption of food in a French Restaurant. From a majority of cases *E. coli* serogroup O111 was isolated, with no other single pathogen being identified. The *E. coli* was putatively classified as EPEC on the basis of its hybrisation with an *eae* (intimin) gene probe, and the lack of detection of virulence factors of other *E. coli* virotypes. In this study we describe the further characterisation of this strain. PCR analysis confirmed the presence of the genes at each end of the locus of enterocyte effacement (LEE), which together with the previously located central *eae* gene implies that the entire 37kbp pathogenicity island is present. The strain adhered sparsely to HEp-2 cells in culture in a diffuse manner, but the FAS test revealed the accumulation of polymerised actin beneath the adherent bacteria. The results confirm the strain as an atypical (diffusely adherent) EPEC, and represent one of the first instances of EPEC being able to cause food poisoning in adults.

MI 09 Survival of toxigenic bacteria in the cot environment

R.O. Jenkins & R.E. Sherburn

School Allied Health Sciences, De Montfort University, The Gateway, Leicester

Infection and toxin production by commonly occurring bacteria, and by *Staphylococcus aureus* in particular, is thought to be a trigger for the physiological events that result in sudden infant death syndrome (SIDS). Mattresses with exposed polyurethane foam and previous use of these types of mattresses by another child have been shown to be significantly associated with increased frequency of isolation of *S. aureus*. The objective of the present study was to compare growth and survival of selected bacteria implicated in SIDS on cot mattress materials.

Good survival capability (>206 days) was shown by *S. aureus*, *Escherichia coli* and *Streptococcus pyogenes* on PU inner-foam and on polyester mattress cover at high RH (75%), but only by *S. aureus* on PU at low RH (25%). Removal of aromatic amine functionality from culture medium containing PU leachate during growth of *S. aureus* suggests that low molecular weight oligomeric molecules arising during PU manufacture, but not linked into the polymer network, are utilizable nutrients. Their presence in PU foams could promote growth and/or prolong survival of potentially harmful bacteria with cot mattresses. Prolonged survival of *S. aureus* on PU at low RH could explain, in the context of the common bacterial toxins hypothesis, the increased risk of SIDS associated with previously used infant mattresses.

MI 10 Biofilm formation in *Campylobacter jejuni* is enhanced by the addition of lysed horse blood

R. Dowling, D. Greenway & R. England

Dept of Biological Sciences, University of Central Lancashire, Preston, Lancashire PR1 2HE

Campylobacter jejuni is the most common bacterial cause of gastroenteritis world-wide. The main transmission route is the consumption of livestock, particularly poultry. Biofilms can provide protection from environmental stresses and antimicrobial agents. *C. jejuni* has been shown to form single species biofilms and can also become incorporated into already established biofilms, which may aid its transmission.

Biofilm formation of five isolates of *C. jejuni* from human faecal samples and livestock was assessed using a modified 96-well microtitre plate and crystal violet method. There was a large increase in biofilm when the *C. jejuni* were grown in Bolton Broth with added 7% lysed horse blood. This increase was not seen when the equivalent of 7% horse serum is added to Bolton Broth, suggesting the active component causing the increase in biofilm is to be found elsewhere in the blood.

MI 11 Post-translational modification of streptococcal proteins

Renata Soares, James Paddick, Susmitha Rao, David Beighton & Karen Homer

Dept of Microbiology, The Dental Institute, King's College London, Guy's Hospital, London SE1 9RT

Streptococcus mutans and *Streptococcus oralis* are aciduric bacteria, capable of survival and growth at low pH. Proteomic analysis (two-dimensional gel electrophoresis and protein identification using liquid chromatography/tandem mass spectrometry [LC-MS/MS]) of bacterial cells cultured at neutral and low pH demonstrated that many proteins

had different isoforms which may arise as a result of post-translational modification. These included the glycolytic enzymes, enolase and glyceraldehyde-3-phosphate dehydrogenase, and HPr, a component of the bacterial PTS. Expression of individual protein isoforms was dependent on the pH of the culture medium. Use of a phosphoprotein-specific dye demonstrated that some of the proteins in cellular extracts were phosphorylated. Enrichment of proteins with potential phosphorylation sites was achieved by gel permeation chromatography and two-dimensional gel electrophoresis. Proteins were digested in-gel with proteases including trypsin, and LC-MS/MS was used to determine potential sites of post-translation modification. Matrix-assisted laser desorption/ionisation and electrospray sources were used to introduce peptides into a linear ion trap/Fourier transform ion cyclotron resonance mass spectrometer which enabled the characterisation of phosphorylation sites. Our data suggest that phosphorylation is a key way in which streptococcal proteins are modified post-translation in response to alterations in environmental conditions.

MI 12 *Balamuthia mandrillaris* exhibit metalloprotease activities

Abdul Matin[1], Monique Stins[2], Kwang Sik Kim[2] & Naveed Ahmed Khan[1]

[1]School of Biological and Chemical Sciences, Birkbeck, University of London, London; [2]Division of Paediatric Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Balamuthia mandrillaris is a recently identified protozoan pathogen that can cause fatal granulomatous encephalitis in humans and animals. However, the pathogenesis and pathophysiology of *B. mandrillaris* encephalitis remains unclear. Since proteases may play a role in the central nervous system pathology, we used spectrophotometric, cytopathic, and zymographic assays to assess protease activities of *B. mandrillaris*. Using two clinical isolates of *B. mandrillaris* (from human and baboon), we observed that *B. mandrillaris* exhibit protease activities. Zymography assays revealed several major protease bands of approximate molecular weights in the region of 40–49KD on SDS-PAGE gels using gelatin as substrate. The protease bands were inhibited with 1,10-phenanthroline, suggesting metallo-type proteases. These proteases exhibited activity over a pH range of 5 to 11 with maximum activity at neutral pH. Among variable temperatures, optimum activity was observed at 37–42°C. *Balamuthia mandrillaris* proteases are able to degrade extracellular matrix (ECM), which provide structural and functional support to the brain tissue. This is shown by degradation of collagen I and III (major components of collagenous ECM), elastin (elastic fibrils of ECM), plasminogen (involved in proteolytic degradation of ECM), casein (breakdown products possess opiate activities) but not haemoglobin. This is the first demonstration that *B. mandrillaris* exhibit metalloproteases, which may play important role(s) in the CNS pathology.

MI 13 Characterization of the carbohydrate moieties of the surface layer proteins from *Clostridium difficile*

Omar Qazi[1], Paul Hitchen[2], Emanuela Calabi[1], Stuart Haslam[2], Anne Dell[2] & Neil Fairweather[1]

[1]Division of Cell and Molecular Biology, Imperial College London; [2]Division of Molecular Biosciences, Faculty of Life Sciences, South Kensington Campus, Imperial College London

Antibiotic-associated diarrhoea (AAD) is caused by infection with *Clostridium difficile*. *C. difficile* is an opportunistic, nosocomial bacterial pathogen, with tissue damage primarily induced by the action of two toxins (TcdA and Tcd B). The mechanism of gut colonisation is not well characterised and may involve bacterial surface associated proteins. Like many other bacteria, *C. difficile* displays a cell surface layer (S-layer). The surface layer of *C. difficile* is composed of two surface layer proteins (SLPs); one of ~ 45 kDa and the second of ~ 36 kDa. The roles of bacterial S-layers are in general poorly understood, but various functions have been proposed including host-cell interaction in pathogens.

Many bacterial species have been shown to exhibit glycosylated SLPs and, in some cases, the glycans have been extensively characterised. SLPs from a number of strains of *C. difficile* were extracted using a variety of techniques and were analysed for the presence of glycans by digoxigenin labelling of oxidised sugars followed by Western blotting. The LMW SLP proteins of some of the *C. difficile* strains analysed were found to be glycosylated. Furthermore, we have utilised glycoproteomics in the structural characterisation of the carbohydrate moieties present and in order to identify the sites of glycan occupancy.

MI 14 Withdrawn

MI 15 Molecular validation of a combinatorial probe panel for genotyping pathogens

Matthew Partington[1], Jonathan West[1], John Perry[2] & Olivier Sparagano[3]

[1]Institute of Nanoscale Science and Technology, University of Newcastle; [2]Microbiology Dept, Freeman Hospital, Newcastle upon Tyne NE7 7DN; [3]School of Agriculture Food and Rural Development, University of Newcastle, NE1 7RU (Tel +44 191 222 5071; Fax +44 191 222 7811; Email Olivier.Sparagano@ncl.ac.uk)

Molecular techniques are paving the way to replace standard culturing diagnostic methods. We have focused on blood stream infection (BSI) diagnostics, with the goal to identify pathogenic bacteria and fungi in blood samples to enable effective antibiotic prescription. In selecting the appropriate molecule for detection there are two important factors; (i) is the molecule present in high copy numbers to obviate amplification chemistries such as PCR, and (ii) is there sufficient sequence variation to discriminate between the different pathogens?

The ribosomal RNA small subunit sequence (16S for bacteria or 18S for fungi), is present in high copy numbers (20,000-100,000 per cell) and present the possibility for identification without PCR. A bioinformatics approach to the second question revealed there is not enough variation for a conventional one target one probe approach. Instead we have developed a novel combinatorial probing method involving the use of a matrix of 24 probes. These will be used solid phase hybridisation to enable the multiplexed identification of 30 different BSI pathogens. Combinatorial probing involves multiple probes which produce a unique hybridisation pattern for each different rRNA sequence.-

The validity of these *in silico* designed probes has been tested using the reverse line blot (RLB), a powerful array system that enables high throughput multiplexed sequence detection, analysing a maximum 43 different samples each with 43 different probes. Briefly, the RLB is a 2-D hybridisation technique akin to Southern blot analysis but instead using a panel of probes, each separated by parallel channels. Further work will involve optimisation of the RLB assay conditions and miniaturisation in the form of a micromosaic assay. Ultimately these can be combined with sample preparation within a micro Total Analysis System (μ TAS) platform for rapid and automated diagnostics at the point of care.

MI 16 Withdrawn

MI 17 Methanolic extract of *Tamarindus indica* Linn. inhibits growth of clinical strains of *Burkholderia pseudomallei* – the first ever herbal activity reported against *Burkholderia pseudomallei*

Esaki Muthu Shankar, Subhadra Nandakumar & Usha Anand Rao

Burkholderia Laboratory, Dept of Microbiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, Tamilnadu–600 113, India

Burkholderia pseudomallei (Pseudomonas pseudomallei) causes melioidosis, a life-threatening infection common among paddy cultivators in south-east Asian countries. No plant materials have been investigated for its activity against *Burkholderia pseudomallei* till date. Therefore, a preliminary study was carried out using disc diffusion and minimum inhibitory concentration methods to evaluate the anti-*Burkholderia pseudomallei* activity of five Indian medicinal plants

documented to have been used for several ailments in the ancient Indian scriptures. The leaf extracts of *Tamarindus indica*, *Lawsonia inermis*, and *Hibiscus rosa-sinensis*, the rhizome extracts of *Curcuma longa* and the seeds of *Vigna radiata* were prepared using methanol as solvent. The disc diffusion and minimum inhibitory concentration methods were used to assess the anti-*Burkholderia pseudomallei* activity of the plants tested. Of the five plant extracts, methanol leaf extracts of *Tamarindus indica* exhibited anti-*Burkholderia pseudomallei* activity starting from disc concentrations of 150 µg by the disc diffusion method. The other plants failed to show any zone of inhibition. Minimum inhibitory concentration assay revealed that the extract was inhibitory for *Burkholderia pseudomallei* upto 125 µg/ml. Our preliminary finding has shown that methanolic extracts of *Tamarindus indica* has anti-*Burkholderia pseudomallei* inhibitory potentials under *in vitro* conditions. Extensive animal studies may be required before investigating the role of *Tamarindus indica* for treating melioidosis.

Keywords *Burkholderia pseudomallei*; *Tamarindus indica*; melioidosis

MI 18 Recovery rates of *Mycoplasma pneumoniae* in induced sputum and throat swab specimens of HIV infected patients at an AIDS Care Centre in Chennai, South India

Esaki Muthu Shankar[1], N. Kumarasamy[2], R. Krishnakumar[1], P. Balakrishnan[2], Suniti Solomon[2] & Usha Anand Rao[1]

[1]Mycoplasma Laboratory, Dept of Microbiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, India; [2]YRG Centre for AIDS Research & Education, VHS, Taramani, Chennai, India

Mycoplasma pneumoniae has been associated with community-acquired pneumonia and mild acute respiratory infections in normal population. The exact incidence of this mollicute in HIV infected patients has never been reported from India. Mycoplasmas have also been reported to act as cofactors in AIDS progression. Numerous authors have reported comparative studies on different pulmonary specimens for isolating *Mycoplasma pneumoniae* from normal population. The present study has been carried out in HIV infected patients to compare the recovery rates of *Mycoplasma pneumoniae* from induced sputum and throat swab specimens of HIV infected patients. Seventy five induced sputum and throat swab specimens were collected from HIV infected patients, who were presenting with underlying pulmonary complaints and whose clinical presentation was consistent with disease caused by *Mycoplasma pneumoniae*, after obtaining informed consent subsequent to approval by the Institutional Review Board (IRB) on human ethics. Patients screened were from the age groups ranging from 18 to 60 years, whose respiratory specimens were cultured on PPLO glucose agar and broth, the later with 1% methylene blue. Presumptive identification of *Mycoplasma pneumoniae* was carried out using guidelines proposed by the *Subcommittee on the Taxonomy of Mollicutes, 1979*. The respiratory specimens from the HIV-infected subjects were analysed for their recovery rates, incidence of other bacterial pathogens, AFB, *Pneumocystis carinii* and their correlation features with CD4+ and CD4 lymphocyte % were also analysed and compared. The detection rate of *M. pneumoniae* was found to be high in patients with depleted CD4 levels. The study has shown that CD4 depletion may enhance mycoplasma infection in the respiratory tracts of HIV infected patients.

Keywords Community-acquired pneumonia; CD4; induced sputum; mollicutes

MI 19 Protective immune response to whole *Leishmania donovani* antigen fractions in Indian Kala Azar patients

Parul Tripathi[1], S. Ray[1], S. Sunder[2], A. Dube[3] & S. Naik[1]

[1]Dept of Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow; [2]Dept of Medicine, Bhu, Varanasi; [3]Division of Parasitology, Cdri, Lucknow, India

Background Human visceral leishmaniasis (VL), also known as Kala-azar (KA) in India is a systemic progressive disease caused by *L. donovani*. In leishmaniasis, T cell responses are correlated with recovery from and resistance to disease and resolution of infection results in life long immunity against the disease.

Objective To identify potential T cell stimulatory antigens of *L. donovani*.

Methods In the present study, we have evaluated whole cell extract (WE) from *L. donovani* (2001 strain, recent Indian isolate), and eleven antigenic fractions (F1-F11, molecular weight range of 139-24.2Kd) by determining their ability to induce in vitro T cell proliferation and cytokine production in peripheral blood lymphocytes (PBMCs) using Lymphocyte proliferation assay and ELISA from patients with history of VL, household endemic contacts and non endemic healthy controls.

Results We have observed positive proliferative response to WE in 21 of the 24 exposed individuals, all 14 cured patients and 7 of the ten endemic contacts and none of the 20 healthy controls. WE activated IFN-γ, IL-12, IL-10 levels were higher in the treated patients and endemic contacts than in healthy controls; IL-4 was not detectable in any of the samples. Further frequent proliferative responses were seen to fractions F1-F4 in the molecular weight range of 139-64.2Kd in both patients and endemic contacts. F1-F11 stimulated variable levels of IFN-γ, IL-12 and IL-10 in patients and contacts. Responses were also seen against fractions that did not induce proliferation. The IFN-γ responses were higher in contacts than in patients while the IL-10 responses were more in patients than in contacts. Low molecular weight fractions were able to stimulate IL-10 responses in treated patients and contacts.

Conclusion These data demonstrate the presence of immunostimulatory antigens in the high molecular weight fractions of whole *L. donovani* antigen. However, these fractions do not stimulate a pure Th1 response and produce variable amounts of IFN-γ and the regulatory cytokine, IL-10. Hence, these high molecular weight immunostimulatory fractions need to be evaluated in greater depth for their possible role as protective antigens.

MI 20 Protective immune responses induced in chickens by outer membrane proteins extracted from different strains of *Escherichia coli*

Mohammad Kazem Sharifi-Yazdi

Dept of Medical Laboratory Sciences, Faculty of Para Medicine, Tehran University of Medical Sciences, Tehran, Iran

Different strains of *Escherichia coli* from human, chickens, and the common strain between human and chickens were isolated and typed with mono-specific antibody. The E.coli strains from each group of human, chicken and common between human and chicken were selected. The polypeptide patterns of selected strains were analyzed and compared with each other by SDS-PAGE. The SDS-PAGE patterns between the strains were very similar, although the densities of some of the peptide bands were different, and some bands were absent in some strains. A similar comparison was made on extracted outer membrane proteins (OMP) using Triton X-100 and sodium dodecyle sulphate on above-mentioned strains. The polypeptide patterns between the strains 078 (chicken strain), 06 (human strain) and 02 (the common strain between human and chicken) were also very similar and two major bands with molecular weights of 44 KD and 25 KD were very distinctive, and were present in all the strains.

MI 21 Withdrawn

MI 22 Assessment of cytokines production in brucellosis

A. Keyhani & A. Rafiee

Dept of Immunology-Medical school-Tehran University, Tehran, Iran

The aim of this study was to investigate the level of some cytokines in patients with brucellosis and to determine the correlation of this parameters with each others and two clinical forms of disease.The study was conducted on 26 subjects,15 patients and 11 healthy individuals with no history of brucella infection. Brucella disease

was identified by increased brucella antibodies in serological tests in addition to compatible clinical symptoms. Diluted whole blood samples were cultured in the presence of either mitogen,heat inactivated bacteria or medium alone.IL-12,IL-10 and Gamma-INF were measured by specific sandwich ELISA. Gamma-INF production was significantly decreased in response to brucella antigen in the chronic groupe of patients.In contrast ,IL-12 production in whole blood culture of chronic group was higher than the acute group of patients.IL-10 production was also augmented in chronic group but without any corellaton to Gamma-INF production. In conclusion the corellation of Th-2 cytokines production and progression of chronic human brucellosis was not demostrated. Nevertheless, diminished production of Th-1 cytokines in chronic group of patients may suggest the T-cells unresponsiveness to brucella antigen which helps prolongation of brucellosis in chronic group of patients.

MI 23 Incidence of tubercle bacillus and other micro-organisms in sputum of volunteered and untreated coughing individuals in Ondo State, Nigeria

F.C. Akharaiyi & **F.O. Omoya**

Dept of Microbiology, Federal University of Technology, P.M.B 704, Akure, Nigeria

Twelve towns in Ondo State were visited monthly for a period of one year and sputum samples were collected from individuals having cough and not on medication or treatment whatsoever. Six hundred (600) samples were collected in each locality from people between age 5–80 years. A total of 7200 sputum samples were examined where, 4127 were Tb positive. Igbokoda and Ikare had highest Tb percentage positive of 6.6 % each follow by Okitipupa and Idanre with 6.5% each. The list was however different in Ondo town with 153 positive having 2.1%. The highest Tb frequency was noted in age group 59–65 with 186 positive out of 1, 037 samples examined, followed by age group 47–58 with 154 positive out of 1,065 samples examined and list in age group 5–20 having 4 positive out of 956 samples. Among the twelve towns surveyed for this study, there was none without Tb infection in the sputum examined. The towns mostly affected were Igbokoda, Ikare, Idanre and Okitipupa with 478, 476, 471 and 466 positive smear respectively. Majority of positive cases were from the farming and trading individuals. Apart from *Mycobacterium tuberculosis*, seven other bacteria and one yeast specie were also isolated.

MI 24 Low health seeking behaviour and inadequate personal hygiene; the risk factors for prevalence of *Gardenerella vaginalis* infection [bacterial vaginosis] among young women in developing countries

Oluwafemi I. Olawuyi[1] & Bello Ominiyi[2]

[1]School of Medical Laboratory Science, University College Hospital, Ibadan, Nigeria; [2]Advocacy for Human Education, Awareness and Development, Ibadan, Nigeria

Introduction *Gardenerella vaginallis* is the most causative agent of bacteria vaginosis, a polymicrobial, non-inflammatory syndrome involving genital tract. The pathogenic development and relationship of the bacillus to the disease, was well studied to establish the hypothesis that it promotes the disease, and that the risk factors for the disease among young women in developing countries are low personal hygiene and inadequate health seeking behaviour towards the infection.

Description The sample size of 2874 respondents of the young women was selected through simple random sampling technique from three different higher institutions located at different major cities in Nigeria, University of Ibadan, Ibadan, Lagos state Polytechnics, Lagos and Ahmadu Bello University, Kano respectively. The demographic data is as followings: Out of 2874 respondents, 550 were below 18 years old, 935 were between 18 and 25, 893 were between 26 and 30, and 496 between 31 and 40 years. The mean age is 25.32.A well developed validated and reliable questionnaire [r= 0.77] was used to collect the data needed for the study and percentage was used to analyze the data. Relative Risk [RR] calculated is 1.6, i.e. RR > 1, indicating that the factors are risk factors, and the Confidential Interval[CI] for RR at 95% Significant level is 1.56< 1.60<1.65 from the formula, CI Lower limit < RR < CI Upper limit. RR and CI are used to validate the instruments.

Lessons learned Table 1 revealed that only 25.34 % of the respondents had knowledge about *Gardenerella vaginallis Infection*, and Table 2 showed that young women in developing countries have lower personal hygiene toward the disease.

Conclusion From the validated result of the study, it was clearly seen that low individual personal hygiene and the inadequate knowledge which reduced the health seeking behaviour towards the *Gardenerella vaginallis* infection are obvious risk factors for the prevalence of the infection among young women in developing countries.

MI 25 Patterns of antimicrobial resistance among bacterial pathogens of children hospitalized in National Hospital for Pediatrics, Hanoi, Vietnam, 2004

Ngo Thi Thi[1], **Paul E. Kilgore**[2], Dang Duc Anh[3], Nguyen Hien Anh[3] & Mary P. Slack[4]

[1]Dept of Laboratory Medicine, National Hospital for Pediatrics, Hanoi, Vietnam; [2]Division of Translational Research, International Vaccine Institute, Seoul, South Korea; [3]Dept of Bacteriology, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; [4]Health Protection Agency, London

Background Antimicrobial resistance is rapidly emerging in Vietnam among both gram-negative and gram-positive pathogens. Recent data suggest that as much as 70% of pneumococcal isolates in Vietnam are fully resistant to penicillin.

Methods From January through December 2004, clinical specimens from sterile and non-sterile sites were tested by standard bacterial culture. Antimicrobial susceptibility testing was performed by disk diffusion. Standard cutoff values for fully resistant, intermediate resistant and susceptible strains were based on NCCLS Performance Standards for Antimicrobial Susceptibility Testing, 12th informational supplement.

Results A total of 6,372 clinical specimens including 2,157 blood cultures. A total of 2,296 specimens were culture-positive for a bacterial species. Common community-acquired invasive bacterial pathogens often responsible for pneumonia, meningitis and sepsis in Vietnam constituted 119 isolates (89 *H. influenzae*, 28 *S. pneumoniae* and 2 *N. meningitidis*). Seventy-two percent of *H. influenzae* strains tested were fully resistant to ampicillin, 77% were fully resistant to chloramphenicol and 47% of strains were resistant to ceftriaxone. Of 27 *S. pneumoniae* strains tested, 56% were fully resistant to penicillin and 85% were resistant to erythromycin.

Conclusions Clinical isolates in the northern region of Vietnam continue to demonstrate substantial levels of resistance to recommended first-line antibiotics. Laboratory capacity strengthening that enables routine MIC testing using practical methods for a high throughput, resource limited institution are urgently needed to support informed clinical choices for drug treatment of serious bacterial infections.

Posters

Physiology, Biochemistry and Molecular Genetics Group

PBMG 01 Some phages like it sweet: glycoproteins in *Streptomyces coelicolor*

Silvia Wehmeier, Anpu Varughese & **Maggie Smith**

Molecular Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD

Bacteriophages use their host's surface macromolecules as receptors to trigger the initial stages of phage infection. Studies of these receptors frequently provide insights on the nature of the host cell wall. Here we present the identification of two genes required for the synthesis of the phiC31 receptor. One of the gene products SCO3154 (renamed *pmt*) is similar to dolichol phosphate-D-mannose:protein O-D-mannosyltransferases (Pmt proteins) found in eukaryotic cells from yeasts to humans. The Pmts transfer mannose from the donor, dolichol phosphate mannose, to serine or threonine residues in the first step in O-glycosylation. Homologues of *pmt* are detected by database searches in other actinomycetes including mycobacteria and corynebacteria. We propose that the actinomycetes use these Pmt homologues to glycosylate proteins using a polyprenol-phosphate-mannose as a mannose donor. *S. coelicolor* strains that lack either *pmt* or SCO1423 (renamed *ppm*) that encodes a putative polyprenol-phosphate-mannose synthase are retarded in growth. We are using proteomics to characterise the putative 'glycoproteome' of *Streptomyces coelicolor*. We are also undertaking a phenotypic analysis of the *pmt* and *ppm* defective mutants to try to reveal the biological role of glycosylation.

PBMG 02 Structure and transcriptional regulation of Flp (fasciclin I-like protein) of *Rhodobacter sphaeroides*: *flp* expression is negatively regulated by the Prr redox-responsive two-component system under aerobic and anaerobic conditions

Robert G. Moody[1,2,*], Eun-Lee Jeong[1,*], Samantha J. Broad[1], Stewart Goodwin[1], Jeff N. Keen[1], Alison E. Ashcroft[1], Michael P. Williamson[2] & Mary K. Phillips-Jones[1]

[1]Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT; [2]Dept of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN; *These authors contributed equally to this work

We have identified a new member of the fasciclin I protein superfamily, Flp, a simple single-domain protein in the photosynthetic bacterium *R. sphaeroides*. Flp (residues 19-155) was expressed in *E. coli* with an *N*-terminal His_6 tag for purification, and the solution structure determined by NMR spectroscopy. Two-dimensional protein analysis of wild type and *prr* mutants suggested that the redox-responsive Prr pathway negatively regulates Flp expression. Reporter studies here confirm this; expression was up to 36-fold higher in PRRA and PRRB strains compared with wild type. Unusually for Prr, regulation occurred under all conditions of aerobiosis – aerobic, semi-aerobic and anaerobic conditions. Presumably sufficient transcriptionally-active PrrA or PrrA~P must occur for efficient repression of this *flp* promoter region even under aerobic conditions. Expression of *flp* was consistently lower in the PRRB strain compared with PRRA under all conditions of aerobiosis, implicating a role for PrrB in *flp* regulation, presumably through phosphorylation of PrrA by this upstream component in this signal transduction pathway, even under aerobic conditions.

PBMG 03 Overexpression and purification of intact VicK and VicR proteins, comprising an essential two-component system in *Enterococcus faecalis*

Victor Blessie[1], Nicholas G. Rutherford[1], David I. Roper[2], Peter J.F. Henderson[1] & Mary K. Phillips-Jones[1]

[1]Astbury Centre for Structural Molecular Biology, University of Leeds, LS2 9JT; [2]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

Enterococcus faecalis possesses 17 two-component signal transduction systems, including VicKR, which shares significant homology with the Vic/YycGF systems required for viability in several Gram-positive bacteria. Hancock and Perego (2004) reported that the VicKR system is likely to be essential for viability in *E. faecalis*. VicK is the membrane-bound sensor kinase component and VicR is the response regulator. To characterise this system in detail, we have overexpressed the encoding genes in *E. coli* to permit subsequent preparation of milligram quantities of purified proteins for *in vitro* studies. In the past, membrane proteins, including membrane-bound sensor kinases, have proven very difficult to overexpress and purify because of their hydrophobic nature. However, using suitable plasmids and conditions based on those developed by us for the successful high level expression of another intact membrane sensor kinase (PrrB), we report here the successful overexpression and purification of intact membrane-bound VicK, as well as the soluble response regulator component VicR.

PBMG 04 Characterization of a putative metal-binding lipoprotein of *Streptococcus agalactiae*

Beverley Bray[1], **Iain Sutcliffe**[2] & Dean Harrington[1]

[1]Dept of Biomedical Sciences, University of Bradford; [2]School of Applied Sciences, Northumbria University

The Group B streptococcus (GBS) is the major bacterial pathogen of neonates. N-terminal lipidation is a major mechanism by which bacteria tether proteins to membranes. Bioinformatic analysis of GBS genomes suggests lipoproteins represent approximately 2% of the predicted proteome including up to 21 putative solute-binding proteins of ABC transport systems. One such protein is homologous to PsaA of *S. pneumoniae*, which is involved in manganese transport. However, other streptococcal PsaA homologues appear to have different cation specificities. Here we characterise the specificity of the GBS homologue (MtaA). Globomycin-treatment and Triton X-114 extractability confirmed the lipoprotein nature of MtaA. Growth of GBS in Chelex-treated medium supplemented with specific divalent cations resulted in differential expression of MtaA. Manganese and iron (II) but not cobalt, copper, nickel or zinc resulted in the down-regulation of MtaA expression. At levels of iron (II) equivalent to normal amniotic fluid (5 μM), MtaA expression was evident. MtaA expression at defined manganese concentrations was decreased in the presence of iron(II). The co-regulation of MtaA expression by manganese and iron probably relates to the need to co-ordinate the intracellular levels of both ions to avoid the generation of iron-derived hydroxyl radicals in the absence of manganese co-factored superoxide dismutase.

PBMG 05 Preliminary studies of recombinant gene expression from the magnetic bacterium *Magnetospirillum gryphiswaldense*

C. Franklin, J.M.L. Bell, C.E. French & F.B. Ward

Institute for Structural and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR

Magnetospirillum gryphiswaldense is an aquatic bacterium, capable of aligning the cell in response to the earths magnetic field and moving along magnetic field lines. The ability to respond to magnetic fields is due to the presence of intracellular magnetosomes, in which magnetite is surrounded by a lipid membrane. For *M. gryphiswaldense* Schuler and colleagues have shown that a number of genes involved in magnetosome formation are clustered together in the same region of the genome and this has been termed 'a magnetosome island'. The process of magnetite formation is under investigation in a number of laboratories.

We are investigating the roles of magnetosome proteins, which are indicated by bioinformatic analysis to be iron binding proteins or iron transporters. A number of magnetosome genes have been cloned including that encoding a 149 amino acid protein with strong homology to the oxygen binding protein hemrythrin, found in invertebrates. No regulatory proteins involved in magnetosome formation have been reported so far. In *Caulobacter crescentus*, another member of the alpha proteobacteria, CtrA is a two component regulator that plays a key role in the cell cycle. *C. crescentus* CtrA is a protein of 231 amino acids containing a receiver domain with a conserved aspartate residue and a DNA binding domain. Based on available *ctrA* sequences we designed degenerate primers and used these to amplify 530 bp and 370 bp fragments from *M. gryphiswaldense*. The derived protein sequences from these fragments showed high similarity to the CtrA sequences from *C. crescentus* and *Magnetospirillum magnetotacticum* MS-1.

We report studies on the expression of the recombinant genes in *E. coli*.

PBMG 06 Putative acyl-homoserine lactone production by *Campylobacter jejuni* reference isolates

S.J. Carmichael, D. Greenway & R. England

Dept of Biological Sciences, University of Central Lancashire, Preston, Lancashire PR1 2HE

Quorum sensing (QS) mechanisms enable bacteria to regulate density-dependent gene expression by the production, secretion and binding of small chemicals or peptides known as autoinducers.

Acyl-homoserine lactone (AHL) mediated QS was originally promoted as a widespread phenomenon in Gram-negative bacteria. However, no known AHL producing bacteria are present in the Epsilon *Proteobacteria* class.

Synthesis of a second group of autoinducer is dependent upon the LuxS enzyme in many bacteria. The genome sequence of *Campylobacter jejuni* NCTC 11168 contains a gene encoding an orthologue of LuxS but does not contain genes predicted to encode any known AHL synthetase.

Putative AHL production by several *C. jejuni* isolates, including NCTC 11168, was assessed through the ability of concentrated conditioned medium to induce β-galactosidase activity in *Agrobacterium tumefaciens* KYC55 (pJZ372)(pJZ384)(pJZ410). These results suggest that AHL mediated QS is present in the Epsilon *Proteobacteria* class.

Initial HPLC separation of *C. jejuni* NCTC 13255 crude extract has provided one active fraction, however the structure and genes regulated by the putative signalling molecule have yet to be elucidated.

PBMG 07 Structural characterization of bacterial glycoconjugates

Paul Hitchen, Maria Panico, Stuart Haslam, Howard Morris & Anne Dell

Imperial College London, Division of Molecular Biosciences, Faculty of Life Sciences, Wolfson Building, South Kensington Campus, London SW7 2AZ

A detailed structural understanding of bacterial surface molecules and the genes involved in their biosynthesis is important to our understanding of the function and integrity of the cell envelope. We are using mass spectrometry as a tool to define the structures of bacterial glycoconjugates in collaborative programmes aimed at defining the biosynthesis and functions of surface molecules implicated in pathogen-host interactions. Specifically, MS glycan-screening methodologies have been used to identify genes involved in the biosynthesis of cell wall arabinogalactan from *Corynebacterium glutamicum*. In other work, we have applied glycoproteomics in the structural characterisation of N-linked glycans from *Campylobacter jejuni* and have demonstrated that *C. jejuni* glycan structures can be transferred onto protein in *E. coli*. We have also elucidated the N-glycan structures generated from mutations to genes in the *C. jejuni* glycosylation locus, which has allowed us to infer the role of individual glycosyltransferases in the N-linked biosynthetic pathway. Similar strategies have been employed to characterise the O-linked glycan modifications on *Neisseria gonorrhoeae* pilin. Using strains carrying defined pilin glycosylation gene mutations, the pilin O-glycan was characterised by MS and gene products involved in the glycosylation pathway were identified. In addition the post-translational modification of pilin with phosphoethanolamine and phosphocholine was identified.

PBMG 08 The functions of multiheme cytochromes in Fe(III) respiration by *Shewanella frigidimarina*

Jennifer S. McDowall[1], Paul S. Dobbin[1] & David J. Richardson[2]

[1]Dept of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ; [2]School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ

Shewanella frigidimarina is a Gram negative facultative anaerobe that can use Fe(III) in the form of insoluble oxyhydroxides as a respiratory substrate. This is enabled by positioning of terminal Fe(III) reductases at the exterior face of the outer membrane where interaction with the cation can occur. Electron transport across the periplasm and outer membrane of *S. frigidimarina* to Fe(III) involves a network of multiheme cytochromes. We are studying the structure, function and regulation of a number of these proteins, including:

i. Ifc_3, a periplasmic tetraheme flavocytochrome *c* that is only synthesised during Fe(III) respiration. We have shown that transcriptional regulation of this protein involves a novel iron-responsive system, and are currently investigating whether an oxygen-responsive FNR homolog in *S. frigidimarina* also plays a role.

ii. SF-90-3876, a periplasmic decaheme *c*-type cytochrome that is synthesised in increased amounts in an Ifc_3-deficient mutant strain. We have purified and spectrophometrically characterised this protein, and are currently investigating its role in Fe(III) respiration by mutagenesis studies.

iii. SF-85-2518, an outer membrane decaheme *c*-type cytochrome that is a putative Fe(III) reductase. We have purified and spectrophometrically characterised this protein, and are currently investigating its contribution to Fe(III) respiration by mutagenesis studies.

PBMG 09 Bacteria capable of antimonate respiration

Louise M. Hartmann & **Richard O. Jenkins**

School of Molecular Sciences, De Montfort University, The Gateway, Leicester LE1 9BH

In recent years the number of bacteria known to respire 'unusual' electron acceptors has expanded. Proteobacteria, *Vibrio,* sulfogens, and methanogens have all been shown to utilise one or more metal(loid) species – such as iron (III), manganate, uranium (VI), selenate, arsenate – as terminal electron acceptors of respiratory metabolism. Considering the toxicological and thermodynamic properties of antimonate (SbV), there appears to be no reason why respiratory reduction of antimonate would not occur within the natural environment.

Gram-positive cocci, isolated using anoxic culture conditions from rural and urban soils and from pond sediments, were demonstrated to reduce antimonate (supplied as sole respiratory electron acceptor), during growth on lactate (supplied as non-fermentable carbon and energy source).Up to 48% of the supplied antimonate was reduced. Addition of an alternative terminal electron acceptor (nitrate or sulfate), or an uncoupler of oxidative phosphorylation from electron

transport (2,4-dinitrophenol), to culture incubations decreased the extent of antimonate-reduction by 43 and 79% respectively. These data indicate that antimonate-reduction occurs *via* respiration and that these bacteria were capable of deriving energy for maintenance and growth through reduction of antimony. This capability could provide a selective advantage in anoxic environments with elevated antimonite concentrations, such as soils/wastes contaminated from semiconductor or fire retardant manufacturing processes, or from mining activities.

PBMG 10 Transformation of *Clostridium* Colombian strains by electroporation to increase cellulolyitic activity

G. Puerto, F.A. Aristizábal, J.M. Bernal & **D. Montoya**

Instituto de Biotecnología, Universidad Nacional de Colombia, Edificio Manuel Ancizar, Ciudad Universitaria A.A.14490

Transformation of solventogenic Clostridia to obtain recombinant strains is an important point to develop economically profitable fermentation processes. This work presents electroporation of two strains of *Clostridium* (*Clostridium sp*. IBUN 22A native strain and *C. acetobutylicum* IBUN IV butanol hyperproducer mutant strain) with a methylated plasmid pMTL500E*licA* which encodes for cellulolytic activity. Transformation efficiency was increased when methylated vectors were used in comparison to previous reports (2.7×10^8 and 3.6×10^8 transformants per microgram of DNA for *Clostridium* IBUN 22A and *Clostridium* IBUN IV, respectively), and plasmid's stability was verified during 100 generations in RCM medium with erythromycine. Solvent production by fermentation using agroindustrial waste Palm Oil Mill Effluent (POME) as sole carbon source with transformants strains was evaluated. Plasmid presence was followed during those new fermentation conditions. Results suggested that acids and solvent production was similar to wild strains. This fact was associated with plasmid lost during the first 24 hours (8 and 15 generations for IBUN 22A and IBUN IV, respectively) and subsequent consumption of lactic acid as carbon source. The electroporation methodology presented in this study is the departure point for the development of stable *Clostridium* transformants industrially attractive for solvent production from waste.

PBMG 11 AFLP fingerprinting of Colombian *Clostridium* spp. strains, multivariate data analysis and its taxonomical implications

C.P. Jaimes, F.A. Aristizábal, Z.R. Suárez, J.M. Bernal & **D. Montoya**

Instituto de Biotecnología. Universidad Nacional de Colombia A.A. 14490, Depto de Farmacia, Universidad Nacional de Colombia

Amplified Fragment Length Polymorphism (AFLP) analysis was used for characterising 13 native Colombian *Clostridium spp* strains. The DNA extraction method was optimised and the use of cetyl trimethyl ammonium bromide (CTAB) and sodium chloride (NaCl) was incorporated. All strains could be typed in these conditions. The AFLP profiles obtained were submitted to multiple correspondence analysis (MCA) and compared with previous pulsed field gel electrophoresis (PFGE) results. The results suggested that the set of native strains could correspond to two new species different to those having been described to date. It is proposed that DNA-DNA hybridisation analysis should be done to produce complementary information for describing the new species.

PBMG 12 Study of chromosomes and megaplasmids of thirteen solventogenic clostridia strains by means of Pulsed Field Gel Electrophoresis (PFGE)

D.M. Quilaguy, Z.R. Suárez, F.A. Aristizábal, J.M. Bernal & **D. Montoya**

Instituto de Biotecnología, Universidad Nacional de Colombia, Edificio Manuel Ancizar, Ciudad Universitaria, A.A.14490

In this work genome size estimation and mega plasmid's presence by means Pulsed Field Gel Electrophoresis (PFGE) for 13 native Clostridia strains is presented. DNA preparation and purification was optimized until obtaining differentiated restriction fragments during electrophoresis. Genomic DNA of the native strains was digested with *Apa*I, *Eco*52I, *Sma*I and *Xho*I and the estimated average for genome size in the native strains was between 4,0 and 4.2 Mpb. High size Plasmids were detected by PFGE and polymerase chain reaction (PCR) was used to establish plasmid pSOL1 presence, which contains important genes for solvent production. Genome size determination for the native strains will be useful to propose and describe them as a new species.

PBMG 13 Inhibition of swarming motility in *Proteus* rods by the synergistic activity of *Ferula gumosa* bioss and *Lavandula officinalis* essential oils

S. Mansouri & S.B. Ashraf Ganjouyeei

Microbiology Dept, Kerman University of Medical Science, P O Box 444, Kerman, Iran

Background A combination of essential oil of *Ferula gumosa* bioss (A) and *Lavandula officinalis* (B) is used in traditional medicine for the treatment of acne in Iran. This combination was reported to have novel anti-swarming activity.

Methods Standard agar dilution method was used through the study.

Results Compound B had antibacterial activity on both Proteus and other bacterial species tested, but could not inhibit the swarming. On brain heart infusion broth (BHI) the cells were in a form of cocci and were non-flagellated. Compound A had neither anti-swarming, nor antibacterial activity .The growth curve was similar to the control without the essential oils and the cells were elongated and flagellated. On BHI supplemented with an equal amount of A and B the growth curve was similar to the control, but the cells were smaller than the control cells, forming short chains, and with no flagella.

Conclusion The synergistic activity of these essential oils could be used to clarify the mechanism of swarming motility in Protues. Since the combination of A and B had no antibacterial activity, the medium supplemented with these compounds could be used for the isolation of bacteria from clinical samples with poly microbial origins.

PBMG 14 Studies on the possible role of the *bgl* operon of *Escherichia coli* in stationary phase

R. Madan[1], R. Kolter[2] & S. Mahadevan[1]

[1]Indian Institute of Science India; [2]Harvard Medical School, Boston, MA

The *bgl* operon of *Escherichia coli* involved in the utilization of the β-glucoside sugars salicin and arbutin is classified as a cryptic genetic system because wild type *E. coli* is unable to utilize these sugars. However, a variety of *cis* as well as *trans* acting mutations are known to activate the *bgl* operon in *E. coli*, thus enabling the organism to transport and utilize these β-glucoside sugars. Evolutionarily, such cryptic genes remain an enigma, as in spite of being silent in the wild type organism, they have not accumulated any deleterious mutations and have not been eliminated by the organism. One possibility is that the seemingly silent genes might be functional under different physiological conditions. One of the questions which is being addressed is whether this apparently silent *bgl* operon could be expressed and thus function under specific physiological conditions and/or whether there are alternative functions carried out by the *bgl* operon apart from β-glucoside catabolism. Unlike the nutrient rich conditions of the laboratory, bacteria spend the majority of their life time under conditions of nutrient starvation and various other stresses such as varying temperatures and pH. We measured the activity of the wild type *bgl* promoter under conditions of nutrient starvation and we show that the activity of the wild type *bgl* promoter is enhanced in the stationary phase of growth when the nutrients are depleted. We further asked whether the *bgl* operon could be playing a role in the stationary phase. Starved *E. coli* populations have been shown to be highly dynamic. As the majority of the population dies because of nutrient depletion and other changes in the medium (such as pH) in the stationary phase of growth, a few members of the population acquire mutations that enable them to survive and grow at the expense of the dying population. We show that Bgl^+ mutants accumulate in the

stationary phase and a strain with an activated *bgl* allele exhibits a selective advantage over a wild type strain in the stationary phase.

PBMG 15 Decolourization using bacteria

Baljeet Singh Saharan[1], Preet Singh Malik[2], Peter Kuschk[3] & Rajender Kumar Sahu[4]

[1]JCD Coe India; [2]JCD Cov India; [3]UFZ Germany; [4]ICT Brazil

Anaerobically treated distillery spent wash is a dark brown effluent with very high Chemical Oxygen Demand (COD) as compare to CPCB disposal standards. 12 bacterial strains were isolated from soil samples obtained from distillery premises through enrichment culture method. All the bacterial isolates were found to be *Pseudomonas* spp. Four bacterial isolates showed considerable decolourization and COD removal efficiency from dilute digested spent wash. Although treated spent wash had considerable sugar contents but it was not readily available to the studied bacterial isolates. Addition of C source in the effluent was found to be essential to have decolourization and COD removal. The effect of different C –sources, N_2 –source, time of incubation, digested spent wash concentration and bacterial isolates % in the effluent on decolourization and COD removal has also been studied.

Keywords Digested distillery spent wash; Basal medium; bacterial identification; Pseudomonas; spp; spectrophotometry; glucose and peptone

Posters

Plenary

PS 01 Molecular analysis of geochemical influence on rock-inhabiting fungal and bacterial communities

Karrie Melville[1], Deirdre Gleeson[2], Nicholas Clipson[2] & Geoffrey M. Gadd[1]

[1]Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, DD1 4HN; [2]Dept of Industrial Microbiology, University College Dublin, Dublin 4, Ireland

Lithic microbial communities are ubiquitous and associated with a wide variety of rocks and minerals. In this research, the contrasting elemental profile of adjacent layers from a sandstone outcrop was examined to investigate whether differences in mineralogical composition influenced fungal and bacterial diversity. Culture-independent molecular approaches were used in combination with geochemical analyses and community-level physiological profiling to assess the effects of mineralogical variation on community composition of micro-organisms inhabiting a sandstone gradient. A DNA-based community fingerprinting approach (ARISA: automated ribosomal intergenic spacer analysis) combined with construction of fungal and bacterial rRNA gene clone libraries was used to assess community structure and to identify some of the constituent microbial populations. Molecular data was combined with X-ray diffraction and X-ray fluorescence and by applying multivariate statistics, we identified those chemical properties that notably influenced community structure. It was found that the sandstone supported a varied microbial diversity and community ribotype profiles differed between mineralogically-distinct sandstones in close proximity to each other. Canonical correspondence analysis (CCA) illustrated relationships between certain ribotypes and particular chemical elements. We can conclude that these molecular and statistical methods provide a powerful tool for resolving rock-inhabiting bacterial and fungal communities, and their relationship to the mineral nature of the rock substrate.

PS 02 Polymerization of fungal phenolic degradation products by metal oxides

Jae Yeon Park & Geoffrey M. Gadd

Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN

Fungal degradation of phenol (and related aromatic compounds) has been well documented. However, the interactions between phenolic degradation products and metals have received little attention. Fungi are able to mobilize metals and metalloids from minerals by several mechanisms, including organic acid and/or proton excretion. Our work proposes an additional mechanism where the fungal metabolism of aromatic compounds could indirectly result in mineral transformations by abiotic reactions with resulting degradation products. We have isolated fungi which have the ability to degrade phenol at high concentrations and which could also dissolve metal oxides during phenol degradation. It was found that catechol, one of the important intermediates of phenol degradation, was polymerized by calcium oxide and cuprite. We can conclude that interactions between organic and inorganic substances are complex and influenced by microbial activity, and these may have important effects on pollutant breakdown and transformation.

PS 03 Scanning electron microscopy techniques for fungal-mineral transformation studies

Marina Fomina, Martin Kierans, Euan P. Burford, Louise McGregor & Geoffrey M. Gadd

Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN

Fungi play a crucial role in geomicrobiological processes, often demonstrating considerable rock and mineral deteriorative potential and are also involved in the formation of a variety of secondary mycogenic minerals. In this study, scanning electron microscopy (SEM)-based techniques were used to study metal-mineral transformations by fungi. We have found that environmental SEM in the wet mode is particularly applicable for observing such interactions in their natural microenvironment with coupled X-ray element mapping revealing sequestration and localization of metals associated with the biomass and as constituents of secondary materials. Cryo-SEM enabled observation of both interior and exterior microstructures and provided structural information on the formation of secondary mycogenic minerals associated with fungal biomass.

PS 04 Mycorrhizal involvement in toxic metal mineral transformations

Marina Fomina[1], Ian J. Alexander[2], Stephen Hillier[3] & Geoffrey M. Gadd[1]

[1]Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN; [2]Dept of Plant and Soil Science, University of Aberdeen, Aberdeen AB24 3UU; [3]Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH

Plant roots and their symbiotic and free-living microbial populations alter the physico-chemical characteristics of the rhizosphere making it different from bulk soil and affecting toxic metal speciation. The mechanisms by which fungi and plants obtain phosphate are of particular interest since solubilization of inorganic phosphates can result in release of associated metals. Zinc phosphate was of least toxicity and the most easily solubilized by axenic ericoid and ectomycorrhizal fungi among tested insoluble cadmium-, copper-, lead- and zinc-containing minerals. Solubilization of toxic metal minerals was related to fungal metal tolerance. Zinc phosphate solubilization by *Paxillus involutus/Pinus sylvestris* ectomycorrhizal associations and protection of host plants against toxic metal mobilized from the mineral also depended on the phosphorus status of the growth matrix. Zinc, copper and lead mobilized from minerals were oxygen-coordinated within the fungal/ectomycorrhizal biomass. 'Heterotrophic leaching' of toxic metal(s) from insoluble minerals comprised acidification and ligand-promoted dissolution mediated by different organic acid anions: if oxalic acid was produced, precipitation of metal oxalates resulted.

PS 05 X-ray absorption spectroscopy (XAS) of toxic metal speciation in fungi

Marina Fomina[1], John Charnock[2], Andrew D. Bowen[1], Fred W. Mosselmans[2], Robert L. Bilsborrow[2] & Geoffrey M. Gadd[1]

[1]Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN; [2]SRS Daresbury Laboratory, Daresbury, Warrington, Cheshire WA4 4AD

The influence of microbiological processes on mineral transformations and metal speciation in the environment is of economic and environmental significance. Fungi can be highly efficient

biogeochemical agents and bioaccumulators of soluble and particulate forms of metals. This project aimed to understand the physico-chemical mechanisms involved in toxic metal transformations and accumulation by fungi and mycorrhizal associations. Because of the amorphous state or poor crystallinity of metal complexes within biomass and relatively low metal concentrations, determining metal speciation in biological systems remains a challenging problem in the case of fungal and mycorrhizal biomass. Synchrotron-based element-specific X-ray absorption spectroscopy (XAS) is an ideal technique for studying element complexation in environmental samples varying from biological to mineralogical in nature. We exposed fungi and ectomycorrhizas to a variety of copper-, zinc- and lead-containing minerals. Our XAS-studies revealed that oxygen ligands (phosphate, carboxylate) played a major role in metal coordination within the fungal and ectomycorrhizal biomass during accumulation of mobilized toxic metals. Coordination of toxic metals within biomass depended on the fungal species, initial mineral composition, nutrients (especially the nitrogen source) and the physiological state/age of fungal mycelium.

PS 06 Thigmotropic responses of fungi in controlled model systems

Andrew D. Bowen[1], Fordyce A. Davidson[2], Robert Keatch[3] & Geoffrey M. Gadd[1]

[1]Division of Environmental and Applied Biology, School of Life Sciences, University of Dundee, Dundee DD1 4HN; [2]Division of Mathematics, University of Dundee, Dundee DD1 4HN; [3]Division of Mechanical Engineering and Mechatronics, University of Dundee, Dundee DD1 4HN

Contour following (thigmotropism) is a well-known phenomenon in the growth and guidance of plant roots, and is also an important factor in the directed growth of animal and plant pathogenic fungi (e.g. *Candida albicans*, *Puccinia graminis*) as well as other species (e.g. *Neurospora crassa*). Current hypotheses propose that these thigmotropic responses aid the search for nutrients on contoured surfaces and growth through porous materials. Our current research seeks to understand fungal colonization of rocks, minerals and building materials and we have utilised micro-engineered structures in an attempt to analyse and ultimately model the growth of fungal hyphae in reaction to topographical features. Using light and confocal microscopy, combined with statistical analysis, we have investigated the thigmotropic responses of fungi to various different chemical and physical environments. We have also shown spatial variations in the levels of contour following within a single fungal colony, which suggests that the intensity of the thigmotropic response depends on the specific stage of hyphal growth.

PS 07 Effects of strontium compounds on fungal growth

Louise McGregor & Geoffrey Gadd

Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN

The surfaces of many nuclear facilities may be contaminated with different radionuclides of which strontium-90 may be an important component. Sr-90 is the most important strontium radioisotope in the environment and has a relatively long half-life of around 30 years. Decontamination of contaminated surfaces is clearly an important objective of the decommissioning portfolio. Strontium is chemically similar to calcium, and this similarity to calcium allows strontium to be localised to the same sites within the human body, e.g. bones and teeth. A novel approach to decontamination is the application of microbial systems and products and it is now recognised that micro-organisms have considerable potential for bioremediation of a variety of contaminants, and some processes are in commercial operation. Fungi have received less attention than bacteria despite these organisms being major biotic components of soil and highly important in organic and inorganic biogeochemistry, including toxic metal cycling. This work has examined the interactions of strontium with selected fungal species chosen for their established properties relating to soil and mineral transformations. It was found that growth responses were variable with inhibitory effects being low and only occurring under specific conditions. Oxalic-acid producing species were able to precipitate secondary oxalate minerals external to the mycelium.

PS 08 Isolation and growth of PAH-degrading fungi

Ji Won Hong & Geoffrey M. Gadd

Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN

Polycyclic aromatic hydrocarbons (PAHs) are common pollutants found in soil and groundwater as a result of human activities such as the combustion of fossil fuels, the incineration of wastes and the accidental spilling of oils. Fungi can metabolize a wide variety of PAHs, converting them either completely to carbon dioxide or to various metabolites. PAH-contaminated soil samples were collected from a variety of sites. To isolate PAH-degrading fungi, serial dilution methods, both by direct and selective enrichment inoculations, were used. A solid PBBM (phosphate-buffered basal medium) supplied with pyrene as a sole carbon source was used as a selective medium for the isolation and cultivation of pyrene degrading organisms. After two weeks incubation, over 70 fast-growing fungal strains were selected. According to their morphologies, ten different isolates were chosen and grown in liquid PBBM and the pyrene degradation rate was analyzed by HPLC. Several isolates were able to degrade >90% of the supplied pyrene after two weeks incubation.

PS 09 Utility of stable isotope probing (SIP) to investigate biodegradation of the biocide polyhexamethylene biguanide (PHMB)

Leon Peter O'Malley

Arch Biocides, P.O. Box 42, Blackley, Manchester M9 8ZS

The Biocides Product Directive (BPD) requires determination of the biodegradability of biocides marketed in the European Union. Therefore, we have attempted to determine biodegradation of the biocide polyhexamethylene biguanide (PHMB). Isolation of potential PHMB-biodegrading micro-organisms has been conducted in enrichment cultures dosed with the biocide as the sole nitrogen source.

As PHMB is highly adsorptive, it has proved difficult to distinguish between losses due to biotic or abiotic processes. To circumvent analytical problems, the applicability of Stable Isotope Probing (SIP) to biodegradation studies was investigated. SIP is currently used to investigate microbial community function. However, this methodology should also be applicable for quantifying biodegradation. We have achieved this by dosing ^{15}N-PHMB to microbial cultures, and extracting ^{15}N-labelled genomic DNA from cultures at the late exponential phase of growth. The percentage ^{15}N content of the isolated DNA was determined by Isotope Ratio Mass Spectrometry (IRMS). By comparison of genomic DNA extracted from identical cultures dosed with equimolar concentrations of ^{15}N from PHMB and a simple nitrogen source (in this case $^{15}NH_4Cl$), we have shown that it is possible to estimate the extent of biocide biodegradation.

PS 10 Anoxic cycling of technetium in aquifer sediments; a multidisciplinary study

J.M. McBeth[1], G. Lear[1], K. Morris[2], I.T. Burke[2], F.R. Livens[1], B. Ellis[3], R.S. Lawson[3] & J.R. Lloyd[1]

[1]Williamson Research Centre, School of Earth, Atmospheric and Environmental Sciences, University of Manchester, M13 9PL; [2]School of Earth and Environment, University of Leeds, LS2 9JT; [3]Dept of Nuclear Medicine, Manchester Royal Infirmary, M13 9WL

The long-lived radioactive isotope technetium-99 is a significant waste product of nuclear reprocessing and energy production which can be removed from solution via microbial reduction of soluble Tc(VII) to insoluble Tc(IV). Progressive microcosm studies with subsurface sediments from the US DOE Field Research Center (FRC, Oak Ridge, Tennessee), with and without added electron donor (acetate, 20 mM),

have shown that Tc(VII) reduction is linked to Fe(III)-reduction. The end product of reduction, identified by X-ray absorption spectroscopy, is $TcO_{2(s)}$. Gamma camera images of Tc-99m movement through sediment columns showed immobilization of Tc-99 correlated with zones of biogenic Fe(II), a potent reductant for Tc(VII), while molecular- and cultivation-dependent analyses of the microbial communities present in the sediments confirmed the presence of Fe(III)-reducing bacteria responsible for these transformations. We also report that nitrate, often a co-contaminant present in nuclear waste streams, controls the rate of Fe(III)-reduction, and hence Tc solubility; 100 mM nitrate completely inhibited the reduction of Fe(III) and Tc(VII).

PS 11 Characterization of the active microbial community present within an aquifer sediment from Cambodia containing elevated arsenic concentrations

G. Lear & J.R. Lloyd

Williamson Research Centre, School of Earth, Atmospheric and Environmental Sciences, University of Manchester

Arsenic contamination of groundwater is a global issue. Recent studies have highlighted the effect of microbial activity on arsenic speciation within subsurface environments, linked to the availability of organic matter which can promote the reduction of sorbed As(V) to more mobile As(III). The aim of this study was to use molecular techniques to characterise the active microbial community of a sediment mobilizing arsenic, following the addition of acetate as an electron donor and proxy for organic matter. Initial RNA analysis revealed a relatively diverse, active bacterial community, dominated by *Novosphingobium capsulatum* (25%), but also including bacteria linked to the reduction of Fe(III) (2%; *Geobacter* spp.) and the oxidation of ammonia (11%; *Nitrospira* spp.). A novel RNA-Stable Isotope Probing (SIP) method was also optimised to identify the organisms directly utilising acetate under As(V)-reducing conditions, within a mixed microbial community. Such studies are vital to increase our understanding of how additions of organic matter, e.g. as sewage or by drawn-down into the aquifer, affect arsenic mobility into water extracted for drinking.

PS 12 Microbial Fe(III) reduction at a low level radioactive waste storage site: microbial population changes and associated effects on subsurface U(VI) mobility

M.J. Wilkins[1], F.R. Livens[1], D.J. Vaughan[1], I. Beadle[2], J. Small[2] & J.R. Lloyd[1]

[1]Williamson Research Centre and School of Earth, Atmospheric and Environmental Sciences, University of Manchester; [2]British Nuclear Fuels plc, Risley, Warrington WA3 6AS

Fe(III) oxyhydroxides can be utilised as terminal electron acceptors by indigenous microbial communities present in disposed radioactive wastes. These micro-organisms may have a critical control on the biogeochemical cycling and hence transport of several environmentally important radionuclides, for example uranium, at the UK's Low-level Radioactive Wastes Repository, located at Drigg in the NW of England.

Microcosm experiments using Drigg sediment and synthetic groundwater were amended with soluble U(VI) and electron donor (acetate). Only a small proportion of the U(VI) sorbed onto mineral surfaces due to the low surface area of the sediments. However, a lowering in the U(VI) groundwater concentration was observed concurrently with microbial Fe(III) reduction, suggesting that reduction to insoluble U(IV) was mediated by dissimilatory Fe(III)-reducing bacteria identified in the sediments. Following the onset of Fe(III) reduction, a large increase in numbers of micro-organisms closely related to *Rhodoferax ferrireducens* was detected in the sediments. The presence of biogenic Fe(II) in the microcosms did not affect the soluble U(VI) concentration, suggesting that U(VI) reduction was mediated enzymatically and not by Fe(II).

PS 13 Identification and culturing of arsenic- and iron-cycling bacteria from two contrasting arsenic-enriched Cambodian sediments

R.L. Pederick, A.G. Gault, D.A. Polya & J.R. Lloyd

School of Earth, Atmospheric and Environmental Sciences & Williamson Research Centre, University of Manchester, Manchester M13 9PL

The microbially-mediated mobilisation of arsenic from sediments by metal-reducing bacteria is gaining consensus as a dominant mechanism for the production of arsenic-enriched groundwaters in shallow reducing aquifer sediments. However, little work has been published on the biogeochemical properties of arsenic-enriched Cambodian sediments.

A combined strategy of anaerobic microcosm- and MPN series-based culturing was used to enrich for and isolate anaerobic bacteria involved in the redox cycling of As and Fe in two contrasting sediments from arsenic-rich Cambodian aquifers. The identity of the bacteria present in the cultures was determined using a range of molecular and culturing techniques.

Results of 16S rRNA gene analysis showed that both sediments have a complex indigenous bacterial community. Furthermore, the two sediments responded in markedly different ways to enrichment culturing. Interestingly, the overwhelming majority of clones isolated aligned best with uncultured bacteria in the NCBI database, indicating the presence of novel As- and Fe-cycling species. Work is underway to characterise the isolated bacterial species.

PS 14 Solid-state biotechnology: nano-spinel synthesis by Fe(III)-reducing bacteria

V.S. Coker[1], Rad Pattrick[1], G. van der Laan[1,2] & J.R. Lloyd[1]

[1]SEAES, University of Manchester; [2]CCLRC Daresbury Laboratory, Warrington

Fe(III)-reducing bacteria can precipitate regular shaped nano-sized crystals of the ferromagnetic spinel, magnetite. Magnetite has the formula $Fe^{2+}(Fe^{3+})_2O_4$ where the Fe^{3+} is split between the octahedral and tetrahedral sites and Fe^{2+} occupies only the octahedral sites. Other elements can substitute for the Fe ions giving spinels of the general chemical formula $M^{2+}O.Fe_2O_3$ which have properties making them ideal for use in high-frequency devices. The objective of this project is to optimise biomineral production to give an environmentally friendly method of spinel manufacture.

Co- and Ni-doped spinels have been synthesised from amorphous oxyhydroxides using *Geobacter sulfurreducens* and *Shewanella oneidensis* using non-growing cells. Characterisation of the site occupancies in the spinels using the synchrotron radiation technique XMCD (x-ray magnetic circular dichroism) has shown that the distribution of the Fe and the dopants between the octahedral and tetrahedral sites varies depending on the formation conditions. Imaging using SuperSTEM has shown that the distribution of the elements is heterogeneous across individual 20nm particles. These features can now be manipulated to optimise the commercial use of these nanoparticles.

PS 15 Biosynthesis of diluted magnetic semiconductor nano-materials

C.I. Pearce[1], R.A.D. Pattrick[1], J.M. Charnock[1,2], J.F.W. Mosselmans[2] & J.R. Lloyd[1]

[1]SEAES, University of Manchester, Manchester M13 9PL; [2]CCLRC Daresbury Laboratory, Warrington WA4 4AD

Diluted Magnetic Semiconductor (DMS) nano-materials have applications in 'spintronic' devices due to their unique magneto-optical and magnetotransport properties. These DMS nano-materials can be synthesised under ambient conditions using a novel biotransformation process, significantly reducing the cost and energy requirements that are associated with existing chemical manufacturing technologies. The anaerobic bacterium *Veillonella atypica* is able to utilise molecular

hydrogen as the electron donor for the reduction of elemental selenium to selenide, via an extracellular mechanism involving redox mediators. X-ray absorption spectroscopy and electron microscopy measurements indicate that, in the presence of suitable transition metal cations, the selenide is precipitated to form nano-sized particles of Mn, Fe or Co-doped ZnSe or CdSe. The integration of these novel cost effective materials into electronic devices has the potential to stimulate the commercialisation of 'spintronics-based' technologies.

PS 16 Bioremediation of heavy metals and radionuclides by *Geobacter sulfurreducens*

Nicholas Law[1], Joanna C. Renshaw[1,2], Iain May[2], Francis R. Livens[2] & Jon R. Lloyd[1]

[1]School of Earth, Atmospheric and Environmental Sciences, University of Manchester; [2]School of Chemistry, University of Manchester

Dissimilatory Fe(III)-reducing bacteria, such as *Geobacter sulfurreducens*, can reduce a range of high valence soluble metals to insoluble forms. Therefore, it may be possible to use these bacteria for *in situ* bioremediation of contaminated land and water.

The objective of this project is to understand the mechanism of metal reduction in this organism. Studies have shown *G. sulfurreducens* can reduce Cr(VI), U(VI) and Tc(VII), but not Np(V). Cr(V) and U(V) were identified as intermediates by EPR and XAS, respectively, suggesting that reduction of these two metals occurs *via* a one-electron transfer process. By using deletion mutants and purified enzymes we have identified cytochrome c_7 and Hyb, a periplasmic NiFe hydrogenase, as two key enzymes involved in the reduction processes. We have also found that *G. sulfurreducens* can remove Hg(II) from solution, potentially via bioreduction.

Mechanisms of these processes are still not fully understood, and the use of purified enzymes and mutants will be combined with other spectroscopic and imaging techniques to provide a clear understanding of the enzymatic pathways.

PS 17 Biogeochemical redox cycling of technetium in estuarine sediments

K. Morris[1], J.R. Lloyd[2], I.T. Burke[1], C. Boothman[2], F.R. Livens[2,3] & R.J.G. Mortimer[1]

[1]School of Earth and Environment, University of Leeds, Leeds LS2 9JT; [2]Williamson Centre for Molecular Environmental Science, School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL; [3]Centre for Radiochemistry Research and School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL

Technetium is a redox active radionuclide that is present as a contaminant at sites where nuclear fuel processing has occurred. Recent studies suggest that Tc(VII) is retained in sediments as anoxia develops due to its reduction to poorly soluble Tc(IV). However, the redox cycling behaviour of technetium when reduction and reoxidation of sediments occurs remains unclear. Here we use sediment microcosms to investigate the biogeochemical cycling of technetium. Removal of $Tc(VII)O_4^-$ from solution occurred during development of Fe(III) reduction in anoxic sediments. The removal mechanism was *via* reductive precipitation of Tc as hydrous $Tc(IV)O_2$. In reoxidation experiments, the behaviour of Tc was strongly dependent on whether air or nitrate was the oxidant. With air, reoxidation of both Fe(II) and sulfide occurred, and approximately 50% of Tc was remobilised to solution as TcO_4^-. With nitrate, reoxidation of Fe(II) and sulfide occurred only in microbially active experiments where Fe(II) oxidation was coupled to nitrate reduction. Interestingly, on nitrate reoxidation, Tc was surprisingly resistant to remobilisation with < 8% Tc remobilized to solution even when extensive Fe(II) and sulfide reoxidation had occurred. This suggests that sediments may retain Tc even if they undergo redox cycling.

PS 18 Technetium solubility during the onset of progressive anoxia

Ian T. Burke[1], Christopher Boothman[2], Jonathon R. Lloyd[2], Robert J.G. Mortimer[1], Francis R. Livens[3] & Katherine Morris[1]

[1]School of Earth and Environment, University of Leeds, Leeds LS2 9JT; [2]Williamson Centre for Molecular Environmental Science, School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL; [3]Centre for Radiochemistry Research, and School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL

Technetium is a fission product that is highly mobile in its oxic form (as $Tc(VII)O_4^-$), but is scavenged to sediments in its reduced forms (predominantly as Tc(IV)). In progressive microcosms, a cascade of stable element terminal electron accepting processes developed as a result of indigenous microbial activity. TcO_4^- removal from solution occurred during microbial Fe(III) reduction, and was essentially complete (>99%) by the onset of SO_4^{2-} reduction. Microbial community analysis revealed a similar and complex microbial population at all three sample sites. At the intermediate salinity site, Paull, a broad range of NO_3^--, Mn(IV)-, Fe(III)- and SO_4^{2-}-reducers were present in sediments including microbes with the potential to reduce Fe(III) to Fe(II). When sterilised sediments were incubated with pure cultures of NO_3^--, Fe(III)- and SO_4^{2-}-reducing bacteria, TcO_4^- removal occurred only during active Fe(III) reduction. X-ray absorption spectroscopy confirmed that TcO_4^- removal in these sediments was due to reduction to hydrous $Tc(IV)O_2$ in both Fe(III)- and SO_4^{2-}-reducing sediments.

PS 19 Anoxic biogeochemical cycling of iron; characterization of a stable microbial consortium coupling anoxic oxidation of Fe(II) to nitrate reduction

C. Boothman[1], I.T. Burke[2], K. Morris[2] & J.R. Lloyd[1]

[1]Williamson Research Centre and School of Earth, Atmospheric and Environmental Sciences, University of Manchester; [2]School of Earth and Environment, University of Leeds

Iron is the second most abundant metal in the Earth's crust (5% by weight), and is most commonly found in the ferrous (II) or ferric (III) oxidation states. While much is now known about the reduction of Fe(III) by specialist anaerobic bacteria, comparatively little is known about microbially mediated iron reoxidation under anaerobic conditions. We have used a suite of geochemical and microbial characterisation techniques to further investigate this process in enrichment cultures inoculated with anoxic estuarine sediments, and supplied with nitrate as an electron acceptor.

Initial microbial characterisation revealed the presence of a complex community, including known Fe(III)-reducing bacteria related to *Geobacter pelophilus*. Upon reoxidation under denitrifying conditions, DGGE analysis showed only minor shifts in the microbial population, although significant stimulation of some species was observed, and maintained after resubculturing. These included several novel bacteria, such as one most closely related to the known Fe(II)-oxidising aerobe *Gallionella ferruginea*. Work is ongoing to further characterise both the microbial community and the mineralogy of these microcosms.

PS 20 The subsurface microbiology of Waikato Basin, New Zealand

Chloe Heywood[1], John Fry[2], John Parkes[1], Joachim Rinna[1], Henrik Sass[1] & Andrew J. Weightman[2]

[1]School of Earth Ocean and Planetary Sciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3YE; [2]School of Biosciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3TL

The Waikato Basin consists of marine shales, siltstones and sandstones interbedded with terrestrial sediments from peats to subbituminous coals. It is hypothesised that the organic-rich terrestrial layers may act

as 'feeders' supporting bacterial activity in the surrounding sediments. During burial and heating organic matter can become gradually more labile to degradation. However, the deeper part of the sequence has been heated to approximately 80 °C leading to the possibility of palaeosterilisation of the deeper sediments (~150 m).

In order to investigate these contradictory propositions most probable number (MPN) counts have been made using anaerobic microtitre plates with media selective for sulphate reduction, iron and manganese reduction, methanogenesis, acetogenesis, general heterotrophy and the utilisation of humic acids. From these MPN enrichments isolates have been obtained for further investigation of the physiological characteristics of the communities. Molecular genetic analysis is being used to monitor the MPN and identify isolates. Volatile fatty acid, anion and gas analyses give information on the metabolism of the cultures. There are significant MPN counts for samples from both above and below the deeply buried section, therefore if palaeosterilisation had occurred the formation has been reinoculated.

PS 21 Relationship between prokaryotes and biogeochemical processes in deep sub-seafloor sediments of the Peru Margin

Gordon Webster[1,2], Barry A. Cragg[2], R. John Parkes[2], John C. Fry[1] & Andrew J. Weightman[1]

[1]Cardiff School of Biosciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3TL; [2]Cardiff School of Earth, Ocean and Planetary Sciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3YE

Studies on sub-seafloor sediments from the Ocean Drilling Program (ODP) have consistently demonstrated the presence of prokaryotic populations and activities in sediments down to depths of several hundreds of metres below the seafloor. Yet little is known about the identity of these populations due to difficulties in enriching/isolating deep sediment bacteria. Here we report a comprehensive investigation of prokaryotic diversity using molecular methods to overcome these problems.

Sediments from the Peru Continental Shelf (ODP Leg 201, sites 1228/1229) were sampled down to 200 mbsf. DNA was extracted, amplified by PCR using 16S rRNA gene primers specific for *Archaea, Bacteria* and bacterial candidate division JS1 and analysed by cloning/sequencing, and DGGE.

Sites were dominated by *Bacteria* from the *Gammaproteobacteria, Chloroflexi* (Green Non-sulphur bacteria) and *Archaea* from the South African Gold Mine Euryarchaeotic Group and Miscellaneous Crenarchaeotic Group. Statistical analysis of the DGGE profiles and comparison with bacterial/geochemical variables revealed relationships between diversity and sediment biogeochemistry. At site 1229, bacterial diversity was related to cell numbers, sulphate reduction, methanogenesis, thymidine incorporation and metal reduction.

This is the first study to show strong relationships between prokaryotes and biogeochemical processes in deep subsurface sediments and suggests a dynamic rather than dormant deep sub-seafloor biosphere.

PS 22 Metaproteomics: studying functional gene expression in microbial ecosystems

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

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

In microbiology, the application of post-genomic techniques has so far been mainly limited to pure laboratory cultures of micro-organisms. Consequently, these studies do not provide information on gene expression in complex mixtures of micro-organisms as found in the Earth's biosphere where they form the backbone of any given ecosystem. The study of environmental microbial communities is hampered by enrichment bias, in which standard culturing techniques select for 'weed organisms' and result in a distorted understanding of microbial structure and function. One way of addressing this situation is to study microbial populations *in situ*, respecting both community structure and natural habitat. This has lead to the recent emergence of metagenomics, the genomic analysis of uncultured micro-organisms. Now, with the availability of extensive metagenomic sequences from different microbial populations, the application of post-genomic techniques, proteomics in particular, to a range of complex microbial communities important to the Earth System is becoming possible. We have recently demonstrated the feasibility of carrying out proteomic investigations on a mixed microbial community from a laboratory-scale activated sludge system that was operated to remove phosphorus from wastewater. This sludge was dominated by *Rhodocyclus*-type polyphosphate accumulating organisms (PAOs). Our aim is to decipher the metabolic details of biological phosphorus removal by applying metaproteomics to the activated sludge system. A comparative study of metaproteomic expression in two distinct activated sludges has already lead to interesting insights into differential protein expression patterns within these communities. With the recent availability of the metagenomic sequences from other activated sludges enriched for *Rhodocyclus*-type PAOs, we have identified a number of proteins that may be directly linked to the metabolic transformations important for phosphorus removal. This study may have direct implications for our understanding of the Earth System since protein expression is a direct reflection of specific microbial activities in any ecosystem.

PS 23 Effects of antibiotics in soil microbial communities

M. Islas, B.J. Reid & P.L. Bond

Centre for Ecology, Evolution and Conservation, School of Environmental Sciences, University of East Anglia

Animal husbandry antibiotics are administered widely as mass medicaments, for disease prevention and growth promotion. These antibiotics and their degradation products are excreted onto pasture or applied to the land. Recently it is suggested there is a direct link between the increased use of antibiotics in agriculture and the spread of antibiotic resistant human pathogens in soil bacteria. Effects in soil microbial communities in response to antibiotics on soil are currently unclear. The aim of this study is to determine effects of Sulfamethazine (SMZ) on soil microbial community composition and the catabolism of the compound. The effects in microbial community composition were determined using molecular analysis by Terminal Restriction Fragment Length Polymorphism (TRFLP). The intrinsic and induced catabolism was determined by ^{14}C-respirometric analysis. Following three months of exposition to SMZ marked changes in the TPFLP profiles were detected indicating the disappearance of bacteria. TRFLP profiles after 6 months SMZ exposure indicated a recovery of bacterial soil communities. Preliminary assessment of TRFLP profiles suggests that the SMZ exposed soil contains bacteria related to known with phenolic and aromatic compound degraders. A 20 day respirometry test indicated degradation of SMZ was 2 fold greater in the soil exposed compared with the control soil.

Acknowledgments Sponsor: Science and Technology National Council, Mexico

PS 24 Compared biogeography of bacterial, archaeal and picoeukaryotic communities in marine sediments

L. Fevat[1], M. Schratzberger[2], D.B. Nedwell[1] & A.M. Osborn[1]

[1]Dept of Biological Sciences, University of Essex, Colchester CO4 3SQ; [2]CEFAS, Lowestoft Laboratory, Lowestoft NR33 0HT

Micro-organisms play a central role in oceanic ecology and regulate the Earth's climate. Some microbial species have been shown to be widespread and dominant across the oceans. Little is known about the biogeography of whole microbial communities, in particular in marine sediments. Importantly, simultaneous comparison of spatial and temporal variations in bacterial, archaeal and picoeukaryotic communities has not yet been made. Here, we used DNA sequencing and T-RFLP analysis of 16S/18S rDNA and rRNA to investigate the structure of total and active bacterial, archaeal and picoeukaryotic communities in offshore sediments from three connected seas around the UK. We found significant differences between the total and the active microbial community structures for the three domains of life. Spatial variation was observed in the total microbial community

structures. In contrast, the active microbial communities showed remarkable spatial homogeneity. Temporal variation in both the total and active microbial community structures over a one-year interval was less apparent suggesting stability of the benthic communities.

PS 25 Sensitivity of stable isotope probing in studying methanotrophic bacteria

M. Tiirola, M. Dumont, H. Schäfer & J.C. Murrell

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

Stable isotope probing (SIP) is a technique for identification of uncultivated micro-organisms that incorporate a particular growth substrate under conditions approaching those *in situ*. SIP involves incubation of an environmental sample with a ^{13}C-enriched substrate, such as $^{13}CH_4$, and the subsequent retrieval and analysis of labelled biomarkers. With DNA-SIP, ^{13}C-DNA is separated from ^{12}C-DNA by CsCl density gradient centrifugation. One drawback of the technique is that long incubation times and unnaturally high substrate concentrations may be needed to retrieve sufficient ^{13}C-DNA for analysis. In this study, the sensitivity of DNA-SIP was studied alongside RNA-SIP. Peat soil samples were exposed to varying amounts (20 -720 µmol) of $^{13}CH_4$ and total nucleic acids were purified. DNA was analysed using length heterogeneity analysis (LH-PCR) and denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rDNA. The sensitivity of DNA-SIP was an order of magnitude greater than previously reported. *Methylobacter* spp. were identified as one of the first methanotrophs to incorporate methane in the peat indicating that they may be dominant methanotrophs in this peatland environment.

PS 26 Bacterial cycling of methyl halides in the marine environment

H. Schäfer[1], M.J. Cox[1], I.R. McDonald[2], P.D. Nightingale[3] & J.C. Murrell[1]

[1]Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL; [2]Dept of Biological Sciences, University of Waikato, Hamilton, New Zealand; [3]Plymouth Marine Laboratory, The Hoe, Plymouth

The trace gas methyl bromide (MeBr) accounts for about 14% of tropospheric ozone depletion. The oceans are the second largest sink in the global cycle of MeBr and bacteria have been implicated in MeBr degradation, but the pathways of MeBr degradation in the marine environment are unknown. We isolated marine bacteria of the *Roseobacter* group that grew on MeBr and methyl chloride (MeCl) as carbon and energy sources. Some contained the *cmuA* gene which encodes the methyltransferase/corrinoid-binding protein CmuA that catalyses the initial step of MeCl oxidation in terrestrial methyl halide-oxidising bacteria. CmuA encoding genes were cloned from two isolates and expression of CmuA during growth on methyl halides was demonstrated by mass spectrometry. Specific PCR primers were used to prepare *cmuA* clone libraries. Analysis of these libraries revealed the genetic diversity of *cmuA* in samples from the Arabian Sea and enrichment cultures, indicating that *cmuA* containing bacteria are widespread in the marine environment. However, *cmuA* was not detected in all marine methyl halide oxidising isolates and it is likely that additional pathways exist that contribute to oceanic cycling of methyl halides and control the flux of methyl halides from the oceans to the atmosphere.

PS 27 The selective toxicity of *o*-cresol and 2,4-dimethylphenol to specific and physiologically distinct anaerobic bacteria

Alex McDowall & Irene Watson-Craik

Dept of bioscience, University of Strathclyde, Glasgow

The anaerobic bacterial toxicity and the fate of cresols and xylenols, which readily enter the groundwater environment from different sources, e.g. leaks at oil and gas works, are not fully understood. Two continuous-flow three-stage (CFTS) systems were employed to analyse the anaerobic toxicity of two phenolic compounds, *o*-cresol and 2,4-dimethylphenol(DMP). The CFTSs, inoculated with return activated sewage sludge and supplied with nutrient medium supplemented with cellobiose (1720mg/l) and sulphate (480mg/l), allowed the spatial segregation of physiologically different bacterial groups, through the imposition of different dilution rates in vessels 1-3. The effects of DMP and *o*-cresol on specific bacterial groups, e.g. Sulphate Reducing Bacteria, acidogens, acetogens and methanogens were then monitored. The toxicity of DMP (75-300mg/l or *o*-cresol (200-500mg/l).to methanogenic and acetogenic activities in Vessels 3 were first assessed. From the analyses of net volatile fatty acid concentrations and methane production it was evident that in Vessels 3 acetoclastic methanogenesis was the major hydrogen sink. Complete inhibition of methane production was recorded at 500mg/l *o*-cresol and 300mg/l DMP. Net acetate concentrations increased, indicating both inhibition of its utilisation and that acetogenesis was less sensitive to the effects of these phenolic compounds.

PS 28 Investigating the molecular basis of pressure-adapted growth using *Photobacterium profundum* as a model system

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

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

The 'pressure loving' γ-proteobacterium *Photobacterium profundum* was isolated from the Sulu trench at a depth of 2.5 km. Although *P. Profundum* grows optimally at 28MPa (15ºC), its ability to grow over a range of pressures (0.1-70MPa) and temperatures (2-20ºC) and its genetic tractability makes it an ideal organism to understand the molecular basis of pressure-adapted growth. A random pool of mini-Tn*5 P. Profundum* mutants were screened for defects in either pressure- or cold-adapted growth. Several of these mutants were identified to have transposon insertions in genes hypothesised to be involved in either lipopolysaccharide (LPS) or capsular polysaccharide (CPS) biosynthesis. We have been characterising the LPS and CPS from these mutants and the parent strain under different growth conditions by SDS/PAGE-silver staining and we are currently also analysing the CPS by transmission electron microscopy. Additionally, we are investigating exopolysaccharide production using calcofluor. The results of this study should greatly improve our understanding of the role of polysaccharides in pressure-adapted growth of bacteria.

PS 29 Diversity of Euryarchaea in termites, guts and their food soils

Kevin Purdy[1], Sarah Donovan[2], Alison Baxter[1], Jolene Thomas[1], Jean-Francois Simon[3], Matt Kane[4] & Paul Eggleton[5]

[1]School of Animal and Microbial Sciences, University of Reading; [2]School of Biological Sciences, University of Plymouth; [3]Institut Universitaire de Technologie de Montpellier (IUT), France; [4]National Science Foundation, Arlington, VA, USA; [5]Natural History Museum, London

Termites are an important component of tropical soil communities and have a significant affect on the structure and nutrient content of soil. Digestion in termites is related to gut structure, gut physico-chemical conditions and gut symbiotic microbiota. Here we describe the use of 16S rRNA gene sequencing and Terminal-restriction Fragment Length Polymorphism (T-RFLP) analysis to examine methanogenic Archaea (MA) in the guts and food-soil of the soil-feeder *Cubitermes fungifaber* Sjostedt across a range of soil types. If they are strictly vertically inherited, then MA in guts should be the same in all individuals even if the soils differ across sites. In contrast, gut MA should reflect what is present in soil if populations are merely a reflection of what is ingested as the insects forage. We show clear differences between the euryarchaeal communities in termite guts and in food-soils from five different sites. Analysis of 16S rRNA gene clones indicated little overlap between the gut and soil communities. Gut clones were related to a termite-derived *Methanomicrobiales* cluster, to *Methanobrevibacter* and, surprisingly, to the haloalkaliphile *Natronococcus*. Soil clones clustered with *Methanosarcina*, *Methanomicrococcus* or Rice Cluster I.

T-RFLP analysis indicated that the archaeal communities in the soil samples differed from site to site, whereas those in termite guts were similar between sites. There was some overlap between the gut and soil communities but these may represent transient populations in either guts or soil. Our data does not support the hypothesis that termite gut MA are derived from their food soil but also does not support a purely vertical transmission of gut microflora.

PS 30 Diversity of life in the Dry Valleys of Antarctica

S.J. Whiting[1], **J.M. Ward**[1], K.D. Bruce[2] & D.A. Cowan[3]

[1]Dept of Biochemistry and Molecular Biology, Darwin Building, University College London, Gower St, London WC1E 6BT; [2]Division of Life Sciences, Franklin-Wilkins Building, King's College London, 150 Stamford St, London SE1 9NN; [3]Dept of Biotechnology, University of the Western Cape, Bellville 7535, Cape Town, South Africa

The Dry Valleys of Antarctica represent one of the most extreme environments on earth and have long been considered a model system for the study of life on other planets. Life within these desert soils, where water contents range from 0.2-2.0% w/w and mean annual temperatures fall below <-20°C, must also contend with desiccating winds, freeze-thaw cycles, and high seasonal UV radiation. Molecular phylogenetic techniques have been used to characterize the diversity of organisms present in Dry Valley mineral soils belonging to each of the three domains of life: Bacteria, Archaea and Eukarya. The cultivated bacteria show a bias towards the *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. The analysis of the uncultivated bacteria show a higher degree of phylogenetic diversity and includes members of phyla with, as yet, no cultivated members. There is virtually no overlap when the cultivated and uncultivated bacteria are compared.

PS 31 Characterization of the *Anabaena* associated microbial consortium: microbial community structure (partially) unveiled

Nermin Adel El Semary, Linda Medlin & Paul K. Hayes

School of Biological Science, University of Bristol, Woodland Road, Clifton, Bristol BS8 1UG (Tel +44 (0) 1179287475))

Microbial community structure has always been underderscribed. *Anabaena*, one of the filamentous heterocystous cyanobacterial genera responsible for waterbloom formation worldwide, received little attention when it comes to defining the type of heterotrophic bacterial types found in association with it. Understanding bloom dynamics requires the identification of all community members to allow further investigation of the possible role played by each contributor. A rapid and efficient culture independent method was developed to characterise heterotrophic bacteria found in association with *Anabaena* filaments isolated from Cotswold Water Park in summer of 2002. The method is based on a subtractive procedure where universal bacterial primers are used to amplify 16S rDNA and then cyanobacteria-specific primers are used to eliminate cyanobacterial sequences from any further analysis. Based on the sequence of the cloned non-cyanobacterial 16S-rDNA we were able to design taxon-specific, fluorescent oligoncucleotide probes: these probes tended to be located within predicted single-stranded loop regions of the ribosomal RNA. *In situ* hybridization with these probes was used to localize the various bacterial taxa on *Anabaena* filaments. These bacterial types included members of Proteobacteria and CFB groups. This approach can be useful in unveiling the composition and structure of complex microbial communities.

PS 32 Microbial life associated with low-temperature alteration of ultramafic rocks

Frida Lise Daae[1], Lise Øvreås[1], Ingunn Thorseth[2] & Rolf Birger Pedersen[2]

[1]Dept of Biology, Jahnebakken 5; [2]Dept of Earth Science, Allegaten 41, N-5007, Bergen, Norway

Microbial life associated with low-temperature alteration of ophiolite-hosted ultramafic rocks located at the island Leka (Norway) has been studied. This site represents an unique, highly alkaline endolitic environment supporting microbial growth. The objective of our study was to describe and identify the microbial community composition associated with fracture filling secondary minerals formed during chemical reaction between the rock and percolating groundwater. Both culturing and molecular techniques were used. Phylogenetic analysis by sequencing of ssu rRNA genes from isolates were performed. To understand which organisms that comprise the unknown community, genomic DNA was extracted from crushed rock, mixed fracture filling minerals, pure brucite and groundwater. Fingerprints of the different communities were generated by using PCR-DGGE with primers specific for *Bacteria* and *Archaea*, the profiles were compared and bands were sequenced. An altered community profile was seen between rock, brucite and water. Heterotrophic bacterial and fungal populations were isolated and sequenced. Fungal isolates were all related to *Ascomycetes*. The isolates from water were different from the isolates found in brucite and in the mixed fracture filling minerals. The energy sources supporting this rich microbial community are still unknown.

PS 33 Associations between indicator bacteria and environmental variables in Jordan River sources

S. Masaphy[1,2] & I. Shpektorov[2]

[1]Migal, Galilee Technology Center, POB 831, Kiryat Shmona, 11016 Israel; [2]Tel Hai Academic College, Upper Galilee, 12210, Israel

Contamination of water sources is recommended to be monitored by various indicator bacteria. Various combinations of local natural environmental variables differently affect the behavior of each population of indicator bacteria, in certain natural water sources. In this study we compared the changes of the bacterial level: total coliforms, fecal coliforms and enterococci in the Banias and the Snir streams, which are the principle sources of the Jordan River. The Jordan sources are considered clean water, which, recently are increasingly used in water tourist activity. The bacterial levels were examined as well as accumulated daily solar radiation, water temperature and water flow. The impact of visitors was also determined. The level of the three types of bacteria increased in both streams during the dry season – June to September 2004. While high association between the fecal coliforms level and local environmental variables was found in the Banias stream, enterococci and the total coliforms did not show a connection to those environmental variables in both streams. However, the enterococci level was highly associated with the level of people visiting the site, especially the in Snir stream. The data suggest that the enterococci bacteria could serve a better indicator for water contamination due to people's activity in the water source examined.

PS 34 Microbial structures as indicators of hydrothermal origin of the Jurassic Mn- and Fe-bearing rocks, Tatra Mountains, Poland

Renata Jach[1] & **Teresa Dudek**[2]

[1]Institute of Geological Sciences, Jagiellonian University, Oleandry 2a, Kraków, Poland; [2]Institute of Geological Sciences, Polish Academy of Sciences, Senacka 1, 31-002 Kraków, Poland (Email nddudek@cyf-kr.edu.pl)

Jurassic Mn- and Fe-bearing rock sequence in the Tatra Mountains, S Poland, form a lens-shaped body, ~2 m thick and a few hundred of meters long. It consists of Fe-rich layer (up to 40 wt% Fe_2O_3), jasperite, Mn-rich layer (up to 50 wt% MnO) and shales. Mineralogical and geochemical data suggest that the origin of the sequence is related to the activity of hydrothermal submarine hot-springs.

Abundant microbial structures occur within these rocks. The Fe-rich layer is composed of encrusting foraminifera *Nubecularia* and rod-like ultracomponents, which represent fossilised cells of ferrous bacteria. The Mn-rich layer contains abundant microbial crusts and oncoids. Microbial crusts, composed mainly of Mn silicate minerals, form discontinued zones up to 25 mm thick, which cover and consolidate grain-like components. Oncoids, usually elongated or globular, are from 3 mm to 2 cm in diameter. Their nuclei are composed of bio-

and lithoclasts or barite crystals. The bacterial ultracomponents in the Mn-rich layer are poorly preserved.

The association of microbial structures with elevated concentrations of Fe and Mn suggests that micro-organisms were likely involved in the formation of Fe and Mn minerals. The assemblage of micro-organisms, which finds its equivalents in the present-day hot-spring deposits, make a strong case for the hydrothermal origin of the studied Jurassic sequence.

Acknowledgments Financial support was provided by the Polish State Committee for Scientific Research (grant no 2PO4D 03 127).

PS 35 Simultaneous resistance of Escherichia coli isolated from clinical samples to heavy metals and antibiotics in Kerman, Iran

S. Mansouri, M.H. Mos-Hafi & R.A. Neamati

Microbiology Dept, Kerman University of Medical Sciences, Kerman, Iran

Background Heavy metal ions are essential for bacterial growth at low concentration, but have toxic effects at higher concentrations. Simultaneous resistance to some heavy metals and antibiotics are reported in some species of enteric bacteria.

Methods Five hundred *Escherichia coli* isolates from clinical samples were tested for tellurite resistance by standard agar dilution method, highly resistant (MIC range ≥ 80 µg/ml, N=11), moderate resistant (MIC range 15-40 µg/ml, N=6) and very low resistant (MIC range ≤ 1µg/ml, N=5) isolates were selected, and their sensitivity to Silver(Ag), Mercury (Hg), Cadmium (Cd), amoxicillin, tetracycline and gentamicin were tested.

Results Resistance to Ag was nearly similar for all isolates, but the MIC level for Cd and Hg was higher in the isolates highly resistant to tellurite (P. value= 0.008, and 0.0008 respectively). No significant difference was observed between the resistance to antibacterial agents and the resistance to heavy metal ions in these bacteria.

Conclusion Simultaneous resistance to heavy metals and antibiotics were not seen in the *E. coli* isolates, however wide distribution of heavy metal resistance especially to tellurite was detected in clinical isolates of *E. coli in* this area.

PS 36 The effect of carbohydrate (EPS and colloidal) enrichment on the bacterial community present in estuarine sediments as determined by T-RFLP analysis

Kelly Haynes[1], Tanja Hofmann[1], Andrew S. Ball[1,2], Graham J.C. Underwood[1] & A. Mark Osborn[1,3]

[1]Dept of Biological Sciences, University of Essex, Colchester, Essex; [2]School of Biological Sciences, Flinders University, Adelaide, Australia; [3]Dept of Animal and Plant Sciences, University of Sheffield, Sheffield

Microphytobenthic (MPB) biofilms present on intertidal mudflats in the Colne Estuary, UK, are dominated by epipelic diatoms, which exude carbohydrate-rich extracellular polymeric substances (EPS). Using a combination of *in situ* sampling, and sediment slurry microcosm experiments, this research has examined how bacterial communities respond to the production of carbohydrate produced within the mudflat system and investigated which bacteria are involved in carbohydrate utilisation Microcosms consisting of 20% sediment slurries were set up with two treatments; colloidal enrichment (230 ug ml^{-1}), and EPS enrichment (100 ug ml^{-1}), using carbohydrate fractions obtained from sediment with high MPB biomass. Microcosms were incubated aerobically at 20°C, in the dark for 14 days. T-RFLP analysis was used to profile bacterial communities at day 0, day 2, day 4, and day 10 for rDNA (total community), and day 0, and day 4 for rRNA (active community), in addition to characterisation of the indigenous total and active bacterial communities in the original sediments. Clear shifts were observed in both the total and active communities between day 0 and day 4, in particular in the EPS enriched sediments. 16S rRNA clone libraries were generated from EPS enriched sediments from day 0 and day 4 in order to identify dominant bacterial groups in the community and/or that demonstrated marked changes in relative abundance in response to carbohydrate enrichments.

PS 37 Temporal and spatial variation of nitrite reductase (*nirS*) gene copy number along the Colne estuary, UK, using Q-PCR

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

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

Denitrification is the biological breakdown of fixed nitrogen in the form of nitrate to atmospheric dinitrogen gas. Nitrate is sequentially reduced to nitrite, nitric oxide, nitrous oxide and finally to N_2 by a series of enzymatic reactions. Denitrification can be studied at a molecular level by examining the functional genes encoding these enzymes. One of the key enzymes in this process is nitrite reductase (encoded by *nirS* & *nirK*), which reduces nitrite to nitric oxide. To investigate the functional potential for denitrification in estuarine sediments, Q-PCR has been used to quantify *nirS* gene copy numbers in the Colne estuary, UK, along a nutrient input gradient from the estuary head, where nutrient inputs are high, to the estuary mouth. Q-PCR reproducibility was first evaluated by quantifying 16S rDNA gene copy numbers present in the same sediments in replicated Q-PCR assays to establish the limitations of Q-PCR and to determine Q-PCR best practice. Q-PCR assays were then developed to quantify *nirS* sequence types in Colne sediments. *nirS* sequences in the Colne were highly dissimilar from previously characterised *nirS* genes and additionally the high variability of *nirS* sequences made the design of 'universal' *nirS* primers and *Taq*Man probes impossible. Therefore, a suite of primers and probes were designed to target and quantify subgroups of *nirS* sequences. Q-PCR analysis revealed a spatial and temporal variation in *nirS* gene copy numbers, which were highest at the head of the estuary and decreased towards the estuary mouth.

Authors

Authors