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Abstracts

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Plenary session

Food, fluids, fingers, faeces and flies: food- and water-borne pathogens

From farm to fork

Government directives and scientific realities

H. Dalton

Defra, London

Abstract not received

Fresh leafy greens and *Escherichia coli* O157:H7: outbreaks, incidence in the environment, source-tracking

Robert Mandrell & Michael Cooley

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There have been 22 outbreaks in the US since 1995 associated with *E. coli* O157:H7 and leafy greens. These and the 2006 multistate food borne outbreak in the US linked to fresh-bagged spinach have raised concerns about leafy green safety and the environments in which they are grown. An ongoing sampling study of watersheds in the Salinas, Central California Coast region, where >70% of the US leafy greens are grown, revealed the incidence of *E. coli* O157:H7 in locations near point sources relevant to understanding why multiple outbreaks associated with leafy greens have occurred. A multiagency investigation of four farm/ranches linked to the 2006 spinach outbreak resulted in one ranch yielding a very significant percentage of O157-positive samples, including the outbreak strain, and the first time a farm/ranch had been identified as the likely source of a leafy green outbreak. Information about *E. coli* O157:H7 will be discussed in the context of it's fitness on plant surfaces, potential environmental risk factors, and source-tracking methods. Contamination of the pre-harvest produce production environment is a dynamic process due to multiple sources yielding multiple strains that can be transported by run-off water, wildlife, and possibly other factors.

Processing pigs to pork: do critical control points exist for *Salmonella*?

Christine E.R. Dodd

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In the production of red meat, few procedures exist to control the spread of zoonotic pathogens between slaughter of the animal and chilling of the final carcass. The implementation of a Hazard Analysis Critical Control Point (HACCP) System relies mainly therefore on the prevention of microbial spread by good hygiene measures. However, pork production is an exception as the particular methods for carcass production involve heat treatments, scalding and singeing, at temperatures which superficially should help control the presence of pathogens such as *Salmonella* on the carcass surface. Despite this, *Salmonella* is an important contaminant of pork carcasses. By using molecular typing to differentiate bacterial isolates obtained at various stages of pork processing, sites which contribute to the final contamination of the carcass can be identified. This has demonstrated both the failure and the potential of these stages such as scalding and singeing to act as critical control points to fully control carcass contamination. A variety of studies has examined the reasons for these failures and demonstrate that reduction of pathogen levels by these procedures are dictated by a range of factors.

Pathogen survival in dairy products

C. Hill

University College Cork, Ireland

Abstract not received

Food-borne disease

Epidemiology of food-borne infection in the UK

R. Adak

Health Protection Agency – Colindale

Abstract not received

Endemic HEV in Europe: prevalence and transmission

Erwin Duizer

National Institute for Public Health and the Environment, Centre for Infectious Diseases Control and the Food Borne Viruses in Europe-network

Acute hepatitis is a public health problem that is caused by a variety of viruses. While Hepatitis E virus (HEV) is recognized as a major cause of viral hepatitis in humans in developing countries, HEV infections in industrialized countries were assumed to be related to travel to endemic regions. However, now there is increasing evidence of locally acquired HEV infections in humans in industrialized countries. In Europe hepatitis E is diagnosed in up to 13% of patients with acute viral hepatitis and anti-HEV-IgG is detected in about 3% of the blood donors.

In Europe, locally acquired HEV belong to genotype 3 and are closely related to the viruses found in pigs. So far, the exact mode of transmission is unknown. Direct transmission from pigs to humans by contact with animals or their excreta is suggested, but not proven. Currently, zoonotic food born transmission of HEV is becoming more likely since the proof of principle was reported in Japan after consumption of raw meat of a Sika deer in 2003 and wild boar in 2005. Since then, the presence of HEV RNA and infectious HEV has been shown in commercially available pig livers in Japan, the USA and the Netherlands.

For Europe, we recommend testing acute hepatitis patients for HEV, regardless of travel history. Furthermore, research to elucidate the risk factors for indigenous HEV infections in industrialized countries is ongoing.

The epidemiology of VTEC in animal reservoirs and implications for human infections

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Enterohaemorrhagic *Escherichia coli* O157:H7 is an important intestinal pathogen of humans with a main reservoir in domesticated ruminants, particularly cattle. By controlling the organism within its reservoir hosts it is anticipated that the risk of human infection can be reduced. Studies will be described that have been carried out to define the prevalence of the organism in ruminant species and to elucidate mechanisms of transmission in the field. The importance of colonised

cattle and the terminal rectum as the principal site for *E. coli* O157:H7 carriage will be discussed. Studies to examine the efficiency of different challenge routes in establishing rectal colonisation and histological examinations revealing intimate bacterial attachment and pathological change at the site will be reported. Several options for the control of *E. coli* O157:H7 in cattle have been proposed but none have been demonstrated to be successful in the field. A novel experimental method to eliminate faecal carriage of *E. coli* O157:H7 will be considered with a combination of a detection method and treatment regimes that could be used in the field to eliminate high level faecal excretion of *E. coli* O157 so greatly reducing prevalence within this host.

Acknowledgements These studies have been supported by: BBSRC, Defra, Food Standards Agency Scotland, the Scottish Executive Rural Directorate and Wellcome Trust.

Gastroenteric viruses: food and waterborne contamination as drivers for evolution

Jim Gray

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Viruses associated with gastroenteritis include rotaviruses, noroviruses, sapoviruses, astroviruses and enteric adenoviruses. With the exception of adenoviruses, the others have RNA genomes and their genetic diversity is associated with the accumulation of point mutations, associated with the error prone nature of viral RNA replication, resulting in the generation of virus variants. Also, recombination or reassortment of viral genes can expand the genetic diversity of gastroenteric viruses co-circulating in the human population, especially, when these events occur during infection with both human and animal viruses. Food and water contaminated with faeces provides the opportunity for infection with more than one related virus and the subsequent recombination or reassortment of viral genes during dual infection of a single cell. Outbreaks of gastroenteritis associated with food or water will be described and the possible public health impacts of virus variants, recombinants and reassortants will be discussed.

Water-borne disease

Science in the fight against water-borne diseases

J.B. Rose

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Abstract not received

Significance of water-borne *Cryptosporidium*

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Cryptosporidium is a genus of coccidian protozoal pathogens of which there are 16 currently recognised species. Members of the genus can cause diarrhoeal disease in a range of human and other hosts. The two species that are most commonly associated with human disease are *C. parvum* and *C. hominis*. Natural infections with *C. hominis* are almost exclusively restricted to humans whilst *C. parvum* is associated with infection in a wide range of hosts, though probably the most important host is the calf.

That contaminated drinking water is a potential transmission pathway has been shown very dramatically is a series of waterborne outbreaks throughout the last three decades. Most such outbreaks have been reported in the UK, though the largest occurred in Milwaukie in the US. Despite the clear link between outbreaks and the water supply, there is still considerable uncertainly about the role of drinking water in sporadic disease. Routine monitoring has shown that *Cryptosporidium* oocysts are frequently detected in treated water. However, rarely is the detection of oocysts followed by human illness.

This paper discusses the disease burden attributable to cryptosporidium in drinking water and also whether or not the detection of oocysts in water supplies necessarily poses a risk to human health.

Survival and transmission of *Campylobacter* in water

Keith Jones

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The following topics will be covered:

Inputs – contamination of surface waters, namely, streams, rivers, estuaries, coastal bathing waters, groundwater and drinking water reservoirs with campylobacters from sewage effluent, grazing livestock, farm slurries and wild animals (including birds).

Survival – the ability of *Campylobacter* to survive the major biocides, temperature, salinity and solar radiation in surface waters. Potential roles for biofilms, sediments and protozoal predation (protection) in survival.

Transmission – the various ways in which campylobacters are transmitted from water to humans and livestock will be discussed with reference to drinking water (bottled water, private water supplies, pristine (?) streams, reservoirs water holes and water butts); recrudescence of livestock drinking from streams, spring, troughs and rivers; and contamination of fresh produce irrigated with irrigation water.

Water-borne viruses

Peter Wyn-Jones

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There is a public perception that swimming in sewage-polluted water may result in disease. Review of reported symptoms and expert opinion suggests that such disease may be caused by viruses. Proving the link between the presence of disease-causing viruses in water and the same virus being the agent of a particular disease incidence or incident is difficult. The reasons for this include the technical problems associated with detecting low levels of viruses in large volumes of water, the difficulty of excluding other potential sources of infection, and the varying persistence of viruses in water, even though viruses generally survive longer in water than bacteria. The principal pathogens of concern include noroviruses and hepatitis A and E viruses. Among the less (or non-) pathogenic viruses detected in samples from waters used for recreation, adenoviruses and enteroviruses are regularly found. Technical problems in virus detection have largely been overcome and it is now possible to detect commensal viruses and some pathogens regularly with confidence in water to the extent that it is feasible to begin research to link viruses in water with observable health effects in target populations, and this could lead to reconsideration of viruses as indicators ofsewage pollution and potentially as an index of associated health risk. Detection of human viruses in sewage also gives us a picture of the viruses circulating in the local populations, and detection of animal viruses provides information on the extent of agricultural run-off into water courses or recreational areas.

Intervention strategies

Microbial ecology of foods: using food structure and chemistry to control bacterial growth

S. Brul

University of Amsterdam, The Netherlands

Abstract not received

Natural products as an intervention strategy

G. Mycock

Unilever, Vlaardingen, The Netherlands

Abstract not received

Potential control of infection by food-borne pathogens through the consumption of probiotics

Gwen Falony & **Luc De Vuyst**

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The human gut is continuously exposed to the threat of being colonised by potentially pathogenic micro-organisms. It has therefore developed an efficient host defence system, promoted by the proper and/or synergistic functioning of the gastrointestinal epithelium and the resident gut microbiota. These two partners form a physical, chemical, and microbial barrier in the gut against microbial pathogens. Probiotics are thought to be able to strengthen every line of colonic defences against invasion by pathogens, thus contributing to human health. These mechanisms include competion for the nutrients available in the gut lumen and for colonization sites on the epithelium. Further, probiotics can directly interfere with growth and even kill pathogens by producing antimicrobial compounds, such as organic acids and antibacterial peptides. This may explain why a large number of *Lactobacillus* strains inhibit the growth of bacterial pathogens, such as the gastric pathogen *Helicobacter pylori,* and food-borne pathogens, such as *Campylobacter, Salmonella*, *Escherichia coli*, and *Listeria monocytogenes*. Last but not least, probiotics may stimulate the innate as well as the adaptive immune response of the host. However, health-promoting effects are strain-dependent and it is obvious that not all probiotic strains possess all possible health-promoting properties. In addition, health-promoting properties of valid probiotic strains are food matrix-dependent.

Intentional food contamination and bioforensic analysis

David K.R. Karaolis

National Biodefense Analysis and Countermeasures Center (NBACC)
Battelle National Biodefense Institute (BNBI)

Bioterrorism and biocrimes are the intentional release or dissemination of biological agents (bacteria, viruses or toxins) with the intent to cause harm or panic. The agents may be used in a naturally-occurring or modified form. Specifically, food bioterrorism is the deliberate act of tampering or manipulating the food or water supply with biological agents or toxins. The diversity of foods and available water supplies as well as its potential economic impact, makes the food and water supply a significant potential target for a terrorist attack. This talk will describe some of the known incidences of intentional contamination of food, the potential of food and water contamination using biological agents, actions which can be taken to prevent and reduce food bioterrorism, and some of the bioforensic techniques available to investigate bioterrorism and biocrime events involving contamination of the food supply.

Hot topic symposium

Post-genomic analysis of microbial function in the environment

Comparative metagenomic analysis of complex microbial communities

P. Hugenholtz

DOE Joint Genome Institute, California, USA

Abstract not received

Application of 16S rRNA arrays in microbial ecology

Gary Andersen

Lawrence Berkeley National Laboratory

A high-density microarray system was developed to accurately measure a broad range of microbial components in air, water, and soil environments. Unique regions of DNA within gene sequences of 16S small subunit of bacterial and archaeal ribosomes are used to identify specific organisms. To assist in probe design, an online tool (greengenes, http://greengenes.lbl.gov) was developed to maintain a comprehensive set of aligned, full-length, chimera-screened 16S rRNA genes from both Archaea and Bacteria and which allows users to exchange taxonomic annotations. A minimum of 11 oligonucleotide probes (25-mers) are used in combination to identify, in parallel, any of over 9,000 distinctive species or taxa on a 500,000 probe, high-density microarray. The combinatorial approach of multiple probes has clear advantages over a single probe for the identification of a target sequence. Universal bacterial and archaeal 16S primers that target conserved areas at the 5' and 3' ends of the 16S rRNA gene are used to amplify 1400–1500-bp fragments for analysis. As an example, the majority of sub-families documented by cloning aerosol amplicons were also detected in 4 of 4 replicate microarray hybridizations. Conversely, the array detected over double the number of sub-families than did the clone library. The phyla *Nitrospira* and *Spirochaetes* were uniquely detected by the array and were verified with specific PCR primers and subsequent amplicon sequencing. Compared with sequencing a 16S rDNA clone library, the microarray was unable to recognize novel prokaryotic families but could identify greater diversity from organisms with similarity to existing sequence. Furthermore, the microarray allowed samples to be rapidly evaluated with replication.

GeoChip: A comprehensive microarray for investigating biogeochemical, ecological, and environmental processes

Zhili He[1], Ye Deng[1], Joy Van Nostrand[1], Christopher Hemme[1], Terry Gentry[2], Weimin Wu[4], Christopher Schadt[2], Liyou Wu[1,2], Baohua Gu[2], David Watson[2], Terry C. Hazen[3], Phil Jardine[2], Craig S. Criddle[4] & **Jizhong Zhou**[1]

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Microarray technology provides the opportunity to identify thousands of microbial genes or populations simultaneously. Recently, a comprehensive functional gene array, called GeoChip, has been developed, evaluated and applied for characterizing microbial communities in natural systems. GeoChip 2.0 contains 24,243 oligonucleotide (50mer) probes and covers >10,000 genes in >150 functional groups involved in nitrogen, carbon, sulfur and phosphorus cycling, metal reduction and resistance, and organic contaminant degradation. It is a powerful generic tool, and can be used for: (i) profiling various environmental samples, such as soil, groundwater, sediments, oil fields, deep sea, animal guts, and etc; (ii) studying biogeochemical processes and functional activities of microbial communities important to human health, agriculture, energy, global climate change, ecosystem management, and environmental cleanup and restoration; (iii) exploring direct linkages of microbial genes/populations to ecosystem processes and functions; and (iv) detecting functional genes and/or organisms in a particular environment. Here, we present two application examples on the dynamics and stability of microbial genes and associated communities during a bioremediation period at the Oak Ridge Field Research Center (FRC) and the Hanford site. GeoChip 2.0 was first used to track the dynamics of metal-reducing bacteria and associated communities for an *in situ* bioremediation at the FRC site in Oak Ridge. Samples were taken from different wells after ethanol injections (After day 166). During the uranium reduction period, both FeRB and SRB populations reached their highest levels at Day 212, followed by a gradual decrease over 500 days. Consequently, the uranium in groundwater and sediments was reduced, and the uranium concentrations in the groundwater were significantly correlated with the total abundance of *c*-type cytochrome genes from *Geobacter*-type FeRB and *Desulfovibrio*-type SRB. Mantel test also indicated that there was significant correlation between the differences of uranium concentrations and those of total c-cytochrome gene abundance or *dsrAB* gene abundance. These results suggested that *Geobacter*-type FeRB and SRB played significant roles in reducing uranium to a level below the drinking standard (<30 µg/L). GeoChip 2.0 was also used to evaluate functional communities at a lactate-fed chromium reduction system at the Hanford site. Extraction well samples showed higher numbers of functional genes than the injection well at the same depth. Within the extraction well, abundance decreased with depth. However, the relative abundance of chromium resistance genes increased with depth in this same well. All results demonstrate that GeoChip is a useful tool for analysis of microbial communities in natural systems.

GeoChip 2.0 is the most comprehensive functional gene array currently available for environmental studies, but due to exponential increases in the number of genes and the number of sequences for each gene, a new generation of such an array (GeoChip 3.0) is in development. GeoChip 3.0 is expected to have the following new features: (i) It is more comprehensive and representative, and covers >37,700 gene sequences of 290 gene families, including the phylogenetic marker, *gyrB*; (ii) The homology of automatically retrieved sequences by key words is verified by HUMMER using seed sequences so that unrelated sequences are removed; (iii) A software package (including databases) has been developed for sequence retrieval, probe and array design, probe verification, array construction, array data analysis, information storage, and automatic update, which greatly facilitate the management of such a complicated array, especially for future updates; and (iv) It includes GeoChip 2.0 probes, and those probes are checked against with new databases.

Metagenomics of nitrate reduction in the hypernutrified Colne estuary

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

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Metagenomic techniques are revolutionising microbial ecology, enabling studies of complex environmental microbial assemblages. In marine sediments bacterial nitrate reduction removes nitrogen from water reducing eutrophication, via conversion of NO_3^- to NH_4^+, N_2O or N_2 during anaerobic respiration. The hypernutrified estuarine system under investigation, (Colne, Essex) receives significant inputs of NO_3^- and NH_4^+. To study these bacterial- and archaeal-driven processes prokaryotic DNA based libraries were constructed. Direct DNA extraction methods isolate eukaryotic DNA and may dominate the resultant libraries due to genome size. In this study a protocol was identified that enriched prokaryotic DNA by indirect DNA extraction methods. These extractions resulted in no compositional biases in the resulting prokaryotic DNA assessed by T-RFLP. The extracted DNA was used for metagenomic library construction and was screened for genes involved in denitrification, nitrate ammonification and anammox bacteria. Several clones containing different functional genes involved in nitrate reduction have been identified and characterised.

Investigation of ocean acidification using metagenomic approaches

Jack A. Gilbert[1], Kate Crawfurd[1], John Woolven-Allen[1], Bela Tiwari[2], Gareth Wilson[2], Dawn Field[2] & Ian Joint[1]

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The dissolution of anthropogenic CO_2 from the atmosphere into the oceans will decrease surface pH by 0.3 units over the next 100 years (IPCC 2007); the phenomenon is referred to as ocean acidification (OA). There are concerns that OA will affect marine microorganisms, with significant impacts on marine biogeochemical cycling. To test this hypothesis, we manipulated six 11,000 litre mesocosm enclosures near Bergen, Norway during May 2006. We analysed the response to OA by measuring organism abundance, nutrient concentrations and physiochemical parameters. In order to determine the functional and phylogenetic changes in the microbial population, we utilised pyrosequencing, fosmid library screening and DGGE sequencing. These analyses have enabled us to elucidate the effects of OA on a wide range of bacterioplankton and to demonstrate the adaptability and resilience of marine microbial populations.

Environmental transcriptome analysis

Víctor Parro

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Transcriptome analysis by DNA microarrays can proportionate an instant picture about the preferential gene expression between two environmental samples. However, this type of analysis is very difficult and complex in natural ecosystems, mainly due to the broad biodiversity and multiple environmental parameters that may affect the gene expression. The necessity of high quality RNA preparations as well as complicated data analysis are also technological limitations. All these drawbacks may be overcome in low biodiversity environments and by applying total RNA amplification techniques. We amplified environmental RNA (meta-transcriptome) from the low prokaryotic diversity extremely acidic and metal rich waters (20 g L^{-1} of iron) for global gene expression by DNA microarrays of the acidophile, strict iron oxidizing bacterium *L. ferrooxidans* (Parro et al., 2007. *Environ Microbiol* 9: 453). Transcriptomic fingerprints were obtained for this bacterium depending of the physicochemical parameters governing the immediate environment, like iron concentration, salinity, metal content, oxidative stress, nutrient limitations, etc. Ecological parameters like plankton-like versus the sessile life style in a biofilm, or the effect of depth in the water column are also being studied. Additionally, we used total environmental RNA for biodiversity assessment by oligonucleotide microarrays. This methodology allows to check specific probes for the 16S and 23S rRNA, as well as any other RNA or functional gene for phylogeny purposes.

Functional genomic analysis of novel regulators in *Pseudomonas* involved in plant–microbial interactions

Patrick Kiely, Jill Haynes, Abdelhamid Abbas, Louise Mark-Byrne & Fergal O' Gara

Biomerit Research Centre, Dept of Microbiology, National University of Ireland (UCC), Cork, Ireland (Tel +353 21 4902934; Email patkiel01@yahoo.com)

It is well accepted that plant signals can influence both the structure and behaviour of the microbial community in the rhizosphere. However, despite this, little is still known about the effects of these signals on both bacterial gene expression and subsequently their role in microbe-plant interactions. In order to investigate this we have profiled the influence of different plant root exudates on the transcriptome of *Pseudomonas aeruginosa* PAO1, using Affymetrix Gene Chips™ (Mark *et al.*, 2005). *P. aeruginosa* PAO1 was chosen as a model strain due to its ability to colonise the rhizosphere and the availability of microarray technology. Subsets of genes differentially regulated in response to the different plant root exudates were identified. One subset of particular interest included genes that encode for putative transcriptional regulators. It was hypothesised that these differentially regulated genes may play a role in microbial behaviour of *P. aeruginosa* in the rhizosphere. To date a number of regulatory genes have been shown to be involved in the competitive ability of *P. aeruginosa* to colonise this complex niche. Functional genomic and proteomic profiling techniques have been used to identify targets of these regulators. Analysis of these targets to date has identified genes with roles in nutrient transport and metabolism and the stress response.

Reference Mark, G.L., Dow, J.M., Kiely, P.D., Higgins, H., Haynes, J., Baysse, C., Abbas, A. Foley, T., Morrissey, J., O'Gara, F. (2005). Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe–plant interactions. *Proc Natl Acad Sci U S A* **102**, 17454–17459.

Genomic and proteomic analysis of bacterial dimethyl sulfide production

J.D. Todd, A.R.J. Curson, R. Rogers, L. Sun, M.N. Nikolaidou-Katsaridou & A.W.B. Johnston

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Dimethyl sulfide (DMS) is an environmentally important gas, its oxidation products causing cloud nucleation and possibly affecting global weather and climate. DMS is generated by bacterial catabolism of dimethylsulfoniopropionate (DMSP), a secondary metabolite made by marine algae and a few angiosperms.

We identified a gene, *dddD*, from the marine γ-proteobacterium *Marinomonas*, which when cloned, conferred DMS production to *E. coli*. DddD is an acyl-CoA transferase (Class III), which may catalyse the formation of a DMSP-CoA intermediate. DddD homologues exist in taxonomically distinct marine and terrestrial bacteria. Within these taxa *dddD* is always dispersed between genes encoding an oxidoreductase, a transporter and a regulator, the type of which varies dramatically. This suggests that *ddd* genes are prone to shuffling by horizontal gene transfer.

Some bacteria that make DMS from DMSP lack DddD, e.g., *Sulfitobacter* EE36. We used proteomics to identify DMSP-induced proteins in *Sulfitobacter*. Such proteins included an acyl-CoA transferase (Class I). Therefore, such bacteria may also make a DMSP-CoA intermediate prior to DMS release.

Community microbial proteomics of various environments

Philip L. Bond[1], Margaret Wexler[2] & Paul Wilmes[3]

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This talk will review the approach of using proteomic analyses to examine protein expression directly from environmental samples (termed metaproteomics). Molecular analysis of environmental samples has vastly increased our understanding of microbial diversity and ecology. However, the next big challenge is to understand details of function in these environments, particularly to link the phylogenetic and functional information. Subsequently, there is increasing emphasis to study microbial communities directly in their natural environments. Escalating sequence information (genomic and metagenomic) provides increasing potential for application of high throughput functional approaches. Transcriptomics and proteomics can be used to detect expression profiles and provide functional insight directly from environmental samples. So far, the metaproteomic approach has been applied to a limited range of environments that include activated sludge, acid mine biofilms, freshwater, soil and faecal samples. The findings of these investigations will be discussed, along with the challenges and future directions of the metaproteomic approach.

Methodological advances in proteomics-based analysis of environmental microbes

P. Wright

University of Sheffield

Abstract not received

Linking phylogeny to function by FISH-MAR

Per Halkjær Nielsen & Jeppe Lund Nielsen

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Microautoradiography (MAR) is an efficient method to obtain reliable information about the ecophysiology of microorganisms at the single cell level in mixed communities. When MAR is combined with fluorescence in situ hybridization (FISH) with oligonucleotide probes for the identification of target organisms specific information about the individual cells and populations directly within in the complex ecosystems can be obtained. Thus, if the identities of specific bacteria have been revealed by clone library analysis/environmental genomics and oligonucleotide probes are designed, it is possible to get important information about their potential role in the ecosystem and/or it can be used to verify functions predicted by the metagenomic information. Investigations onto specific probe-defined microorganisms have provided information about which organic substrates that can be taken up, autotrophic or mixotrophic activity, activity under various electron acceptor conditions, uptake of inorganic phosphate, and response to inhibitors. Also quantitative kinetic parameters such as cell-specific uptake rates and substrate affinity constants can be obtained. FISH-MAR can also be used to investigate degradation pathways of organic matter in a microbial community. Examples from natural ecosystems and from wastewater treatment systems will be given and the newest developments in the FISH-MAR technique will be presented.

Stable isotope probing in microbial ecology: from methane to metagenomics

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Stable isotope probing (SIP) offers microbial molecular ecologists a chance to link the function of microorganisms with their phylogeny. Using this technique it is possible to label cellular constituents of microorganisms which are active in soil with highly enriched ^{13}C substrates. These ^{13}C labelled constituents can then be separated from non-labelled macromolecules from inactive microorganisms and used as template in PCR with both phylogenetic (16S rRNA) and functional gene probe primer sets. DNA-, RNA and PLFA-SIP techniques have been used to study the diversity and activity of methane oxidising bacteria in a number of environments including landfill cover soils, peat and forest soils. The merits and limitations of DNA-SIP including issues of substrate concentrations, sensitivity, incubation time and the analysis of ^{13}C labelled DNA with DGGE and clone libraries for methanotroph 16S rRNA genes and functional genes such as *pmoA* and microarray analyses will be discussed. DNA-SIP techniques can also be used in environmental metagenomics studies in proof-of-principle experiments to recover methane monooxygenase gene clusters directly from environmental soil DNA samples. Examples of our use of DNA-SIP, whole gene amplification and metagenomics in studies on methane oxidation and methylotrophy will also be discussed.

Application of Raman spectroscopy in environmental microbiology

A. Whiteley

Centre for Ecology and Hydrology, Oxford

Abstract not received

Metagenomics and metaproteomics of polysaccharide-degrading micro-organisms

Michael J. Cox, Jennifer L. Edwards, Clive Edwards & Alan J. McCarthy

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Metagenomic libraries have previously been used successfully to identify polysaccharide hydrolases in bulk coastal and estuarine water (Cottrell, 1999, *Appl Environ Microbiol* 65, 2553–2557) by analysis of fosmid libraries constructed from DNA extracts cloned in fosmids and screened for expression in a heterologous host. Here, we are concentrating on cellulose and chitin, which are present and abundant in the marine environment as part of the particulate organic carbon pool, and using an integrated 'omic' approach to examine the presence and diversity of polysaccharide degrading microorganisms that colonise baits *in situ*.

We are using functional genetic markers and analysis of expression to screen the metagenomic fosmid libraries and hope to access a more complete range of glycosyl hydrolases. These are an excellent target for the development of metaproteomic techniques in diverse samples as they can be identified by zymography and then concentrated by cellulose and chitin affinity chromatography prior to separation by 2D gel electrophoresis.

Thus metagenomic and proteomic investigation coupled with traditional isolation and characterisation of microorganisms from the bait samples will provide a comprehensive primary analysis of the degradative population in marine biofilms. Stable isotope probing with 13C cellulose as substrate will be used to provide definitive evidence for the implication of specific taxa in the primary degradation of particulate organic carbon in the sea.

Combining PLFA-SIP with molecular methods for better understanding of ecosystem functions

Brajesh K. Singh

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Stable isotope probing (SIP) has been used widely to analyse active microbial populations involved in particular ecological functions. In

this approach the soil/water sample is treated with ^{13}C labelled substrate which is incorporated in cellular components by the active microbial populations such as PLFA, DNA and RNA. Both PLFA-SIP and DNA/RNA-SIP have been criticised for different reasons. The PLFA-SIP approach is preferred when low amount of labelling and quantitative information are needed but it has low phylogentic resolution and, therefore, identification of the active populations at genera or species level is not possible. For DNA/RNA-SIP, high amount of ^{13}C incorporation in microbial populations is essential to separate heavy fraction from light nucleic acid. Exposure of soil microbial community to unrealistic amount of substrates and nutrient amendments has been demonstrated to have effects on the observed active populations. Hence results obtained from laboratory incubation experiment may not represent the active population in the field.

Here, I demonstrate and discuss that a combination of PLFA-SIP and molecular methods (cloning and sequencing of functional genes and gene expression) may provide a better understanding of ecosystem functioning in natural conditions.

Lab on a Chip PCR: linking function and phylogeny at the single cell level

J.R. Leadbetter

California Institute of Technology, USA

Abstract not received

The CAMERA project (Environmental Metagenomics and Meta-analysis)

P. Gilna

University of California at San Diego, USA

Abstract not received

Cells & Cell Surfaces/Microbial Infection Groups joint session

Mechanisms of diarrhoeal disease

Enteric bacterial toxins

Patrice Boquet

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This lecture will describe the different mechanisms by which bacterial toxins interfere with the integrity of the intestinal mucosa leading to diarrhoea. In particular mechanisms involving the modification of Rho GTPases by bacterial toxins will be exposed in detailed. Some new data concerning *Clostridium difficile*, the most common cause of bacterial diarrhoea in hospitalized patient, will be presented. Indeed, several outbreaks due to the PCR ribotype 027 and associated with increased disease severity and death have been reported worldwide. The 027 strain is characterized 1) by the presence of a deletion in the negative regulator TcdC gene, that controls the TcdA and B production, leading to a prematurely terminated protein thus unable to control negatively the production of toxins and 2) by the presence of a third toxin named CDT that belongs to the iota binary toxin family. Beside the 027 strain there are others *C. difficile* strains that also harbour the CDT toxin and various deletions/mutations of the TcdC gene. We have evaluated by quantitative PCR in different strains of *C. difficile* isolated in our clinical laboratory harbouring the CDT toxin and various TcdC deletions/mutations the real impact of this gene on toxin production.

Intracellular mediators and pathogen-induced alterations in intestinal electrolyte and fluid transport

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Enteropathogenic *E. coli* are the causative agent of endemic diarrhea in developing countries. The underlying cause of diarrhea is multifactorial with disruption of tight junctions, decreased barrier function and altered ion transport among other effects. EPEC alter electroneutral ion exchange including sodium-hydrogen exchange (NHE) activity, chloride/hydroxyl, and sodium glucose co-transport (SGLT1) activity. A decrease in sodium and chloride absorption causes an imbalance in ion levels which is the theoretical equivalent to altered chloride secretion. NHEs are present as multiple isoforms in the human intestinal epithelia including NHE1, NHE2 and NHE3. Each isoform plays a different role in terms of altering sodium uptake as well as intracellular pH at the level of the cell or the tissue. The activity of NHE3 is decreased upon infection with EPEC resulting in less sodium absorption. NHE3 is thought to play a role in regulating sodium absorption throughout the intestine rather than at the cellular level as for NHE1 and NHE2. Studies in knock-out mice show that only those without NHE3 activity develop diarrhea suggesting that only this isoform regulates water balance in the intestine. EPEC cause an increase in NHE1 and NHE2 activity with the change in NHE2 activity depending on activation of PKC alpha and epsilon. The activation of these isoforms is thought to represent an ameliorative response by the host cell. The combined loss of NHE3 activity and apical chloride/hydroxyl exchange is though to contribute to EPEC-mediated diarrhea in combination with other factors including altered barrier function and decreased SGLT1 activity.

Secretory, inflammatory and invasive diarrhoea – linking clinical patterns and mechanisms

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Although acute diarrhoeal illnesses are often described as being watery and non-invasive, or inflammatory and invasive, there is poor correlation between the clinical disease patterns and the underlying bacterial mechanisms at work. This will be illustrated by looking at varying clinical presentations in different human host groups, and at the underlying bacterial pathogenetic mechanisms for several microorganisms. In particular, diarrhoeal disease caused by *Vibrio cholerae* 01 will represent the classical secretory diarrhoeal illness, and the diarrhoeal and enteric disease syndromes caused by *Salmonella* spp. will be discussed.

Development of a polarised IVOC system to study colonisation of human intestinal mucosa by EPEC

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In vitro organ culture (IVOC) represents a gold standard model to study enteropathogenic *E. coli* (EPEC) infection of human intestinal mucosa. However, the optimal examination of the bacterial–host cell interaction requires a directional epithelial exposure, without serosal or cut surface stimulation. In order to overcome standard system limitations, a polarised IVOC system was developed using modified Snapwell inserts in which a biopsy sample is sandwiched between two Perspex disks with a central aperture. Incubation of small intestinal biopsies showed good tissue survival for 8 hours with intact surface epithelium. Bacterial leakage around biopsy edges was negligible after apical infection with EPEC, opening up the potential to quantify colonisation of mucosal surfaces as a system read out. EPEC small intestinal colonisation was considerably enhanced in the Snapwell system compared to conventional IVOC, and evidence of bacterial detachment, as seen in *in vivo C. rodentium* mouse infections, was identified. Real time RT-PCR for IL-8 and hBD-2 was achieved demonstrating the ability to look at host gene responses. We have established a polarised system which allows the study of mucosal events under restricted apical infection, bringing the *ex vivo* model closer to the *in vivo* situation.

Diarrhoea as a manifestation of serious systemic infection

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Diarrhoeal diseases remain a substantial cause of global morbidity and mortality. An appreciation of mechanisms underlying the pathogenesis of diarrhea is important for all those involved in research or treatment of these conditions. Advances in cellular and molecular science have greatly enhanced our understanding of how these pathogens lead to infection, diarrhoea and death. Such understanding is crucial to the development of strategies to manage infection in individuals and provide insights into novel treatment strategies and vaccine development. Adherence to mucosal surfaces, penetration and tissue invasion, release of toxins and evasion of host immune defenses are some of the key pathogen determinants leading to symptomatic disease. Host factors including age, nutrition, immune status and co-existent disease are of prime importance in determining the range of pathogens, severity and outcome of infection. Diarrhoea is also a common manifestation of some severe systemic infections where the

GI tract is not the primary focus for example severe bacterial sepsis or staphylococcal toxic shock syndrome. Here diarrhea is the result of the profound effect of sepsis on normal gastrointestinal physiology. I will provide an overview of how these cause diarrhoea focusing in more depth on specific mechanisms used by common pathogens.

Mechanisms of parasite-induced diarrhoeal disease

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Giardia lamblia is a leading cause of parasite-induced diarrhoeal disease worldwide. Trophozoites reside in an "off-shore" location within the lumen and at the epithelial surface of the small intestine, but do not invade the mucosa. Symptomatic infections are characterized by diarrhea, abdominal pain, and general malaise, yet half of all infections are asymptomatic. The host response to infection is devoid of inflammation, indicating that inflammatory mediators are not important in causing diarrhea. The existence of giardial enterotoxins has been postulated, but they remain poorly defined. *Giardia* infection induces small intestinal hypermotility, which develops in a delayed fashion relative to peak parasite burden and is dependent on adaptive immune defenses. Hypermotility promotes giardial clearance and may contribute to diarrhoea through diminished nutrient and electrolyte absorption. Diffuse epithelial microvillous shortening occurs in giardiasis, which leads to disaccharidase insufficiencies and can further limit nutrient and electrolyte uptake. Infection also causes epithelial barrier dysfunction related to the loss of claudin expression and increased apoptotic cell loss. These events are accompanied by impaired sodium-dependent glucose absorption and enhanced electrogenic anion secretion. Together, current evidence suggests that *Giardia*-induced diarrhoea is primarily caused by malabsorptive mechanisms, although epithelial barrier break-down and secretory processes may also contribute.

Lactobacillus plantarum reduces *S. typhimurium* invasion in a novel 3-D porcine jejunum model

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Recent advances in 3-D cell culture have led to 'next generation' tissue culture models to study microbial pathogenesis. However, there is a paucity of porcine cell lines available for research use. With the recent characterisation of the porcine jejunal cell line IPEC-J2, it has been possible to create a novel porcine 3-D tissue culture model utilising the rotary wall vessel system, to study microbial pathogenesis in swine.

In our laboratory the porcine tissues generated using the above model were evaluated using a variety of microscopy techniques to study cellular morphology, brush border, M cell formation and cellular differentiation. Additionally, the 3-D IPEC-J2's were used to elucidate how a porcine probiotic *L. plantarum* could mitigate against *S. typhimurium in vitro* by quantification of adhesion and invasion (A&I) by confocal microscopy and a time course study using scanning electron microscopy.

The studies to date concluded that *L. plantarum* significantly reduces invasion of *S. typhimurium* into 3D IPEC-J2 aggregates in a competition (A&I) assay. Interestingly, probiotic *in vitro* protection assays indicate that probiotics may modulate *S. typhimurium* pathogenesis.

Role of 5-HT and Enterochromaffin cells in rotavirus disease

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Abstract not received

Role of the enteric nervous system for viral and bacterial-mediated diarrhoea

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Fluid loss from the intestinal tract is the cardinal symptom of secretory diarrhoea. A decreased water uptake and an induction of fluid secretion underlie the abnormal fluid loss. The attenuated fluid absorption usually reflects a decreased rate of net electrolyte uptake by the villus epithelium. Secretion from the crypts is in most cases induced by an activation of enteric nervous secretory reflex(es), since the diarrhoeal agents fail to reach the crypt epithelium. The participation of enteric nerves has been demonstrated by means of pharmacological agents, such as tetrodotoxin, lidocaine and hexamethonium, influencing nervous function. These drugs attenuate fluid secretion in most types of acute diarrhoea such as those caused by cholera toxin, *E. coli* enterotoxins, *Rotavirus* and live invasive strains of *Salmonella typhimurium*. Furthermore, luminal cholera toxin has been shown to activate enteric neurons using immunohistochemical methods. Destroying one of the enteric plexuses markedly decreases the ability of cholera toxin to induce intestinal fluid secretion. The picture that emerges from these studies is that agents that represent a potential "threat" to intestinal mucosa and the organism evoke a fluid secretion via one and the same nervous secretory reflex. Hence, it should be possible to treat different types of acute diarrhoea with one drug. The secretary response represents an innate immunity mechanism since the potential "noxious" agent is "diluted". Furthermore, the secretion is often accompanied by a nervously induced increased propulsive motility attempting to remove the secretory agent. The involvement of nerves in diarrhoea implies new potential sites of action for pharmacological treatment.

Salmonella AvrA coordinates suppression of immune and apoptotic defenses via JNK pathway inhibition

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Introduction Certain bacteria secrete effector proteins that enter the gut epithelia and usurp cell signaling pathways. We have investigated the activity of the secreted *Salmonella* AvrA protein using both *Drosophila* and murine models.

Methods We created transgenic *Drosophila* harboring *avrA* stably integrated into the genome. The *avrA* coding sequence is under an UAS promoter which allows for ectopic expression in *Drosophila* using the UAS/GAL4 system.

Results Flies expressing AvrA failed to mount an immune defense against gram negative bacterial challenge as exhibited by higher mortality and abrogated upregulation of anti-bacterial peptides. Further analysis showed that AvrA inhibits the phosphorylation of JNK in response to infection. This was also demonstrated by genetic crosses showing that AvrA rescued an ablated rough eye phenotype caused by constitutive JNK expression. We were able to recapitulate AvrA mediated inhibition of JNK phosphorylation in human HEK293 cells. Furthermore, murine infection with mutant AvrA$^-$ *Salmonella* exhibited increased intestinal apoptosis compared to wild type *Salmonella* SL3201.

Conclusion We suggest a potential strategy of *Salmonella* infection by inhibition of the proapoptotic JNK pathway.

Salmonella enterocolitis: subversion of host defenses by bacterial virulence factors

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Salmonella Typhimurium is a common cause of enterocolitis. Work on tissue culture models has identified a substantial number of *Salmonella* virulence factors. However, it had remained unclear whether/how these virulence factors contribute to a "real" infection. We have developed a streptomycin pretreated mouse model to study *S.* Typhimurium virulence factors and the components of the mucosal immune system which contribute to acute enterocolitis. Here, I will discuss the role of mucosal dendritic cells. Intestinal dendritic cells (DCs) are believed to sample and present commensal bacteria to the gut-associated immune system to maintain immune homeostasis. How antigen sampling pathways handle intestinal pathogens remains elusive. Conditional DC depletion experiments revealed that intestinal virulence of Δ*invG S.* Typhimurium critically required DCs for invasion across the epithelium. 3 days after intragastric inoculation, a MyD88-dependent mucosal inflammatory response was mounted. Using bone marrow chimeric mice, we found that pathogen transport by DCs across the intestinal epithelium is a discrete – and MyD88-independent – step of the mucosal innate immune response to infection in-vivo. This defines the function of mucosal DCs in the genesis of acute *Salmonella* enterocolitis.

Dissecting the pathogenesis of the food-borne bacterium *Campylobacter jejuni* through identification of secreted virulence proteins

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Campylobacter jejuni, a motile Gram-negative bacterium, is a leading cause of bacterial gastroenteritis worldwide. To cause disease in humans, *C. jejuni* invade the cells that line the human intestinal tract. While the bacterial flagellum is essential for motility, recent work indicates that the flagellum also serves as a type-three secretion system (TTSS) for the export of *C. jejuni* virulence proteins. The TTSS proteins have been designated the *Campylobacter* secreted proteins (Csp). A subset of the Csps, termed *Campylobacter* invasion antigens (Cia), are required for maximum host cell invasion. Exploiting the genetics and TTSS of *Yersinia enterocolitica*, we have developed a screen and identified approximately seventy Csps. Culture of *C. jejuni* with the bile salt deoxycholate induces the expression of some of the Csps, and alters the kinetics of *C. jejuni*-host cell invasion. The secreted proteins will be analysed *in silico* to identify putative effector proteins that may alter host cell pathways and confer an invasion and/or intracellular survival advantage to *C. jejuni*. Determining the functional roles of the Csps will increase our understanding of *C. jejuni*-mediated enteritis.

Enteropathogenic *Escherichia coli* (EPEC): induction of non-inflammatory osmotic diarrhoea? Paradigms and new insights on host–pathogen interaction

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Enteropathogenic *E. coli* [EPEC] colonisation of the small intestine is linked to a non-inflammatory diarrhoeal disease that afflicts millions, and kills ~200,000 infants each year. Disease is dependent on a ~35 kb chromosomal Locus of Enterocyte Effacement (LEE) 'Pathogenicity Island' that encodes i) a Type Three Secretion System (TTSS), ii) EPEC secreted proteins (Esp) 'translocators', iii) injected 'effector' proteins and iv) the Intimin outer membrane protein. The TTSS/translocator proteins function to generate a 'molecular syringe' to directly 'inject' LEE and non-LEE-encoded (Nle) effector proteins into target cells. Characteristic histo-pathological changes associated with disease are i) intimate binding to enterocytes, ii) loss (effacement) of absorptive microvilli, iii) formation of actin-rich pedestal-like structures beneath the adherent bacteria, iv) changes in mitochondrial morphological, v) disruption of epithelial barrier function, vi) rapid onset of diarrhoea, and vii) an unexpectedly weak inflammatory response. This talk will concentrate on our work with the Caco-2 small intestinal epithelia model that has define complex co-operative roles of LEE effectors in processes i) to vi) (above), and Nle effector for vii). Our findings provide new insights, paradigms and plausible mechanisms to explain how EPEC induces a rapid watery diarrhoea and minimises inflammation.

Clinical Microbiology Group / Society for Anaerobic Microbiology joint session

Anaerobe 2007: Changing perceptions and patterns of anaerobic infection

Antibiotic resistance in *Bacteroides* spp: mechanisms and clinical impact

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Bacteroides occur normally in the human gut, but some species, notably *Bacteroides fragilis* and *Bacteroides thetaiotaomicron,* are opportunistic pathogens, causing abscess formation and sepsis outside of this environment. Post-operative *B. fragilis* infections following bowel perforation are of particular concern. These can prolong hospitalisation time of patients, involve intensive and costly antibiotic therapy, and may even be fatal. Several groups of antibiotics are active against *B. fragilis* including nitroimidazoles, carbapenems, chloramphenicol, combinations of β-lactam drugs with a β-lactamase inhibitor, and certain quinolones. However, resistant organisms are emerging with accompanying clinical consequences. Metronidazole is a drug of choice used to treat *B. fragilis* infections. The inactive prodrug enters the bacterial cells, is activated by anaerobic reduction of the nitro group, and then damages DNA causing strand breaks. Resistance may occur through active metronidazole efflux, prevention of drug activation, and enhanced DNA repair. Modulations in metabolic electron flux or modified nitroreductase activity can also affect metronidazole activation. The genetic bases of these systems and other antibiotic resistance mechanisms in *Bacteroides* spp will be reviewed in the context of susceptibility testing, emerging resistance surveillance, and therapeutic approaches.

Repair of metronidazole induced DNA damage in *Bacteroides fragilis*

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Repair of DNA double-strand breaks (DSBs) in *Esherichia coli* has been accepted as the paradigm for recombinational repair in bacteria. Processing of DSBs is initiated by the helicase and exonuclease functions of the RecBCD complex. In many other bacteria, such as Gram positives and some members of the α-proteobacteria, DSBs are re-sected by the AddAB (RexAB) complex that has analogous function to RecBCD but no significant homology to the *E. coli* proteins. Pre-synaptic processing of DSBs has not been studied within the broader context of bacterial diversity. The *Bacteroidetes/Chlorobi* are an evolutionary distinct group which diverged prior to the split of the Gram positives and the classical Gram negatives. *Bacteroides fragilis* is an obligate anaerobe which is a resident bacterium of the human gastrointestinal tract but is also an important opportunistic pathogen. The drug of choice for treatment of anaerobic infections is metronidazole, which causes DSBs in the bacterial chromosome following reduction of the prodrug. We have identified distantly related homologues of the r*exA* and *rexB* genes in the *B. fragilis* genome, which implies that the AddAB/RexAB system may be more widespread in eubacteria. The genetical and biochemical roles of the *B. fragilis* RexAB homologues in repair of DNA damage will be described.

Bacteroides endotoxin: an overlooked mediator of inflammation?

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Bacterial pathogen associated molecular patterns (PAMPs), the agents responsible for triggering inflammatory Toll-like receptor (TLR) signalling, are present in the atherosclerotic artery, the arthritic joint and the blood of septic patients. It has been proposed that translocation of commensal derived PAMPs from the gut into the circulation may contribute to these pathologies. We show that ligands of TLR2 are more abundant in the human gut than ligands of TLR4 or TLR5. Accordingly, while enterobacterial species were found to be able to induce signalling via TLR4, whole heat killed cells, endotoxin and lipid-A of the prominent Gram-negative gut-resident *Bacteroides fragilis* stimulated TLR2 but not TLR4-dependent signalling. We found that while venous endothelial cells are unresponsive to *B. fragilis* endotoxin, coronary artery endothelial cells are responsive to *B. fragilis* endotoxin via differential expression of TLR2. All previous measurements of plasma endotoxin concentrations have used the limulus assay callibrated with *Escherichia coli* endotoxin, though we found that this assay underestimates *B. fragilis* endotoxin concentrations by a factor of around 2,200. Re-analysis of human plasma using *B. fragilis* endotoxin callibrated limulus assay suggests that *B. fragilis* endotoxin may approach clinically relevant concentrations under certain circumstances.

The adaptive response of *Bacteroides fragilis* to oxidative stress plays a role in virulence

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The intestinal anaerobe, *Bacteroides fragilis*, is resistant to the effects of oxidative stress. Survival requires a global transcriptional response and de novo protein synthesis which protect cells against toxic oxygen radicals and reprogram metabolism to meet the new demands of an oxidizing environment. There is mounting evidence that this Oxidative Stress Response (OSR) is important for opportunistic infections that occur when the organism translocates to extra-intestinal sites. *In vitro* transcriptional and protein profiling indicate that the OSR has multiple layers of regulation which 1) establish a rapid, acute response to minimize the immediate effects of reactive oxygen species and 2) during prolonged exposure induce an extended response that directs metabolic activity to supply reducing power for detoxification and restore lost energy generating capacity. Integral to the metabolic response is the down-regulation of gene expression related to translation and biosynthesis that occurs as oxidative stress increases in severity. The acute response is mediated by the redox regulator, OxyR, which directly controls gene-products that remove reactive oxygen species. Loss of the acute response leads to a more oxidized cytoplasm even during brief treatment with low concentrations of oxidants and these strains are more sensitive to oxidants *in vitro*. Significance of the acute response in vivo was demonstrated by comparison of wild-type strains to *oxyR* mutants in an intra-abdominal abscess model of infection in mice.

Antibiotic resistance and the role of glycans in diabetic foot infections

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Background and objective Diabetic foot (DF) infections, affecting 4 million people worldwide, are major long-term complications of Type 2 DM. In DF anaerobes and aerobes exist in synergy forming biofilms which affect progression of ulcers. Glycan-lectin interactions allow organisms to adhere to host cells or each other to form biofilms in which antibiotic resistance and sensitivity changes.

Methods 50 pus samples from the DF patients (UCLH) were analysed for glycan profile and antibiotic resistance. 22 lectins were utilised to identify the glycans of cell exudates and lipopolysaccharide.

Results Glycan profiles of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Finegoldia magna, P. bivia, P. prevottii, P. assachrolyticus, P. anaerobicus, and P. micros* were obtained. Significant differences were seen with some organisms and not others.

Of 64 isolates of *F. magna*, some were penicillin (2), tetracycline (26), erythromycin (17) and clindamycin (7) resistant. Sensitivity was noted to cefotoxacin (12), imipenem (12), augmentin (12), pipertazobactam (12) and metronidazole (12).

Conclusion The resultant data obtained has increased our knowledge of biofilm formation and organism interactions.

Propionibacterium acnes: an important pathogen?

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Propionibacterium acnes is found predominately in the sebaceous gland-rich areas of adult skin. Although linked to inflammatory acne and other types of infections (e.g. medical devices), *P. acnes* is still considered a relatively non-pathogenic member of the resident human microbiota. Its presence in clinical samples is still likely to be dismissed on the grounds of contamination rather than true infection, which may lead to an underestimation of its association with various conditions. The publication in 2004 of the completed *P. acnes* genome sequence highlighted the potential of this organism to cause disease. In particular, a wide range of putative virulence factors, including exocellular enzymes and cell surface proteins with predicted host-interactive properties, have been found. This paper will consider whether *P. acnes* should be taken more seriously as an important opportunistic pathogen. On the basis of studies conducted in our laboratory we will discuss the association of *P. acnes* with prosthetic hip infections and consider how recent developments in *P. acnes* phylogeny may impact on our understanding and study of the organism's capacity to cause disease. Finally, we will look at the variable expression of immunoreactive surface proteins on *P. acnes* and its implications for inflammatory acne and other conditions.

Is acne an infection?

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The role of commensal skin propionibacteria in inflammatory acne is considered in relation to background knowledge of this multifactorial disease. The dissemination of antibiotic resistance in propionibacteria is now of global concern, indicated by rising prevalence and increasingly by compromised clinical outcomes. The molecular mechanisms of resistance and the epidemiology of propionibacterial resistance to macrolide, streptogramin and lincosamide (MLS) antibiotics as well as the tetracyclines is reviewed. These data suggest considerable heterogeneity among propionibacterial populations. A model is proposed in which acne can be considered as an infection of individual pilosebaceous follicles where response to antibiotic treatment is dependent on the follicular level of antibiotic achieved during treatment and on the presence or absence of resistant organisms in each follicle. Therapeutic strategies are suggested that may reduce the problem of resistance and enhance therapeutic outcomes. The key point is made that while acne is not an infectious disease in the sense that it can be passed on from one person to another, evidence is presented to suggest that resistant propionibacteria certainly can be disseminated in this way.

Actinomyces – gathering new threads of evidence

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Application of molecular biological techniques has revolutionised the classification and identification of the genus *Actinomyces*. Consequently, a clearer picture is emerging of the natural habitats and clinical entities associated with specific *Actinomyces* spp.

Arguably, of greatest importance is the recognition of actinomycosis as a major factor and indicator of poor prognosis in both infected osteoradionecrosis and bisphosphonate-associated osteonecrosis of the jaws.

Among recently described species, *A. graevenitzii* has been reported almost exclusively from oral and respiratory sites and may be a causative agent of actinomycosis. Conversely, *A. turicensis, A. funkei, A. radingae* and *A. europaeus* are isolated commonly from superficial soft tissue infections and may form part of the normal flora of lipid-rich areas of the skin. *A. neuii* is associated with post-operative endophthalmitis and abscesses of the breast and axilla.

Isolation and identification of *Actinomyces* spp. by conventional methods remains problematic, hence clinical cases may be under-reported or mis-identified. Diagnosis is commonly belated and based solely upon histological findings. Development of direct detection methods may greatly aid patient management and further elucidate clinical associations.

Multilocus sequence typing scheme for *Propionibacterium acnes*

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A multilocus sequence typing (MLST) scheme has been developed for the characterisation of *Propionibacterium acnes* in order to study its epidemiology, population and evolutionary biology, and validated using a diverse collection of 123 *P. acnes* isolates, from different clinical sources across 10 countries. 38 sequence types (STs) were identified, with 49% of the total isolate collection belonging to ST-6. MLST grouped strains into distinct clonal complexes that correspond to previously identified *P. acnes* types I and II and previously unidentified novel groups. It is likely that recombination has contributed to the recent evolution of *P. acnes* strains. From the population of organisms examined there was no obvious correlation between different STs and site of isolation, disease association or geographical origin; indicating that *P.* acnes is a widespread opportunist.

Identification of biomarkers in *Clostridium difficile* using mass spectral profiles

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C. difficile, long associated with pseudomembranous colitis resulting from antibiotic usage, is increasing significantly as a nosocomial pathogen. Its pathogenic mechanisms are not fully understood but emphasis has been placed on the role of its toxins. In particular, its enterotoxin and cytotoxin (*toxin A and B*) are thought to be responsible for the diarrhoea and inflammation, although their relative contributions are unknown. In order to gain a more holistic profile of other proteins of this diverse species, we are utilising comparative proteomics of strains that span a 30 year period. Intact cells are being examined directly by MALDI-TOF MS while cytosolic/membrane-bound proteins are being analysed using a range of ProteinChips in SELDI-TOF-MS. Results to date indicate a highly coherent species but there are marked differences in protein expression among isolates. Spectral profiles indicate a range of surface-associated mass ions that reflect its intraspecific diversity. Data mining techniques are being used to dissect out unique biomarkers which are being characterised further by LC/MS/MS.

Taxonomy, evolution and identification of *Bacteroidetes*/*Chlorobi* and *Firmicutes* (clostridia) species based upon novel molecular signatures

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Most of the higher taxonomic groups within Bacteria (viz. phyla, orders, Classes, families etc.) are presently defined on the basis of their branching patterns in the 16S rRNA trees. In most cases, no biochemical or molecular characteristic is known that is unique to these groups. The availability of genome sequences for all major groups within Bacteria provides a huge and unprecedented resource for discovering novel molecular characteristics that are unique to different taxonomic groups. Our phylogenomic work is aimed at identifying two different types of rare genomic changes (RGCs) that are distinctive characteristics of various higher taxa within bacteria. The first kind of RGCs consists of conserved inserts and deletions (i.e. indels) in widely distributed proteins that are specific for various taxonomic groups. The second types of RGCs are whole proteins (or ORFs) that are uniquely found in particular groups or subgroups of bacteria, but nowhere else. Our recent work has identified large numbers of conserved indels and whole proteins that are specific for the *Bacteroidetes* or *Chlorobi* groups of species or which are uniquely shared by them. Several other RGCs that are uniquely shared by the *Bacteroidetes* and *Chlorobi* species, as well as *Fibrobacteres*, provide evidence that species from these three groups shared a common ancestor exclusive of all other bacterial phyla. We have also identified many conserved indels and whole proteins that are specific for either all *Firmicutes* or various sequenced *Clostridia* species. These RGCs provide novel and more definitive means for circumscribing and identifying species from these groups in clear molecular terms and for understanding their evolution. Because of the group specificities of these RGCs, functional studies on them should also lead to discovery of novel biochemical and physiological characteristics that are unique to these groups of bacteria.

Anaerobic infections in the 20th and 21st centuries

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Enormous strides were made in our understanding of anaerobic bacteria and their role in disease in the 20th century. Included were advances in anaerobic culture techniques, major advances in taxonomy, studies on virulence factors in anaerobes, the recognition of defects in host defenses which predispose to infection with anaerobes, and clinical descriptions of anaerobic infections and their importance. Antimicrobial agents became available and antimicrobial susceptibility testing evolved; resistance of anaerobes to certain antimicrobial agents became recognized as a problem, as did suppression of certain elements of the indigenous flora leading to colonization with potential pathogens. The introduction of molecular techniques has led to vast new amounts of information regarding taxonomy and inter-relationships between various organisms. Studies of ecology of anaerobes and other bacteria at various sites in humans and animals has been an important advance. The 21st century is barely under way, but already we have seen serious medical problems by a new variety of *Clostridium difficile* and by *Clostridium sordellii* and *Clostridium novyii*. The sophistication of molecular procedures and availability of new equipment to facilitate studies of this type have led to improvement in speed and accuracy in identification of anaerobes and to improved studies of epidemiology and virulence.

Exploiting clostridial genome information

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Despite their industrial and medical importance, our general understanding of the biology of the genus *Clostridium* is minimal. This ignorance hinders our ability to counter the diseases they cause, and to exploit their beneficial properties, e.g., in the generation of biofuels and as delivery vehicles in cancer therapy. Both the improvement of commercial important strains, and the development of effective countermeasures against pathogens, can only be approached in a rational manner if the fundamental physiological processes that operate in the target organism are fully understood. Genomic research provides an opportunity for rectifying current deficiencies. However, the effective exploitation of the amassed data has been precluded by a woeful inadequacy in procedures for insertional gene inactivation. We have developed a mutagenesis system specifically for clostridial hosts, the ClosTron, based on the mobile group II intron from the *ltrB* gene of *Lactococcus lactis*. Integrants are readily selected on the basis of acquisition of resistance to erythromycin, and are generated from start to finish in as little as 10 to 14 days. Unlike single crossover plasmid integrants, the mutants are extremely stable. The procedure is highly efficient and reproducible, and is revolutionizing functional genomic studies in clostridia. Its application to various clostridial species will be described.

Characterisation of a putative quorum sensing two – component system of *Propionibacterium acnes*

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P. acnes, a predominant resident micro-organism of human skin, is thought to be involved in the development of acne. Quorum sensing (QS) is the regulation of gene expression in response to cell density. Gram-positive bacteria often employ peptides and two-component systems (TCSs) to facilitate QS regulation. Characterisation of the potential QS signal transduction pathway of *P. acnes* may help to identify new drug targets for acne. Here we identified a novel putative QS-TCS in the *P. acnes* genome with divergently transcribed genes for the sensor and regulator. These were cloned into pGEX-6P1 and pET30b, respectively, and overproduced and purified from *E. coli* BL21(DE3). Using an *in vitro* phosphorylation assay we demonstrated the sensor could transfer the phosphoryl group to the regulator.

Moreover, transcriptional analysis of the sensor and peptide genes showed they are likely to be transcribed together. Although transcribed divergently in the genome, these studies suggested that their functions are linked.

Preliminary studies on the genomics and proteomics of *Clostridium difficile*

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In order to investigate the microevolution of *C. difficile* over the last 40 years, we are undertaking the sequencing and annotation of the complete genomes of representative strains from 1970s to 2007 while simultaneously data mining the proteome of large numbers of strains for biomarkers using various forms of mass spectrometry. Comparative sequence analysis of a representative strain from 1979 (B, 4.4 MB) and 2006 (A, 4.1 MB) to that of the fully sequenced strain 630 genome revealed that 86.8% and 75% respectively were mapped. Differences in the number of putative ORFs encoding adhesins, proteases and toxin related proteins were identified. While a larger number of motility, surface and phage related proteins were characterised in strain B (2,247) a larger number of hypothetical proteins (3,015) were recorded in strain A. Proteomic analysis to characterise the expressed complement of characteristic chromosomal components of each genome, as well as elucidating the structural configuration on each genome, are currently in progress.

Changing molecular epidemiology of *Clostridium difficile* at a UK hospital 2001–2007

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C. difficile infection is the major cause of hospital-acquired diarrhoea in the UK and can result in life-threatening complications. Disease is associated with antibiotic use and results from production of exotoxins. Molecular typing of isolates by PCR ribotyping (16S–23S rRNA spacer region) has identified >100 ribotypes in the UK. PCR ribotypes were determined for *C. difficile* isolates from Southampton University Hospitals Trust (SUHT) in 2001, 2005 and 2007 (n=335). Antimicrobial susceptibilities to 12 antibiotics were measured by E-test.

Prevalences of the commonest ribotypes 001, 027 and 106 were 73%, 0% and 0% in 2001, 0%, 8% and 58% in 2005 and 7%, 51% and 22% in 2007, respectively. They had similar antimicrobial susceptibilities and were resistant to erythromycin and moxifloxacin, unlike less common ribotypes.

Substantial changes in the molecular epidemiology of *C. difficile* at SUHT have occurred since 2001. The predominant ribotypes in 2001, 2005 and 2007 were 001, 106 and 027, respectively. The increased prevalence of ribotype 027 in 2007 is consistent with the recent UK-wide spread of this hypertoxin-producing strain.

Clostridium difficile and nanotechnology

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Clostridium difficile, like many Bacteria and Archaea, produces an S-layer on the external face of the vegetative cell. S-layers are proteinaceous, two-dimensional para-crystalline arrays that surround the cell, and are attached to the underlying cell wall via non-covalent forces.

A remarkable feature of S-layer proteins is their ability to self-assemble *in vitro* to form regularly spaced arrays of defined morphology. There is now considerable interest in the exploitation of S-layers for nano-technological applications, from construction of novel nano-surfaces incorporating metallic components or semi-conductors, to novel 'S-liposomes' displaying defined arrays of active biological molecules. Our laboratory is a member of an EU 'NASSAP' consortium – Nano Arrayed Systems based on Self Assembling Proteins – that aims to exploit bacterial S-layers and other self assembling bacterial systems to develop new materials for medical and industrial applications.

The *C. difficile* S-layer is composed of two proteins; the low molecular weight S-layer protein (LMW-SLP) and the high molecular weight S-layer protein (LMW-SLP). I will describe a series of experiments that probe how these two proteins interact to form a structure capable of self-assembly.

Clostridium difficile: a clinical and epidemiological update

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A highly virulent variant of *Clostridium difficile* is emerging throughout Europe. This strain is characterized as toxinotype III, North American pulsed-field type 1 (NAP1), restriction-endonuclease analysis group type BI and PCR-ribotype 027. Two months before the first outbreak of *Clostridium difficile* PCR ribotype 027 in The Netherlands was discovered in June 2005, 17 hospitals participated to a surveillance study of the incidence of *C. difficile*-associated disease (CDAD). The median incidence rate of CDAD was 16 per 10 000 patient admissions (2.2 per 10 000 patient-days) and varied from 1 to 46 per hospital. Type 027 was identified in 10 patients from 1 hospital during an unrecognized outbreak. We concluded that the incidence rate of CDAD in The Netherlands is considerably lower than in Canada and USA but that the emerging 027 type can spread unnoticed. After the first two outbreaks due to *C. difficile* PCR ribotype 027 in The Netherlands, a national surveillance was initiated to investigate the spread and the epidemiology of CDAD. In the following 18 months period, 1175 faeces samples from 863 patients were received from 50 healthcare facilities. Of these patients, 218 (25.3%) had CDAD due to type 027, and 645 (74.7%) had CDAD due to other types. Type 027 was more frequently present in general hospitals than in academic hospitals (OR 4.38, 1.60–12.0). Outbreaks were observed in 10 hospitals and in 1 nursing home. The clinical syndrome and affected patients differ with CDAD due other types and this can be of benefit for an early recognition. Patients with type 027 were significantly older (OR 2.18, 95% CI 1.43–3.33) and used significantly more fluoroquinolones (OR 2.88, 1.01–8.20). Clear trends were observed for more severe diarrhoea (OR 1.99, 0.83–4.73), higher attributable mortality (6.3% vs. 1.2%, OR 3.30, 0.41–26.4) and more recurrences (OR 1.44, 0.94–2.20). Type 027 was found in 20 (18.3%) of 109 hospitals in The Netherlands with a geographic concentration in the Western and central part. Using a new developed MLVA subtyping technique for *C. difficile* type 027, we could demonstrate transfer of specific types between hospitals. All 027 strains isolated sofar in The Netherlands were resistant to new fluoroquinolones and to erythromycin, but susceptible to clindamycin. Since 2005, individual countries have developed surveillance studies to the spread of type 027 in their country. *C. difficile* type 027 caused outbreaks in the UK (since 2003), the Netherlands (since 2003), Belgium (since 2003), France (since 2006), and has also been detected in Austria (2006), Japan (2005), Ireland (2005), Switzerland (2006), Luxembourg (2006), Poland (since 2003) and Denmark (2006). Preliminary data indicated that type 027 was present in 3 of more

than 1500 historical isolates collected in Sweden between 1997–2001. We conclude that *C. difficile* type 027 has been detected in an increasing number of European countries, which is either because more countries have started surveillance surveys or because type 027 is spreading rapidly.

Clostridium difficile in the clinical setting: management and antibiotics

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Abstract not received

Cwpv, a variable cell wall protein of *Clostridium difficile*

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The surface of *C. difficile* is covered in a paracrystalline array of two surface layer proteins (SLPs) derived from a single gene, slpA, but there are also other proteins present on the cell surface layer. The high-molecular weight SLP incorporates three copies of the Pfam04122 cell-wall binding domain, as do twenty-eight other proteins encoded within the *C. difficile* 630 genome. One of these, designated CwpV as it is variable between strains, contains three Pfam04122 domains in its N-terminal domain, and has nine almost identical 120 amino acid repeats at its C-terminus. These repeats are present in six of twelve strains examined, and have highly conserved sequences, although the numbers of repeats varies from four to six. We have analysed *cwpV* in two hyper-virulent strains from the Quebec and Stoke Mandeville outbreaks, R20352 and R20921 respectively. In these strains, the N-terminal domain of CwpV is highly related to the other strains. However, the C-terminal domain has eight almost perfect 79 amino acid repeats which appear to be completely unrelated to those found in *C. difficile* 630 and in the other strains analysed, in both amino acid sequence and predicted secondary structure.

cwpV is transcribed and expressed throughout growth in *C. difficile* 630, as demonstrated by reverse-transcription PCR and Western immunoblotting of total cell lysates. The presence of CwpV at the cell surface of *C. difficile* 630 has been shown by immunostaining; FACScan analysis; immunogold transmission electron microscopy; and proteomic analysis of the cell wall. Although implicated by the observed repetitive structure, no adhesion or haemagglutination properties have yet been found and the function of this protein is still to be elucidated.

Detection of anaerobic bacteria in cystic fibrosis pulmonary exacerbations

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The lungs of clinically stable Cystic Fibrosis (CF) patients are colonised by a range of potentially pathogenic anaerobic species. The aim of this study was to determine whether anaerobes are also present in the sputum of CF patients with an acute pulmonary exacerbation. Sputum samples were collected from 32 adult CF patients admitted for treatment of an acute pulmonary exacerbation. Induced sputum samples were also collected from 20 healthy volunteers who do not have CF. Anaerobes from a range of species including *Prevotella, Veillonella, Propionibacterium* and *Actinomyces* were detected in high numbers from all patients prior to commencing antibiotic therapy. Anaerobic bacteria were also detected at the end of antibiotic treatment and for 22/32 (69%) patients they were present in lower numbers. Anaerobes were present in induced sputum samples from control patients in much smaller numbers. These results indicate that anaerobes are present within the CF lung during an acute exacerbation of pulmonary infection.

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Microbial diversity in therapy resistant periapical lesions

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In most instances endodontic infections respond well to conventional endodontic therapy. However, bacteria can survive within the root canal system and/or within the periapical tissue. In such cases, healing of the periapical lesion is compromised.

20 surgically removed apical lesions from therapy-resistant apical periodontitis were examined for the presence of bacterial DNA using PCR targeting the 16S ribosomal RNA (rRNA) gene, followed by cloning and sequencing. Bacterial DNA was detected in 17 of 20 samples (85%). Sequences of *Fusobacterium* spp. *Prevotella* spp., *Bacteroides* spp., *Porphyromonas* spp., *Campylobacter* sp., *Peptostreptococcus* spp., *Selenomonas* sp., *Corynebacterium* sp., *Propionibacterium propionicus*, *Treponema denticola*, *Tannerella forsythia*, *Porphyromonas endodontalis*, *Streptococcus mitis*, *S. intermedius* and *P. nigrescens* were detected. More than 90% of the samples contained uncultivable bacteria. In conclusion; a wide range of bacteria was found to colonise periapical lesions on therapy-resistant teeth. These bacteria may contribute in impeding healing of these lesions.

Anaerobic bacteria in cancer treatment

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To increase cancer treatment potential, substantial resources are currently being devoted to development of new tumour-specific anti-cancer therapies. One way to selectively target tumours involves the use of non-pathogenic anaerobic bacteria, predominantly *Clostridium* spp., as gene delivery vehicles. The concept of this approach has been around for more than 50 years, and is based on the unique physiology of solid tumours which is often characterized by regions of hypoxia and necrosis. Systemically injected spores from a variety of clostridial strains in tumour-bearing animals have been shown to localise and germinate only in hypoxic and necrotic regions of solid tumours. The application of clostridia as delivery vehicle has proven to be highly feasible and safe. Importantly, treatment of experimental tumours with genetically engineered clostridia has yielded significant therapeutic effects. This therapeutic efficacy is due not only to the fact that clostridia show a high selectivity for hypoxic tumour areas, but also because these hypoxic areas are considered one of the most important barriers to current cancer treatment options. The approach may thus act in a complementary way to current radiotherapy and chemotherapy treatments.

An overview of the current status of anaerobic bacteria use in cancer treatment will be provided.

Education & Training Group session

Risk assessment and recording in microbiology: ask the experts

Risk assessment in microbiological teaching and practice

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The Health and Safety Executive recognises the range of challenges faced by educational establishments, and their academic and technical staff, relating to the health and safety of their students. In Science, and in particular within the field of microbiology, these challenges can be manifold. This short presentation aims to clarify some of the issues surrounding the legal duties of educational establishments towards students and to provide some practical advice on how these duties might be discharged. The importance of risk assessment for microbiological work and its extension to individual students will be covered, as will the meaning of the term 'relevant training' as it applies to practical work and work placement activities.

Risk assessment in industrial microbiology – more than just HACCP

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Abstract not received

Practical work: accurate recording and reporting

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Recent changes in teaching at UK Universities include: increased numbers of students without a commensurate increase in resources; enrolments in courses where microbiology is a subsidiary subject, such as pharmaceutical science or forensic science; the wider access agenda with to increasing numbers of students arriving from non-standard backgrounds such as Access courses. Even in those students arriving with A-levels the change from the 2 year A-level to the AS/A2 split has had consequences. All the same, one is expected to help produce graduates who are competent for employment or graduate study.

Here we report the introduction of industrial-style record books into first- and second- year microbiology Modules on the Pharmaceutical and Cosmetic Science degree at DMU. Clear learning outcomes and transparent assessment criteria are linked directly to Module Handbook and textbook in order to drive the development of independent work in students, especially the skills of laboratory reporting and recording. This has helped to prepare students for industrial placement, for their final year laboratory project, and, eventually, for employment.

Environmental Microbiology Group / Virus Ecology Group joint session

Ecology of viruses

Evolution and taxonomy of microbial viruses

Phage ecology and evolution

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I will present a broad overview of issues of phage ecology and evolution. Phage ecology, minimally, may be differentiated into organismal, population, community, and ecosystem ecologies. In addition, phage ecology considers what I prefer to describe as phage environmental microbiology: measures of the occurrence and diversity of phages especially within otherwise undisturbed ecosystems. Phages also may be employed to experimentally test more-general ecological theory that is applicable (in principle) to a greater diversity of organisms than just phages, viruses, or parasites Approaches toward phage ecology can be descriptive, experimental (in vitro, in situ, or in silico), or theoretical. Consideration of phage evolution are in many ways less cohesive than those of phage ecology, and this is true in part because the traditional concerns of evolutionary biologists (e.g., adaptive landscapes or Muller's ratchet) are less directly applicable toward increasing our understanding of phage biology than are the traditional concerns of ecology (e.g., predator-prey interactions). Nevertheless, I will consider five somewhat cohesive issues of phage evolution: Phage relatedness, phage origins and evolutionary diversification, actual mechanisms of phage evolution (as summarized as deviations from Hardy-Weinberg assumptions), phage evolutionary ecology (that is, phage evolution from the perspective of phenotype), and phage experimental evolution.

Archeal viruses and their ancestry to eukaryotic and bacterial viruses

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Archaea and their viruses are poorly understood when compared to the Eucarya and Bacteria domains of life. We combined crystallography and electron cryomicroscopy to determine the structure *Sulfolobus turreted icosahedral virus* (STIV), an archaeal virus isolated from an acidic hot spring (pH 2–4, 72–92°C) in Yellowstone National Park. The STIV major capsid protein (MCP) structure, determined by crystallography, is clearly related to the MCP of adenovirus and nearly identical to the MCP structures of the eukaryotic PBCV-1, and the bacteriophage PRD1. Combining cryoEM analysis of the 1000Å particle and the MCP x-ray coordinates revealed key capsid interactions that are akin to those observed in adenovirus and PRD1 as well as the means of anchoring the MCP into an internal viral membrane. The structural evidence presented argues strongly for a common ancestor for these virus groups.

The origin of viruses and the transition from the RNA to the DNA world

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Recent progress in comparative genomic and structural biology, together with the discovery of unusual viruses in Archaea and giant viruses in Eukarya, have rejuvenated the discussion on the nature and origin of viruses. Viruses are definitely living organisms that have played a major role in the evolution of modern life. Many scientists now believe that they even predated the last common ancestor of all extant cellular orgnisms (*the Last Universal Common Ancestor, LUCA*). Viruses are today the major component of the biosphere and cellular genomes are continuously visited by viruses/plasmids coming from a hidden viral reservoir of huge magnitude. Interestingly, know viruses (the tip of the iceberg) already exhibit a much greater diversity than cells in the nature of their genomic material and in their mechanisms of genome replication. Combining this observation with odd data from comparative genomics, this suggests in particular that present cellular genomes and mechanisms of DNA replication could be a subset of those invented in a primordial virosphere of DNA viruses infecting RNA cells. If true, viruses could have played a major role in the transition from ancient cellular RNA genomes to the DNA genomes of modern cells (they might have 'invented' DNA), and possibly in the establishment of the three cellular domains of life (the three viruses, three RNA cells hypothesis).

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Evolution of host physiology in response to phage attack

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Bacteria are under constant threat of infection by bacteriophages giving rise to strong selective pressures to acquire resistance to phage. Phage resistant host strains arise easily on selection for phage resistance and these are invariably receptor mutants. Our studies on the phage receptor in *Streptomyces coelicolor* have revealed the presence in the actinomycetes of a general protein O-glycosylation system. However in nature, bacteria are usually resistant to phage infection because of the presence of specialised phage defence systems such as restriction/modification. We are working on a novel type of bacteriophage defence system, the phage growth limitation (Pgl) system, originally discovered in *S. coelicolor*. The Pgl phenotype is characterised by the ability of Pgl^+ hosts to support a phage burst on initial infection but subsequent cycles are severely attenuated. Our current model indicates that three proteins, PglY (an ATPase), PglX (a methyltransferase and a putative endonuclease) and PglZ (an inhibitor of the endonuclease) form a complex that is controlled by a fourth protein, PglW (a protein kinase). It is proposed that the complex can switch between a methylating complex and an endonucleolytic complex depending on the status of the infecting phage DNA. An additional complexity of the Pgl system is the observation that it is subject to phase variation.

Morphological diversity and abundance of viruses in soils

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Viruses of soils could be of great importance because they may play an important role in the ecology and evolution of soil biological communities by being able to transfer genes from host to host. As a significant cause of microbial mortality they can also affect the population dynamics of environmental microbiological communities. We have isolated virus-like particles from different soils in the Dundee area. A wide range of virus morphotypes including tailed, polyhedral (spherical), rod-shaped, filamentous and bacilliform virions were observed by electron microscopy. We have made the first observations and characterisation of viruses from different functional soil domains surrounding plant roots: rhizosphere, rhizosheath and soil bulk. In spite of differences in the diversity and abundance of bacterial and fungal communities in these domains, no obvious morphological differences in virus populations were found. Two soils collected from the dry valleys in Antarctica were also sampled and again a wide range of virus-like particles were observed.

Virus–environment–host interactions

The dissemination of Shiga toxin genes by lambdoid bacteriophages

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Phage conversion of the host phenotype is usually associated with integration of a temperate phage followed by stable replication of the prophage and expression of imported genes. Conversions to pathogenicity or enhanced virulence achieve notoriety, and the emergence of novel pathogens in the form of shigatoxigenic *E. coli* (STEC) strains such as 0157:H7 is one of the best documented examples. Since the first description of this potentially fatal zoonotic disease in 1982, STEC strains have been isolated, counted and typed to provide information on survival, gut colonisation, shedding and transmission. The lambdoid bacteriophages that carry the Shiga toxin genes (*stx*) expressed by the *E. coli* host are by comparison neglected. Although *stx* genes can be carried by very different lambdoid bacteriophages, there is evidence that short-tailed Stx-phages are the most prevalent. Experiments with a model phage have demonstrated that, unlike lambda, this Stx-phage can overcome the endogenous immunity system to insert multiple copies of its genome into *E. coli*, and through recognition of a highly conserved, universal and essential gene product on the outer membrane, ensure its distribution across populations of Gram negative bacteria. Comparative phage genome sequencing and typing is beginning to inform the ecology of these viruses with the aim of establishing their epidemiological significance and identifying the carriage of genes that impinge upon lysogen fitness.

Viral control of bacterioplankton diversity and production: lytic vs temperate lifestyle – impact of viruses on bacterial community structure

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Viruses can influence the diversity and production of bacterioplankton and this influence also depends on the viral lifestyle. Lysogeny dominates where host abundance is low or decay rates of free viruses are high and lytic infection dominate in the opposite environments. In support of that, experimental and sequence data suggest that viruses are typically lytic in temperate surface waters. Several studies have shown that viruses can influence the community composition of *Bacteria* and *Archaea*, however, in some experiments, rather the identity than the number of detectable phyotypes was affected. Both viruses and flagellates seem to increase richness (number of detectable phylotypes) and decrease production. When both types of predators were present, bacterial richness was stimulated in all experiments and production was always repressed. Predator control of the dominants in the outcome of competition for nutrients could explain that, since it should increase richness (by allowing less competitive species to increase in abundance) and simultaneously decrease production (by reducing the fast growing dominants). Overall, there is an unbalance in the studies of lytic and temperate lifestyles in aquatic communities, which reduces our progress in understanding the ecological role of viruses.

Viruses in Antarctic lake systems

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Antarctic lakes are extreme ecosystems with simple truncated food webs dominated by micro-organisms. The lakes range from hypersaline to freshwater and display a wide range of physical/chemical conditions. The saline and brackish lakes of the Vestfold Hills have high concentrations of virus particles (mean 54.3 to 94.3×10^9 l^{-1}) that overlap and exceed the range reported for marine systems and overlap the range reported for lower latitude freshwaters, while in the ultra-oligotrophic freshwaters viral particle numbers (mean 0.53×10^9–0.74×10^9 l^{-1}) are below the minimum values reported for freshwater lakes elsewhere. Viruses showed no clear seasonal pattern in the saline lakes over an annual cycle, while in the freshwater lakes peaks were seen in summer and late autumn. In all of the lakes studied in the Vestfold Hills lysogeny occurred in winter and spring (between 32% to 73% lysogenic bacteria) with the lytic cycle predominating in summer. Burst sizes were low (mean 4, range 2–15), however the percentage of visibly infected cells was high (average 22–34%) compared to lower latitude lakes (range 0.6–4.2%). The evidence suggests that viruses may play a significant role in carbon cycling in these microbially dominated ecosystems.

Phages and cholera

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Factors which enhance the water-borne spread of bacterial epidemics and sustain the epidemic strain in nature are unclear. A distinctive epidemiological feature of the epidemic diarrhoeal disease cholera is its appearance with seasonal regularity in endemic areas, such as the Ganges Delta region of Bangladesh and India. Recently, prevalence of lytic bacteriophages acting on epidemic strains of *V. cholerae* (cholera phages) in the environment, and their amplification in cholera patients have been shown to remarkably influence the dynamics of cholera. Between January 2001 and November 2003 in Dhaka, Bangladesh, the number of cholera patients increased when the concentration of cholera phages in water decreased. Similarly, cholera epidemics tended to end concurrent with large increases in the concentration of these phages in the water. To further understand this phenomenon, the dynamics of *V. cholerae*-phage interaction was studied during the entire course of a cholera epidemic in Dhaka City. The changing prevalence in the environment of the epidemic *V. cholerae* O1 strain and a particular cholera phage (JSF4) to which the bacterium was sensitive, was measured every 48–72 hours for a period of 17 weeks. The incidence of phage excretion in stools of 387 cholera patients during the course of the epidemic was also monitored. This study showed that the peak of the epidemic was preceded by high *V. cholerae* O1 prevalence in the environment and was followed by high JSF4

phage levels as the epidemic ended. The build up to the phage peak in the environment coincided with increasing excretion of the same phage in the stools of cholera patients. Thus, in-vivo phage amplification in cholera victims likely contributed to increased environmental phage abundance, which led to decreased prevalence of the epidemic *V. cholerae*, and hence the collapse of the epidemic. Understanding the complex interactions of multiple environmental and biological factors including the role of phages may lead to the development of a unified model to explain cholera dynamics.

The role of prophages in the evolution of *Citrobacter rodentium*

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Citrobacter rodentium is a natural mouse pathogen and an important model organism for infections caused by the human pathogens, enteropathogenic and enterohaemorrhagic *Escherichia coli*. During annotation of the newly sequenced *C. rodentium* genome, we identified six prophage-like elements, some of which we have shown to be active phages. Comparative genomic analyses showed that these prophages displayed similarities with Mu and P2 family phages, and some appear conserved among other enteric pathogens. Other prophage regions appear specific to *C. rodentium*, and many of the prophages carry 'cargo' genes that may be involved in pathogenicity. Several of the prophage-like elements are predicted to have disrupted core functions due to their insertion in the host genome, for example insertion into flagella operons may be associated with *C. rodentium*'s lack of motility. Prophages have played a crucial role in the evolution, virulence and overall diversity of many bacterial pathogens and *C. rodentium* is no exception.

Viruses and biogeochemical cycling

Viruses and the sulphur cycle

Gill Malin

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The volatile compound dimethyl sulphide [$(CH_3)_2S$; DMS] plays a vital role in the global sulphur cycle as the major vehicle for the transfer of sulphur between the sulphur-rich oceans and terrestrial ecosystems. Furthermore, DMS oxidises rapidly in air to form sulphate aerosol particles that influence the chemistry of the atmosphere and cool the Earth's climate directly or by acting as cloud-condensation nuclei. The primary source of DMS is dimethylsulphoniopropionate (DMSP) a compatible solute found in some marine phytoplankton. In living phytoplankton cells this compound may function as a grazing deterrent, in overflow metabolism under conditions of unbalanced growth and as an antioxidant. When cells die due to autolysis, grazing or viral lysis some of the DMSP released is released as DMS due to the action of algal or bacterial DMSP lyase isozymes. In this talk I will focus on the link between viruses and clouds by reviewing what we have learnt so far from studies on viral lysis as a mechanism for the release of DMS, including research on viral infection and lysis of axenic cultures and mesocosm populations of *Emiliania huxley*.

Viruses and the carbon cycle

Martha R.J. Clokie

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The presence of photosystem genes *psbA* and *psbD* in bacteriophage genomes necessitates that we must consider their contribution to the carbon cycle. These genes encode for D1 and D2, the two proteins that form the major protein dimer at the heart of photosystem II where light energy is transformed into chemical energy. It has been established that photosynthesis genes are a common feature of cyanophage genomes. *PsbA* and *psbD* are found in phages which infect marine *Synechococcus* and *Prochlorococcus* strains, and potentially in other cyanobacterial phages too as both genes occur in oceanic viral metagenomic data sets.

D1 is rapidly turned over in healthy plant and cyanobacterial cells. It appears that phages encode this gene in order to boost photosynthesis during the infection cycle to increase the number of phage particles released from an infected cell. Thus carbon fixation is in infected *Synechococcus* is driven through phage encoded proteins. To characterise this relationship I have employed a model system, which uses the *psbA* and *psbD* containing myovirus S-PM2 to infect the marine *Synechococcus* strain WH7803. Results from transcriptomic analysis will be interpreted in the context of cyanobacterial abundance and infection rates.

Viruses of the Archaea

David Prangishvili

Molecular biology of the Gene in Extremophiles Unit, Institut Pasteur, rue du Dr Roux, 25, 757245 Paris Cedex 15, France

I will summarize current knowledge on the diversity of double-stranded DNA viruses infecting the Archaea and provide a unifying view on them. It will be highlighted that archaeal viruses present a particular assemblage of species, the majority of which are fundamentally different in its morphotype and genome from known double-stranded viruses of the other two domains of life, the Bacteria and Eukarya.

Viral ecology in marine sediments: benthic processes on a microscale

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Research in aquatic viral ecology has almost entirely focused on pelagic ecosystems, and has verified that viruses significantly influence pelagic microbial mortality, diversity and biogeochemical cycling. Surprisingly, the role of viruses in one of the largest biospheres on the planet, marine sediments, has until recently remained largely unexplored. Benthic viral ecology is, however, a rapidly expanding research area, and recent studies have demonstrated that benthic viral communities are highly diverse and dynamic players in benthic ecosystems. One cm^3 of surface sediment contains 10^8–10^{10} viruses; a 10–100 fold higher viral density than in the upper lying water column. With estimated turnover rates of 0.5–5 days, viruses may therefore constitute a significant loss factor for benthic bacteria (up to 40% of bacterial production). The accumulating data on benthic viruses thus suggest that viruses are important and integrated components of the benthic biosphere. Despite these progresses, we still lack even basic information of the distribution and dynamics of viruses on different spatial and temporal scales as well as an understanding of how virus-host interactions are regulated in the complex physical and chemical microenvironment of sediments (e.g., retarded transport coefficients, steep concentration gradients of physical and chemical parameters, smaller spatial scales of distribution, higher organism densities). This presentation provides an overview of the current knowledge in the field.

Cyanophages active against bloom-forming freshwater cyanobacteria

Li Deng & Paul K. Hayes

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Cyanobacteria are important members of phytoplankton communities both in marine and freshwater environments. Over the past two decades, some work has shown this to be true for cyanobacterial communities that are infected with cyanophage. There is increasing evidence that lateral gene transfer within cyanobacterial communities and populations has a role in generating novel phenotypes. Very little is known about the role of cyanophage in regulating freshwater cyanobacterial population development and structure. In this study, a total of 35 cyanophages able to infect bloom-forming freshwater cyanobacteria from the genera Microcystis, Anabaena and Planktothrix were isolated from Lake Zürich, Switzerland and lakes in the Cotswold Water Park, U.K. The collection of isolated cyanophages encompassed a variety of morphotypes, including the first filamentous cyanophage. Some cyanophages isolations were found to have a very broad host range and were able to infect Anabaena, Microcystis and Planktothrix. The ability to infect a wide range of host taxa extends the potential reproductive period for lytic propagation, and also has implications for the transfer of genetic information between deeply separated cyanobacterial lineages.

Viral genomics, metagenomics and post-genomics

From PCR to microarrays: What can a molecular toolbox tell us about the ecology of coccolithoviruses

Willie Wilson

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The virus genus *Coccolithovirus* is a group of large, double stranded DNA viruses that infect the globally important marine coccolithophorid *Emiliania huxleyi*. Coccolithoviruses are now known to be one of the causative agents of *E. huxleyi* bloom demise. We have developed diagnostic molecular tools to analyse the dynamics of coccolithoviruses and their hosts during natural blooms. We have recently sequenced the 407,339 bp genome of one coccolithovirus and revealed that only 14% of the predicted genes confer any significant database homology. The genome encodes a range of unexpected genes never previously observed in a virus, some perhaps providing insights into the ecology of coccolithoviruses. Most notably are those involved in biosynthesis of ceramide, a sphingolipid better known for its role in apoptosis induction. Microarray analysis of genes on the virus genome is starting to provide clues to the propagation mechanism of this unusual group of viruses. A lot to fit in, it's sure to be a roller coaster ride.

Genomics and ecology of viruses infecting harmful bloom forming algae

Keizo Nagasaki, Yuji Tomaru, Yoko Shirai, Yoshitake Takao & Hiroyuki Mizumoto

Fisheries Research Agency, Japan

More than 20-viruses infecting marine eukaryotic microalgae have been isolated so far and characterized at different levels of resolution. HcRNAV is an icosahedral virus infecting the bloom-forming dinoflagellate Heterocapsa circularisquama, which harbors a linear 4.4-kb ssRNA genome. Based on the phylogenetic analysis of the RdRp domain, this virus apparently differs (deeply branching) from any other viruses ever known (including the other ssRNA viruses infecting marine microalgae). The HcRNAV population is composed of multiple ecotypes which differ in intraspecies host specificity; i.e., the dominant ecotypes differ in strain-specific infectivity between them. Results of genome comparison and protein tertiary structure prediction supported the hypothesis that the nanostructural difference on the viral surface between the two ecotypes is crucial for determining the infection specificity. Viral RNA transfection experiments also suggested the intraspecies host specificity is regulated at the virus entry process. Furthermore, northern hybridization analysis showed the intracellular viral RNA replication efficiency may affect host cell lysis extent. Continuous field survey has demonstrated that H. circularisquama population dynamics is apparently affected by HcRNAV infection; still, detailed interpretation of the field data awaits further study because viral impact on the algal population is assumed to be highly complicated; not only quantitatively but also qualitatively.

Viral metagenomics

Mya Breitbart

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Viruses are the most abundant biological entities on the planet, and play important roles in biogeochemical cycling, horizontal gene transfer, and defining bacterial community composition. However, we are only beginning to understand the identity and diversity of viruses in the environment. Addressing this issue is difficult because there are no conserved genes that are shared in all viruses, and viruses must be cultured on hosts, many of which cannot be cultivated using standard techniques. Metagenomic sequencing has recently been used to examine the identity and diversity of viruses in a variety of environments, including seawater, marine sediment, soil, and human feces. The majority of metagenomic sequences are not similar to those in the current databases, suggesting that environmental viruses represent the largest reservoir of unknown sequence space. In addition, mathematical modeling based on the distribution of overlapping sequence fragments from shotgun libraries suggests that viral communities are incredibly diverse, with over 10,000 viral genotypes in one kilogram of marine sediment, and hundreds of thousands of viral genotypes in the world's oceans. Comparative analyses of viral communities from different environments suggest a shared global pool of viral diversity, with local environmental conditions enriching for certain viral types through selective pressure.

To lead or to follow?: The delicate evolutionary dance of oceanic cyanobacteria and their viruses

M.B. Sullivan, M. Coleman, L.R. Thompson, S. Kern, A.S. Defrancesco, P. Weigele, K. Huang & S.W. Chisholm

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The primary production of vast regions of the oceans are numerically dominated by the unicellular cyanobacteria *Prochlorococcus* and *Synechococcus*. The genomes of these ecologically successful cells range from the smallest known oxygenic phototrophic genomes found in high-light adapted *Prochlorococcus* to the much less streamlined genomes of the low-light adapted *Prochlorococcus* and marine *Synechococcus*, the latter of which contain more signatures of past interactions with parasitic genetic elements (e.g., integrated viral genomes and mobile elements) than the former. While our understanding of the relative contributions of various mechanisms of genome evolution is in its infancy, it has become clear that oceanic cyanobacterial viruses (cyanophages) play a critical role in both shaping and benefiting from host genome content. Remarkably, this is true even for central metabolic processes such as photosynthesis, where it appears that cyanophages play a role in driving the evolution of globally distributed photosystems in the oceans. Here we will use the

lessons learned from cyanophage–host systems to explore (1) how phage-driven genetic diversification of 'host genes' might influence important global biogeochemical cycles, and (2) the variability observed across available cyanophage isolate genome sequences as well as wild cyanophage genome fragments.

Global gene expression of the 'photosynthetic' cyanophage S-PM2

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The cyanophage S-PM2 infects marine *Synechococcus*, a globally important primary producer. The sequencing of the genome of S-PM2 revealed a similarity to that of the phage T4, as well as a large number of ORFs that are database orphans. It is not known if, or when these orphan phage genes are expressed during an infection cycle or what their role is. By the design and development of a microarray, the expression of all S-PM2 genes was monitored during an infection cycle. The results indicated phage genes could be divided into five clusters each having a discrete expression profile. One of these clusters contained a number of genes that have no known function, yet are expressed very early during infection, with these genes located together on the genome. The localisation of groups of genes on the genome with similar expression profiles was found to be widespread through the genome. With a number of homologues of host genes expressed during the infection cycle. The results allow a greater insight into how S-PM2 infects its host.

Eukaryotic Microbiology Group session

Eukaryotic microbial pathogens, attack and counter attack

Molecular networks used by *Candida albicans* to sense the host, co-ordinate metabolism and communicate with itself and others

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Infections caused by the fungal pathogen *Candida albicans* are a cause of death in patients whose immune system is not fully functional as a consequence of cancer, age, or AIDS. *C. albicans* is non-virulent in healthy individuals and is part of the normal intestinal flora. *C. albicans* undergoes a series of characteristic morphological changes important for its ability to cause damage to the infected host. These changes are controlled by a combination of host environmental cues and molecules secreted by the fungus itself. In host niches with mixed microbial populations, fungal cell shape can also be under the control of factors secreted by bacteria (for example *Pseudomonas aeruginosa*). Understanding fungal pathobiology requires studying how *C. albicans* senses and metabolically adapts to its host, how it interacts with the host's immune system, but also how fungal-bacterial and fungal-fungal interactions impact on microbial virulence. Host-pathogen, fungal-bacterial and fungal-fungal interactions are phenomena that lie at the heart of Candida virulence and environmental sensing. Identifying the underlying molecular principles may identify novel treatment options.

The cell wall in the survival and virulence of *Aspergillus fumigatus*

J.P. Latgé

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A common characteristic of all fungal cells is that they are surrounded by a cell wall. Fungal cell walls are mainly composed of polysaccharides. Two of them, β1-3 glucan and chitin constitute the common fibrillar skeleton core of the cell wall of all fungal species. This fibrillar core is further decorated with different polysaccharides which composition varies with the fungal species. In the opportunistic human fungal pathogen *Aspergillus fumigatus*, this amorphous alkali-soluble material is composed of β1,3 glucan and galactomannan. Cell wall composition and structure is also depending on the morphotype and the conditions of growth.

The cell wall of *Aspergillus fumigatus* is a protective skeleton for this mould and a source of pathogen-associated molecular patterns (PAMPs); both aspects being directly associated to the pathogenic life of the fungus. At least, three of the cell wall polysaccharides are involved in host immune response. Moreover, specific conidial and mycelial cell wall components can be associated with fungal survival in the host. This is specially true for the extracellular matrix recently analysed in *A. fumigatus* that has been shown to glue the hyphae together and protect the fungus against external stresses. The impact of the cell wall and associated structures during disease establishment will be discussed.

The evolution of virulence in the human pathogenic fungus *Cryptococcus*

Hansong Ma[1], Ferry Hagen[2], Teun Boekhout[2] & **Robin C. May**[1]

[1]School of Biosciences, University of Birmingham, Birmingham B15 2TT; [2]Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Cryptococcus neoformans and the related species *Cryptococcus gattii* are the causative agents of cryptococcosis, a fatal infection of the central nervous system. Since other *Cryptococcus* species are non-pathogenic, it is important to understand how and why pathogenicity has evolved in these two species.

Following inhalation, cryptococcal cells are engulfed by phagocytic cells of the innate immune system. We and others have previously demonstrated that *Cryptococcus* can exploit this intracellular niche in order to proliferate within and eventually disseminate throughout the host. We now show that intracellular parasitism of macrophages is a major determinant of virulence in this organism. By *in vitro* analysis, live cell imaging and phylogeny construction we demonstrate that macrophage parasitism is both strain specific and rapidly evolving. Finally, we suggest that sudden 'shifts' in this ability may lead to disease outbreaks, such as that which is currently occurring on Vancouver Island.

Effect of protein mannosylation on immune responses to *Cryptococcus neoformans*: the yin and the yang

Stuart M. Levitz

University of Massachusetts Medical School, USA

C. neoformans is a major pathogen in persons with deficient T-cell immunity. *C. neoformans* mannoproteins (MP) are a heterogeneous group of antigens that elicit cell-mediated immune responses in mice and humans. MP share a C-terminal serine/threonine (S/T) rich region, that is the site of heavy O-linked mannosylation, followed by a glycosylphosphatidylinositol (GPI) anchor that presumably serves as a cell wall attachment site. The extensive mannosylation plays an essential role in immune stimulation by targeting MP to mannose receptors (MR) on antigen-presenting cells (APC). MR blockade with mannosylated ligands reduces uptake of MP and inhibits T-cell activation. Dendritic cells (DC) are the immunodominant APC responsible for immune stimulation. The kinetics of MP capture by DC are rapid, MR-dependent and result in co-localization with MHCII. However, MP neither stimulates IL–12p70 release nor upregulates DC maturation markers. Moreover, MP vaccination elicits only partial protection against *C. neoformans*. Studies are in progress examining methods of eliciting protective responses by administering MP combined with Th1-inducing adjuvants such as CpG. Thus, DC provide the crucial link between innate and adaptive immune responses to *C. neoformans* via a process by which MR efficiently recognize MP. However, a protective T cell response appears to require additional stimuli.

Genetic and genomic analysis of iron acquisition, signaling and virulence in *Cryptococcus neoformans*

James Kronstad, Won Hee Jung, Guanggan Hu, Kristin Tangen & Anita Sham

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Cryptococcus neoformans is a basidiomycetous yeast that causes life-threatening infections in immunocompromised individuals. We have used serial analysis of gene expression and microarrays to characterize transcriptome changes for *C. neoformans* under a variety of conditions including iron deprivation in culture, loss of cAMP signalling functions and infection of pulmonary and central nervous system sites. These studies and follow-up functional analyses revealed that iron sensing and acquisition are critical components of cryptococcosis. For example, we found that the transcription factor Cir1 controls the expression of genes of the iron regulon and all of the known major virulence factors

including polysaccharide capsule, melanin and the ability to grow at host body temperature. In parallel, we are systematically evaluating the role of genes encoding putative iron uptake functions including siderophore transporters such as Sit1 and the high-affinity iron permeases Cft1 and Cft2. Finally, our studies have also revealed interesting links between iron sensing and the cAMP pathway in *C. neoformans*.

Macrophage 'hijacking' by the fatal human pathogen *Cryptococcus*

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

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Cryptococcosis is a fatal fungal disease of humans and other mammals that occurs following infection by either of two yeast species: *Cryptococcus neoformans* or *Cryptococcus gattii*. Following inhalation, cryptococci are engulfed by phagocytic cells, but previous studies by our group and others have demonstrated that they are then able to proliferate inside these cells, thus acting as intracellular parasites. We now show that cryptococci can also manipulate the host macrophage in order to mediate an exquisitely controlled 'escape' process. This expulsive process, which we have termed 'vomocytosis' can occur either into the extracellular milieu or, remarkably, into neighbouring host cells, thus resulting in direct cell-to-cell transmission. After vomocytosis, both the host macrophages and the expelled cryptococci appear morphologically normal and continue to proliferate. Vomocytosis therefore represents an important mechanism by which pathogens are able to escape from phagocytic cells without triggering host cell death and thus inflammation. Moreover, direct cell-to-cell spread of cryptococci would allow the pathogen to remain concealed from the immune system and protected from antifungal agents, thus achieving long-term latency.

From attachment to invasion: infection associated genes of *Candida albicans*

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The human pathogenic fungus *Candida albicans* can cause a wide range of infections including oral thrush and life threatening systemic candidosis. However, the mechanisms by which *C. albicans* invades and persists within mucosal epithelium or organs such as the liver are not clear. We characterized the cellular and molecular mechanisms of epithelial-fungus interactions and liver invasion using reconstituted human oral epithelium (RHE), infected organs from animals and clinical samples. To identify *C. albicans* genes that are expressed during invasion of epithelial cells and liver tissue, we used genome-wide transcriptional profiling *in vivo* and *ex vivo*. By analysing the different phases of infection from attachment to tissue penetration in a time course experiment and in patient samples, and by comparing the profiles of an invasive with those of a non-invasive strain, we identified genes and transcriptional patterns which are associated with the invasion process. This includes genes involved in metabolism, stress, nutrient uptake, as well as transcriptional programmes regulating morphology and environmental sensing. We identified several novel infection-associated genes with unknown function. One gene, up regulated in both RHE infection and patients, named *EED1*, was essential for maintenance of hyphal elongation. Mutants lacking *EED1* showed transient cell elongation on epithelial tissue, which enabled only superficial invasion of epithelial cells. Once inside an epithelial cell, Δ*eed1* cells could proliferate as yeasts or pseudohyphae but remained trapped intracellularly. Our results suggest that the adaptive response and morphology of *C. albicans* play specific roles for host-fungal interactions during mucosal and systemic infections.

NAD+ regulation of virulence in *Candida glabrata*

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The opportunistic yeast pathogen *Candida glabrata* adheres to host epithelial cells via a large family of surface expressed adhesins encoded by the *EPA* genes. Many of the *EPA* genes are encoded at sub-telomeric loci and are transcriptionally repressed by chromatin silencing machinery. One particular silent *EPA* gene, *EPA6* is is expressed during murine urinary tract infection (UTI). Expression of *EPA6* in the urinary tract is due, at least in part, to limitation for vitamin B3 (nicotinic acid (NA) a precursor of NAD^+). *C. glabrata* is an NAD^+ auxotroph, and as a result relies on environmental vitamin precursors as its source of NAD^+. In the simplest model, under limiting environmental NA concentrations, NAD^+ levels fall, abrogating function of the NAD^+-dependant histone deacetylase protein Sir2, resulting in de-repression of the silent sub-telomeric *EPA* genes.

To further characterize the role of NA limitation in regulating gene expression, we have used *C. glabrata* microarrays and have identified genes induced by NA limitation, and which are repressed by Sir2 or the related histone deacetylase Hst1. We are currently characterizing the relative contribution of different Hst1 and Sir2-regulated genes to overall virulence and the overall impact of NAD^+ metabolism on virulence.

Ferritin: a novel source of iron for *Candida albicans* during oral infection?

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The iron sequestration by host iron-binding proteins provides a natural resistance to infections, known as "nutritional immunity". Inside oral epithelial cells iron is stored in the iron-storage protein ferritin. Extracellular iron found in saliva is mostly bound to lactoferrin. Therefore, iron availability is extremely limited in the oral cavity. In this study, we aim to determine the natural iron sources for the human pathogenic fungus *Candida albicans* during oral infection. We demonstrated that this pathogen can grow on agar at physiological pH with ferritin as the sole source of iron while the non-pathogenic fungus *Saccharomyces cerevisiae* does not. To obtain iron from ferritin *in vitro*, the fungus needs the iron reductive pathway and must acidify the medium. Hyphae, but not yeast cells of *C. albicans* can bind ferritin *in vitro* and during infection of epithelial cells. This tight binding seems to be necessary for the usage of iron from this protein. To our knowledge, this is the first observation which suggests that ferritin can be used as an iron source by a pathogenic fungus during infection.

Candida albicans: phylogenetics and virulence

Frank C. Odds, Donna M. MacCallum & Mette D. Jacobsen

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Strain differentiation of *Candida albicans* isolates by multi-locus sequence typing (MLST) has enhanced our knowledge of the phylogenetics of this important opportunistic fungal pathogen. Two-thirds of all isolates, worldwide, belong to one of five clades of closely related strain types. It therefore becomes possible to investigate whether, as with many bacterial pathogens, there are specific associations between subsets of strains and their virulence properties.

We are studying gene expression profiles and phenotypic properties of a set of 44 *C. albicans* isolates of known virulence in a mouse model of invasive *Candida* infection. Strains of high and low mouse virulence are found within all four major clades. However, to date there are clade differences in the ability of strains to form biofilms on plastic surfaces and in the numbers of repeat sequences in the *ALS* gene family, which encodes surface adhesins. The presentation will update the status of this ongoing project.

MLST also permits investigation of genetic variation among individual cells in the population of *C. albicans* isolated from a single clinical sample. The data show that many individuals, whether carriers or patients, harbour populations in which variants that have lost heterozygosities in portions of individual chromosomes coexist with their heterozygous counterparts. A propensity for continuous genetic variation of this nature may provide *C. albicans* with the diversity needed for rapid adaptation to changing environmental microniches in the absence of a full meiotic sexual cycle.

Further characterization of the murine model of invasive *Candida albicans* infection

Donna M. MacCallum & Frank C. Odds

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The mouse model of invasive *C. albicans* infection is the most popular model used to analyse virulence of this fungus. Previous work has identified a number of key time points in the infection process (MacCallum & Odds, 2005). These include 5–10 hours, when the fungus is cleared from the bloodstream; 24 hours, when fungal numbers begin a rapid increase in the kidneys; and 48 hours, when approximately 50% of blood samples test positive for *C. albicans*.

In this study, the characterization of the model is expanded upon by examining organ histology, measuring cytokine levels in different organs and examining *C. albicans* cellular morphology.

To test the model, three strains with differing virulence were used as the infecting strain. Preliminary results of the study are presented here.

Interkingdom signaling between two microbial pathogens: transcriptional responses from *Candida albicans* to *Pseudomonas aeruginosa* secreted factors

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Effects of *P. aeruginosa* signaling molecules on eukaryotic organisms are well documented with recent research concentrating on interactions between *P. aeruginosa* and *C. albicans*. Instrumental in these interactions are 12-carbon molecules including those produced by bacteria (e.g. 3-oxo-C12 HSL). Our work concentrates on such interactions where, unlike previous studies with well-characterised strains, we use *P. aeruginosa* clinical isolates recovered from CF patient sputum. We found significant differences in types, levels and timing of HSL molecules produced by different strains. This is likely to significantly impact on interactions with *C. albicans* in the CF lung. In particular, it seems likely that mixtures of secreted bacterial signals/metabolites have different effects on the yeast. Supporting this hypothesis, we find that some of our strains affect *C. albicans* morphology whereas others do not. We investigated, by microarray analysis, effects that concentrated, sterile supernatants from a selection of isolates had on *C. albicans* gene expression. Surprisingly the supernatants produced by the different strains induced (109) and repressed (126) a large number of common genes, despite the differential effects on *C. albicans*. Some of these genes are associated with stress response e.g. drug efflux systems, whereas others are limited to specific functions such as cell surface adhesion and ergosterole biosynthesis. The significance of these changes is being investigated. Analyses of the effects of the supernatants that inhibit the yeast-hyphal transition indicate that several pathways are being affected. We are exploring this further using time course RT-PCR and targeted mutants.

Dissecting pathogenicity in oomycete pathogens

Pieter van West

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Oomycetes are serious pathogens of animals and plants. We study fundamental molecular processes involved in pathogenicity and development of fish pathogenic oomycete *Saprolegnia parasitica* and the plan pathogen *Phytophthora infestans*. We use molecular methods ranging from genomic to proteomics to dissect pathogenicity determinants that are important for both organisms.

In *P. infestans*, the appressorial stage of the interaction is one of the first points in which direct contact between the pathogen and the plant occurs via the formation of specialised infection structures, incl. the appressorium, penetration peg, and the infection vesicle. We have isolated proteins that are abundant in appressoria using proteomics, one of which is a cellulose synthase. Three other cellulose synthase genes were identified within our EST collections. Real time RT-PCR reveals that all four genes are up-regulated in germinating cysts with appressoria. Gene silencing studies of the cellulose synthases perturb the morphology and structure of appressoria. Also culturing germinated cysts in the presence of cellulose synthase inhibitors resulted in similar changes in appressoria structure and morphology. Pathogenicity studies employing *P. infestans* zoospores in the presence of the cellulose synthase inhibitor resulted in greatly reduced infection rates.

Functional genomics of plant infection by the rice blast fungus *Magnaporthe grisea*

N.J. Talbot

University of Exeter

Abstract not received

Regulation of the cell orientation response: relationship to tissue invasion by *Candida albicans*

Alexandra Brand[1], Anjalee Vacharaksa[2], Cheryl Gale[2] & Neil A.R. Gow[1]

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Candida albicans hyphae are observed in organs derived from models of deep-seated infection and may provide a mechanism for tissue penetration and invasion. In plant fungal pathogens, the ability of hyphae to respond directionally to the topography of host surfaces is a key element in pathogenesis. *Candida albicans* hyphae are also able to respond directionally to external cues. We are interested in the molecular mechanisms that regulate this re-orientation response and have presented a model suggesting that calcium influx and signalling may provide directional cues to the growing hypha. We have recently

identified cell polarity determinants that may act as downstream effectors of calcium signalling. Their deletion or mutation attenuated the directional response to external cues. In the *rsr1/bud1* mutant, this phenotype was particularly severe and correlated with reduced tissue penetration *in vitro* and *in vivo* and attenuated virulence. Hyphal directionality mechanisms could therefore be involved in pathogenesis in *C. albicans*.

New insights into the cell biology of *Cryptococcus neoformans*

Arturo Casadevall

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C. neoformans virulence strategy includes a capsule, melanin, and an unusual intracellular pathogenic strategy. Recent studies reveal unexpected complexity in the architecture of the capsule and polysaccharide structure. Melanin is a cell wall-associated pigment that has an important role in virulence and may have novel functions in capturing electromagnetic radiation for biological energy. *C. neoformans* is a facultative intracellular pathogen which a unique replication strategy characterized by shedding of polysaccharide-filled vesicles into the host cell cytoplasm and the ability to exit macrophages without damaging the host cell. The constellation of these observations suggests a remarkable story in cell biology.

Antigenic variation in malaria involves a single highly structured switching pathway

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Abstract not received

MPM2 positive phosphoproteins and trypanosome cell division

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The microtubule cytoskeleton is essential for directing cellular morphogenesis in the African trypanosome *Trypanosoma brucei*. During the cell cycle various cytoskeletal, structures, including the flagellum, ensure the orderly segregation of duplicated organelles and accurate positioning of the cleavage furrow. The monoclonal antibody MPM-2 has been widely used in mammalian systems and recognises a diverse range of proteins in mitotic cells. In trypanosomes however, we have shown that MPM2 specifically recognises phosphoproteins located within cytoskeletal structures essential for faithful cytokinesis. Using a proteomic based approach we have identified two of these MPM2 positive phosphoproteins and shown by RNAi that ablation of these proteins results in a specific cell division defect characterised by unequal apportioning of organelles between daughter cells. Immunolocalisation and GFP tagging studies have revealed that both proteins bind to cell body microtubules and we are currently investigating whether these proteins act to stabilise/destabilise microtubules during cell division.

Differentiation and virulence of *Leishmania*

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Parasites of the genus *Leishmania* are trypanosomatid parasitic protozoa that cause a spectrum of diseases, termed the leishmaniases. *Leishmania* are transmitted by sandflies as extracellular, flagellated promastigotes that differentiate into metacyclic forms prior to inoculation into the mammalian host. Transformation into proliferating intracellular aflagellated amastigotes then occurs within the phagolysosome of host mononuclear phagocytes. The recent completion of genome projects for several trypanosomatid species, including *L. major*, *L. infantum* and *Trypanosoma brucei*, has allowed comparative genomic analysis to identify potential virulence factors. Most *L. major* genes have orthologues in the other Tritryp genomes. However, there are "*Leishmania* restricted" genes, some of which are likely to account for the specific lifestyle of the parasite and its ability to survive and replicate in host macrophages. An example is the macrophage migration inhibitory factor (MIF). Structural, biochemical and immunological analysis of recombinant *L. major* MIF proteins, together with expression analysis of the endogenous proteins, reveals they have evolved diverse characteristics that can in some, but not all, aspects mimic host MIF. Other examples of *Leishmania* virulence factors under investigation and discussed in this presentation include peptidases and their natural inhibitors.

A cellular design for pathogenicity in African trypanosomes

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Bloodstream from African trypanosomes possess a surface coat comprising of one type of glycoprotein. Although only one type of variable surface glycoprotein (VSG) is expressed by any one cell at any one time there are around 1000 VSG genes and antigen gene switching allows the immune evasion and maintenance of the parasitaemia. A major issue is how this allelic exclusion is controlled. Our work reveals a novel nuclear location for the expressed VSG gene with particular transcription factor also being located to sub-nuclear domains. The VSG protein is trafficked to the cell surface by vesicles traffic into and out of the parasite. This single portal is the flagellar pocket – an internal sub-domain of the surface formed around the single flagellum. Endocytosis operates within the pocket to "cleanse" the cell surface of antibody and recycle VSG. Electron tomography has been used to provide a full 3D description of this flagellar pocket. This architecture reveals how this intricate cellular design is central to the pathogenicity potential of this parasite.

Cell surface glycoconjugates and Trichomonas vaginalis pathogenesis

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Trichomonas vaginalis is the protozoan parasite responsible for trichomoniasis, the most common non-viral sexually transmitted infection. Attachment of this extracellular parasite to host cells is required to establish infection, yet neither host cell receptors nor parasite surface molecules involved in attachment have been identified. A candidate molecule for host-pathogen interactions on the surface of *T. vaginalis* is its lipophosphoglycan (LPG)-like molecule. To study the importance of LPG in infection, mutants with altered LPG were generated and examined. These mutants were shown to differ in the monosaccharide composition of their LPG, relative to wild-type parental parasites. LPG mutants were severely deficient in adhesion and cytotoxicity to ectocervical cells, indicating a role for LPG in parasite-mediated cytotoxicity. We have further investigated the possibility that *T. vaginalis* LPG utilizes the S-type lectin, galectin-1, as a host receptor. Wild-type*T. vaginalis* was shown to bind to galectin-1 in a lectin-specific manner, whereas LPG mutants did not bind, consistent with a LPG:galectin mediated interaction. Further studies aimed at determining whether *T. vaginalis* is capable of utilizing galectin-1 as a host cell receptor will be discussed.

Fermentation & Bioprocessing Group session

Measurement and monitoring in bioprocesses

The bioprocess defines the product – what should you measure and when

R. Dennett

National Biomanufacturing Centre, Liverpool

Abstract not received

Improved bioreactor

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For some years we have been developing batch vessels with variable geometry cooling jackets (Coflux®). In this design, the heat transfer area is used as a temperature control parameter (as opposed to varying the jacket temperature). This allows the heating or cooling flux can be held constant, even when the process heat load is changing.

Variable geometry jackets represent a significant design improvement for bio reactors. They offer better temperature control, greater energy efficiency and they deliver more uniform heat flux. The most compelling benefit however relates to heat monitoring. For many years, heat measurement has been used for studying bio processes at the lab scale. Heat is a very effective tool for monitoring cell populations and bio mass. On large vessels however, heat measurement has always proved difficult to perform. This relates to an underlying conflict between temperature control and heat measurement. By using a variable area cooling jacket to control temperature, the heat noise associated with temperature control can be eliminated. This permits very sensitive heat measurements on large bio reactors with non intrusive instruments.

A strategy for implementing PAT during early development of monoclonal antibody production from mammalian cells

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PAT implementation during early development of biopharmaceuticals offers significant benefits developing an initial understanding of the links between critical process measurements and product quality attributes. Apart from these benefits, a PAT philosophy increases the value of data acquired during early development. Care must be taken to balance the strategy taking into account new project throughput and project attrition rates. This talk will focus on the practical aspects of implementing this strategy. As an example, the discussion will use a new multiplexed PLS modelling approach to FT-NIR monitoring of concentration in mammalian cell cultures.

PAT (Process Analytical Technology) methodologies in anti-infectives biomanufacturing

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The significant impact of PAT in biomanufacturing is far from realised mainly due to the insufficient use of PAT in a systematic and process wide perspective. Here we describe how information obtained with several at-line and on-line PAT monitoring tools on different process steps can be used in a consolidated way and/or in feed-forward mode to understand and better control the overall bioprocess.

Several industrial case studies will be presented in an articulated way, making them a general roadmap for other bioprocesses. The economic case in favour of the use PAT in the fermentation part of an industrial bioprocess will be made.

Keywords PAT in Biomanufacturing, API production processes, multiplexing NIR solutions

Application of selected ion flow tube mass spectrometry, SIFT-MS, to the analysis of volatile compounds from cell and bacterial cultures

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Selected ion flow tube mass spectrometry, SIFT-MS, is a highly sensitive on-line real time technique for the analysis of breath, air and liquid headspace samples. It combines soft ionisation using selected precursor ions H_3O^+, NO^+ and $O_2^{+\bullet}$ with fast-flow tube and quantitative mass spectrometry and allows on-line analyses of volatile compounds (e.g. alcohols, ketones, aldehydes, ammonia) down to the parts per billion level. Recently, it has been utilized to monitor the trace metabolites in the headspace of cell cultures and bacterial fermentations. The enormous potential of SIFT-MS has been shown by a study of the kinetics and isotope patterns of ethanol and acetaldehyde production by *Saccharomyces cerevisiae* in aqueous glucose and glucose-6,6-d_2 solutions. Further, acetaldehyde has been shown to be released by the lung cancer cell lines SK-MES and CALU-1 in culture with respective production rates of 1×10^6 and 1.5×10^6–3×10^6 molecules/cell/min. Most recently, volatile compounds emitted by bacterial cultures grown from cough and sputum samples provided by cystic fibrosis patients have been studied that show HCN to be a specific marker for *Pseudomonas Aeruginosa*. The results of these studies will be presented and discussed and the use of SIFT-MS to monitor processes within a bioreactor will be raised.

In situ multi-wavelength fluorescence spectroscopy as effective tool to simultaneously monitor spore germination, metabolic activity and quantitative protein production in recombinant *Aspergillus niger* fed-batch cultures

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The production of a mutant green fluorescent protein (S65TGFP), controlled by the maltose inducible glucoamylase promoter, was followed *in-situ* in fed-batch cultures of recombinant *Aspergillus niger* using multi-wavelength fluorescence spectroscopy. Disturbance of quantitative product analysis by interfering fluorescence signals was resolved by using a set of defined combinations of excitation and

emission wavelengths (λ_{ex} / λ_{em}). This technique resulted in excellent linearity between *on-line* signal and *off-line* determined S65TGFP concentrations. Spore germination was detectable *in situ* by monitoring the back scattered light intensity. Moreover, flavin-like fluorophores were identified as the dominating fungal host fluorophores. The time-dependent intensity of this fluorophore, potentially fungal flavin-containing oxidoreductase(s), did not correlate with the biomass concentration but correlated well with the fungal metabolic activity (e.g. respiratory activity). Other fluorophores commonly found in microbial cultures such NADH, pyridoxine and the aromatic amino acids, tryptophan, phenylalanine and tyrosine did not contribute significantly to the culture fluorescence of *A. niger*. Thus, multi-wavelength fluorescence spectroscopy has proven to be an effective tool for simultaneous *on-line* monitoring of the most relevant process variables in fungal cultures, e.g. spore germination, metabolic activity, and quantitative product formation.

Fluorometric and microscopic methods for bioprocess monitoring

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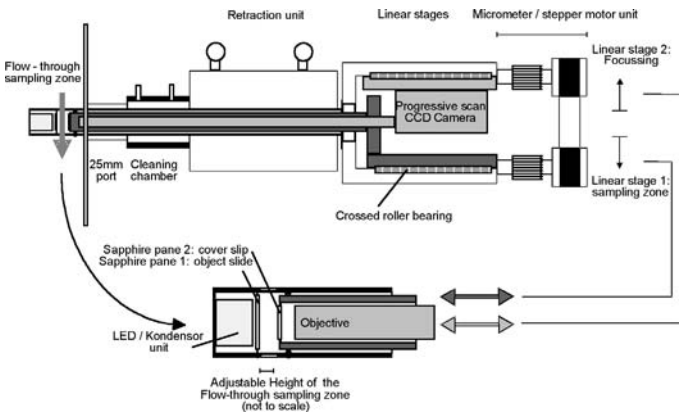
Optical sensors offer the possibility to monitor non-invasively bioprocesses on line and without any time delay. Several optical sensor systems are described in the actual sensor literature, however only few of them are commercially available. The class of optical sensors can be subdivided in the classes of optical chemo-sensors (such as dissolved oxygen or pH probes), spectroscopic sensors (such as NIR, FTIR or fluorescence probes) and image analysis systems (such as *in situ* microscopy)

The principles of the so called 2-D-spectrofluorometry will be presented for non-invasive monitoring of bioprocesses, its modelling and for down-stream process analysis. This techniques opens the possibility to monitor all fluorescent compounds within 30 sec in emission and excitation scans (from 380 to 600 nm each). Thus concentration profiles of intra- and extracellular components can be analysed and be appropriate models, and prediction of relevant bioprocess variables such as biomass and educt/product concentrations is possible.

Within this presentation the design and the principles of an *in situ* microscope will also be discussed. Here the set-up and the different functions will be described in detail and several applications will be presented in the monitoring of yeast, BHK, CHO, microcarrier cultivations and crystallisation processes. The needs of an intelligent image analysis will be discussed for the different application examples.



Set up of the in situ microscope

Control of fed-batch fermentation using off-gas analysis

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Fed-batch cultivation of *E. coli* for the production of plasmid DNA offers many advantages over batch growth. Not only does fed-batch fermentation allow higher biomass concentrations but it also reduces the specific growth rate of the culture. Lower growth rates are advantageous for plasmid DNA production as lower growth rates increase the specific plasmid yield as plasmid replication exceeds the rate of cell division. Various methods for control of exponential fed-batch have been reported. The strategies vary in their complexity and equipment requirements. Possibly the most simple method would consist of manual measurement of growth rate and adjustment of the addition of a limiting substrate. Direct, online, measurement of the limiting substrate concentration and automatic adjustment of the feeding rate is also possible.

Off-gas analysis is a non-invasive method of monitoring the dynamics of a fermentation. The CO_2 and O_2 concentration can be measured using relatively inexpensive hardware. Data acquisition technology can be used to manipulate data in real time on a computer. Derived variables can then be used for control purposes using industry standard Proportional-Integral-Derivative control techniques. By using these methods and introducing some more advanced control methods such as data filtration and gain scheduling we have demonstrated that it is possible to automatically control a fed-batch fermentation.

Individuals behave differently – flow cytometry and cell sorting for fully understanding bioprocesses

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Accurate measurements relating to cell proliferation and viability are essential if informed decisions about a fermentation process are to be made, since performance will depend largely upon cell number and individual cell physiological state. The advantages of using cytometric techniques over the more traditional microbiological analyses are well documented and the development of multi-parameter flow cytometric techniques has led to the functional classification of the physiological state of single celled micro-organisms. This classification is based on either 1) the presence or absence of an intact fully polarised cytoplasmic membrane and the transport systems across it or 2) energy dependent/independent intracellular enzyme activities. Using all of these techniques it is possible to resolve an individual microbial cells physiological state, beyond culturability, to include metabolic activity enabling assessment of population heterogeneity and dynamics. In this work we use cell sorting to fully understand the function of three well-known flow cytometric techniques for measuring bacterial cell physiological state, namely PI/DiBAC4, PI/DiOC6(3) and the RedoxSensor™ Green kit (Molecular Probes/Invitrogen) during a batch culture of the model organism *Bacillus cereus*.

Food & Beverages / Systematics & Evolution Groups joint session

Molecular detection of food and water pathogens

Overview of molecular detection methods

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The analysis of food and water for the presence of bacterial, viral, and parasitic pathogens is critical for ensuring public health. The traditional approach for bacterial pathogen detection involves enrichment in a suitable growth medium, followed by plating onto selective agar(s) and confirmation of presumptive bacterial isolates using a number of biochemical and serological tests. However, traditional methods are labor intensive and time consuming. The need for a more rapid assessment of the microbial safety of food and water has led to the development of molecular methods, particularly polymerase chain reaction (PCR)-based assays, for pathogen detection. An important advance is the development of real-time PCR based on the detection and quantitation of a fluorescent reporter in a closed-tube format. Real-time chemistries allow for detection of amplification while the reaction is occurring, thus post-PCR processing for amplicon identification is not required. Applications of PCR assays include detection of specific food-borne pathogens in different types of complex matrices, simultaneous detection of more than one pathogen in a single sample, pathogen typing, and species identification. Real-time multiplex PCR assays, involving amplification of two or more target sequences simultaneously in one reaction, have the potential to decrease cost, time, and effort in pathogen detection. Nanotechnology, as it applies to diagnostics, is a rapidly developing molecular analysis technology that is driving the development of nanofluidic lab-on-a-chip systems. Microminiaturization of nucleic acid-based techniques will have a significant impact on food and water testing, potentially enabling the assays to be performed using portable instruments in non-laboratory settings.

Development of a multiplexed PCR-microsphere array for the rapid detection of pathogens from foods

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Detection and characterisation of foodborne pathogens using conventional culture and phenotypic identification, is time consuming and laborious. PCR-based methods are more rapid, specific and sensitive. This study aimed to develop a PCR-microsphere array to detect *Campylobacter coli*, *Campylobacter jejuni*, *Clostridium perfringens*, verotoxigenic *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* from foods. Microsphere array technology facilitates the design of automated, high throughput, multiplexed suspension arrays, which detect PCR products by direct hybridisation to DNA probes immobilized on microspheres. A nine-plex PCR-microsphere array has been developed and optimised for specificity and sensitivity. Currently, the assay is being applied to the detection of the target organisms in naturally contaminated and artificially spiked foods, in comparison to conventional culture-based methods. This PCR-microsphere array detects six important foodborne pathogens in a rapid, multiplexed format, allowing for the timely reporting of laboratory results.

The development of a reverse transcriptase real time PCR assay for the detection of *Salmonella* in fresh meat

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The EU requires that fresh meat is examined for the presence of *Salmonella*. Testing may be carried out by a cultural method (ISO 6579) taking 3–4 days for positive clearance of samples. The meat industry thus has an urgent need for an alternative and validated rapid method. This study focused on the development of a RNA based real time PCR assay for detection of viable *Salmonella* from fresh meat. A novel gene target in *Salmonella* was identified and sequence specific FRET hybridisation probes were designed. The developed method involves an initial enrichment prior to the extraction and amplification of the target gene. A range of *Salmonella* serovars and non-*Salmonella* strains (typical meat microflora) were tested in the newly designed assay to determine the inclusivity and exclusivity of the assay. The method has also been shown to successfully detect *Salmonella* from inoculated meat carcasses swabs.

Standardisation and evaluation of molecular detection assays

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Recognizing the need of standardization, in 1999 the EC launched a research project FOOD-PCR, including 35 institutes and companies, which aimed to validate and standardize the use of diagnostic PCR for detection of bacterial pathogens in foods. Standard PCRs were devised for 5 major foodborne pathogens. The methodology was focused on 3 primary sample types. The consortium continued as FOO-PCR2 as part of MedVetNet. The objective was to implement PCR-based methods, such as real-time PCR or microarray-PCR, for both detection and verification of food-borne pathogens, through international, multi-center harmonization and standardization of methods. We establishment five groups, a PCR database, preparation of European draft standards, hands-on workshops for harmonisation of methodologies, SOPs for collaborative trials, and exchange of scientists and students. The microaaray group looked into the possibilities to harmonize similar international efforts and draft a standard guideline for a simple, single-dye approach to routinely screen *Salmonella* isolates for the presence of genes encoding for sero-group, fimbrial antigens, pathogenic properties, antibiotic resistance determinants, phage susceptibility (Malorny *et al.*, 2004). Such draft guideline could be used, in the first instance, by large reference laboratories to set up uncomplicated and fail-proof microarray tests for a comprehensive characterization of *Salmonella* isolates. This could be useful for early identification of new outbreak strains and to study the epidemiology of *Salmonella* on genotypic level. The information used will be open to the public. Diagnostic companies could exploit commercial potential of the guideline by providing smaller laboratories with kits. A Nordic project is currently looking into the possibility of ring-trial assessment of the platform. It will provide risk managers with an additional tool for *Salmonella* control. The aspects of discriminatory power, reproducibility, cost-effectiveness, ease-of-use, etc. are under evaluation. The work continues in the large European IP project including 47 partners (www.biotracer.org).

Detection and characterisation of *Cryptosporidium* sp. seeing the whole picture through evidence-based methodology

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Monitoring for *Cryptosporidium* oocysts is an important element of public health risk assessment for water, with an on going shift away from "end product" to raw water monitoring as part of the implementation of water safety plans. Characterisation of the species present is key information in determining the significance of the health risk when oocysts are detected. This information is not provided by standard methods.

We have developed and adopted a robust evidence-based molecular characterisation approach; Oocysts are detected using standard methods and DNA is extracted from processing pellets or microscopy slides using a freeze-thaw, spin column protocol. *Cryptosporidium* DNA is then characterised using repetitive PCR and sequencing of at least two gene loci, one of which appears to preferentially amplify species important in human health (COWP) and the other providing detailed information about these and other non-anthroponotic species which may be present (ssu rRNA) in samples containing multiple species.

Catchment-based studies validated this approach, which provides a benchmark for further innovation in test development.

An assay for the botulinum toxins that requires functional binding and catalytic domains within the neurotoxin

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The botulinum neurotoxin family (BoNTs) comprises seven serotypes (A-G) which cause botulism food poisoning and are a potential bioterrorism threat. Increasingly, these serotypes are being further subdivided into subtypes which are not necessarily detected by antibodies raised against another subtype. The BoNTs exert their potent action by cleaving proteins within the nerve ending at specific residues. Serotypes B and F cleave Vesicle Associated Membrane Protein (VAMP). Types A & E cleave Synaptosomal Associated Protein of 25kDa (SNAP-25).

A novel capture method has been employed to retrieve active toxin from contaminated media which uses synaptosomes. After capture, the bound toxin can be incubated in the presence of its substrate. The cleaved substrate can then be detected using serotype-specific antibodies raised against the unique cleaved product of each toxin serotype. Synaptosomes have a number of advantages over antibodies as a capture phase. All BoNT serotypes and subtypes are captured and very small samples volumes may be used for the detection of biologically active toxin in complex media such as serum.

Real time epidemiology

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The characterization of pathogenic micro-organisms is essential for effective surveillance. Strain characterization or "typing" informs short term surveillance such as case cluster/outbreak recognition and in the medium to long term national and international epidemiology and effective intervention and control strategies for disease prevention. Traditional so-called phenotypic typing methods are largely based upon techniques such as serotyping, phagetyping and antimicrobial sensitivity testing which require viable organisms and gene expression. The development of molecular techniques provides direct access to the genome of the organism and improved typability and discrimination. Some molecular methods provide molecular fingerprints for epidemiological investigations whereas techniques like sequence typing are rapidly becoming established as the new gold standards for strain characterisation. The advantages of molecular fingerprinting or typing techniques are their standardisation and accessibility, often combined with their electronic portability. These powerful technologies are based upon rapid nucleic acid amplification and expanding global databases. These developments inform the design of improved methods for direct pathogen detection combined with strain specific information in real time, thereby providing information for action and more effective intervention and control of infectious diseases.

Molecular detection of water-borne pathogens

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During the last twenty years, several high profile water-borne or water associated outbreaks of disease have hit the headlines within and outside of the UK. Their impact is directly related to the size of the population exposed to the contaminated water. Confirmation of possible outbreaks of waterborne infection requires microbiological evidence that the water was the route of transmission. Testing for indicator organisms has been traditionally used for routine monitoring of the microbiological safety of water. However, indicator organisms have not been appropriate for some pathogens and molecular techniques would be most suitable for their detection.

An important challenge in modern water quality monitoring is the rapid, high-throughput, specific, and sensitive detection of waterborne pathogens. The introduction and subsequent improvements of molecular based detection methodologies have changed the "conventional detection" of microbial pathogens and introduced a new era of "rapid quantitative detection" technologies. Using this technology, waterborne pathogens can be tracked down within hours of sampling in different environmental samples that may contain and transmit them to humans, thereby reducing the morbidity and mortality associated with disease outbreaks.

The complex nature of environmental samples will continue to challenge its application in the testing laboratory and drive further improvements to these methodologies.

Development of a rapid selective mPCR assay to identify *Salmonella enterica* serovar Typhimurium and Heidelberg

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Salmonellosis is one of the most common infectious foodborne diseases in the world, both in animals and humans. Traditional culture based methods for the detection of *Salmonella* are labourious and costly. Rapid detection and serovar identification would dramatically decrease the time required to identify *Salmonella* outbreaks and potential health risks. We have developed and optimised a multiplex PCR (mPCR) assay to rapidly and selectively identify *Salmonella* Typhimurium and Heidelberg. Comparative genomic analysis allowed for the identification of potential serovar-specific regions. A primer pair targeting a putative inner membrane protein amplified a 199 bp product from both Typhimurium and Heidelberg isolates, whereas, a primer pair targeting a putative cytoplasmic protein amplified a 759 bp product specific to Typhimurium. We are currently validating the use of this assay in variety food matrices.

Mechanical detection technologies for food- and water-borne diseases

Anja Boisen

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Micrometer sized diving boards (also called cantilevers) can be used for rapid detection of food and waterborne diseases in food and water. Basically, a biochemical reaction at a cantilever surface can be monitored as a bending of the cantilever, due to a change in the surface stress. Furthermore, highly sensitive mass detection can be achieved using resonating cantilevers. We have developed cantilever-based sensors with integrated read-out, which hold promises as fast and cheap 'point of use' devices as well as interesting research tools. The detection technique involves no labelling of the molecules by fluorescent, magnetic or radioactive markers and bulky detection schemes like laser scans, CCD imaging or radiography are avoided. The cantilever-based sensor principle has a wide range of applications in real time local monitoring of chemical and biological interactions as well as in the detection of specific DNA sequences, proteins and particles. We will present design, fabrication and applications of some of our recently developed mechanical sensors.

Rapid detection and differentiation of *Campylobacter jejuni*, *C. coli* and *C. lari* in food samples using multiplex real-time PCR

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Thermophilic C*ampylobacter* species are the most common cause for food associated bacterial gastroenteritis worldwide. Therefore rapid and reliable methods for their detection and differentiation are required. A multiplex real-time PCR assay based on four differently labelled TaqMan® probes was established combining two previously published PCR assays for *C. jejuni* and *C. coli* with a newly developed detection assay for *C. lari* and an internal amplification control. Each assay is based on another gene. The method identified more than 100 different *Campylobacter* target strains correctly. Exclusivity was tested with 31 non-target *Campylobacter* strains and 43 other bacterial species. The detection limit was 1 to 10 cfu per 25 g food for all three species. More than 200 various food samples (chicken, duck, turkey, mussels and fish) were investigated after a pre-enrichment step of 48 hours parallel to the conventional diagnostic method including cultural and biochemical identification. By providing reliable results within two days compared to 7–8 days for the conventional diagnostic method, the presented multiplex real-time PCR is a good alternative to accelerate routine analysis and therefore enhance food safety.

Physiology, Biochemistry & Molecular Genetics Group session

The physiology of non-growing microbes

Regulation of growth to non-growth transition

Messenger RNA interferases encoded by toxin – antitoxin loci are particularly abundant in slowly growing prokaryotes

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Prokaryotic chromosomes code for stress-response-elements known as toxin-antitoxin (TA) loci. TA loci consist of two genes in a transcriptional unit. Most TA loci encode mRNA cleaving enzymes that are inhibited by a cognate protein antitoxin. The RelE type of mRNA interferases cleaves mRNA positioned at the ribosomal A-site whereas the MazF type cleaves mRNAs site-specifically, apparently independently of the ribosomes. RelE and MazF are activated by amino acid starvation and other metabolic stresses.

The model organism *E. coli* K-12 contains at least seven *bona fide* TA loci (four *relBE*, two *mazEF* and one *hicAB*). Using a comprehensive bioinformatic approach, we identified ≈1200 TA loci in 220 totally sequenced prokaryotic genomes (200 Bacteria and 20 Archaea) divided between 8 gene families (Gerdes and Pandey, 2005 and unpublished). Our phylogenetic survey shows that TA loci are particularly abundant in slowly-growing free-living prokaryotes whereas intracellular bacteria have few or none. For example, *Mycobacterium tuberculosis* has 60 TA loci, whereas *M. leprae* has none. *Sulfolobus solfataricus* has ≈30 *vapBC* loci and transcription of many of these was stimulated during heat shock (Tachdjian & Kelly, *J. Bacteriol.* 188, 4553, 2006). Models for how mRNA interferases can help the cells survive environmental stresses will be discussed.

Nucleoid-associated proteins and bacterial physiology

Charles J. Dorman & Tadhg Ó Cróinín

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Gram-negative bacteria such as *Salmonella enterica* and *Escherichia coli* express up to twelve distinct members of the family of nucleoid-associated proteins. The best-studied of these include the factor for inversion stimulation (Fis) and H-NS. Each of these proteins makes a key contribution to the management of the gene expression profile of the cell as the bacterium moves from one stage of growth to another. In many cases, these contributions are antagonistic. This suggests a homeostatic regulatory mechanism in which the relative abundances and activities of global regulators operate to modulate the gene expression programme of the cell.

Control of actinobacterial dormancy and resuscitation by the Rpf family of secreted proteins

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Many bacteria persist in an apparently non-growing state in their natural environment. For example, latent tuberculosis, which may affect as much as one third of the world population, results from the ability of the causative organism, *Mycobacterium tuberculosis*, to persist within the human body, sometimes for decades, as an asymptomatic infection. The persistence of non-growing organisms probably also accounts, at least partially, for the extensive bacterial diversity that is detectable in environmental samples by molecular methods but not by culturing. (However, perhaps it is the microbiologists in this case, rather than the organisms they attempt to study, that are uncultured.) In spite of the obvious importance of persistent non-growing organisms, relatively few laboratory-based investigations of their physiology have been published, undoubtedly because they are very difficult to study. A notable exception is provided by *Micrococcus luteus*, an organism that loses culturability during prolonged stationary phase in laboratory batch culture. Dormant *M. luteus* cells may be resuscitated by adding sterile supernatant from an actively growing culture. A protein called the resuscitation-promoting factor (Rpf) is responsible for resuscitation activity. Homologues of Rpf form an extended protein family that is found throughout the actinobacteria and functionally similar proteins are also present in the firmicutes. Because of their potency it was originally surmised that these proteins might have some kind of signalling function. More recent work indicates that they exert their function at the level of the bacterial cell wall, probably via muralytic activity. Our current understanding of how these proteins may control the culturability of their producer organisms will be discussed.

Investigation of the regulation of the alternative sigma factor σ^B in the human pathogen *Listeria monocytogenes*

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The Gram-positive food-borne pathogen *Listeria monocytogenes* exhibits the capacity of surviving harsh environmental conditions, which is achieved in part by the activation of a group of genes known collectively as the *sigB* (σ^B) regulon. Access to this regulon is provided by the alternative sigma factor, σ^B, but the factors that influence the activation of σ^B are poorly understood in *L. monocytogenes*. This project seeks to elucidate the molecular mechanism involved in regulating the activity of σ^B, in particular how RsbW (a putative anti-σ factor) and RsbV (a putative anti-anti-σ factor) act to control the availability of σ^B. We have generated polyclonal antisera against each of the proteins under investigation. These antisera have been utilized in western blotting experiments aimed at quantifying cellular levels of RsbV, RsbW, and σ^B. Results have indicated that σ^B levels drop in stationary phase despite increased activity of σ^B and the ratio of the putative anti-σ^B factor RsbW to σ^B increases under the same growth conditions. As σ^B availability is largely dependent on the phosphorylation state of RsbV, experiments have been performed that assess the cellular abundance of RsbV and RsbV-P under similar growth conditions. Future work will assess putative protein-protein interactions that exist between RsbV, RsbW, and σ^B. Data obtained will provide insight into the mechanisms that regulate the activity of σ^B of *L. monocytogenes* in response to environmental challenges.

Regulation of σ^s expression by CspA paralogues

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Salmonella enterica sv. Typhimurium (*S.* Typhimurium) requires the action of protective cold shock protein A paralogues to adapt to and

multiply at low temperatures. These proteins overcome the translational blockage that occurs during temperature downshift by binding to and destabilising RNA secondary structure.

The general stress σ subunit, RpoS, plays an important role in adapting cells to low temperatures, oxidative stress and stationary phase. Under such conditions, RpoS acts as an 'emergency co-ordinator', inducing transcription of stress response genes. RpoS is regulated post-transcriptionally by at least three small RNAs: DsrA, RprA and OxyS. The process requires Hfq, a bacterial Sm-like protein, which stabilises sRNAs and enhances RNA-RNA interactions.

The present study investigates how CspA paralogues regulate RpoS expression in *S.* Typhimurium. The phenotypes of mutants with deletions in genes encoding CspA paralogues, Hfq and/ or RpoS are examined during various RpoS-regulated processes; in addition the levels of σ^s are determined. A model is proposed to explain how the system may operate.

Development

Controlling *Bacillus subtilis* complex colony architecture using the regulator DegU

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DegU functions as a co-regulator of multicellular behaviour exhibited by *B. subtilis*. We have proposed a model where entry into different multicellular behaviours is triggered along a gradient of DegU phosphorylation (DegU~P). Previously it had been demonstrated that genetic competence is regulated by unphosphorylated DegU. In contrast, swarming motility is activated by very low levels of DegU~P, complex colony architecture requires low levels of DegU~P, and high levels of DegU~P inhibit the former three processes and are required for exoprotease production. The experimental findings that support this proposal indicate that the DegSU two-component regulatory system functions as a rheostat rather than a molecular switch to co-regulate the physiological response exhibited by the bacterium. We are in the process of identifying genes that are regulated by DegU and required for complex colony architecture development, an analogy for biofilm formation. One such gene that we have already identified through comparative genome-wide transcriptional profile analysis is *yvcA*, which encodes a putative membrane bound lipoprotein. Transcription of *yvcA* is activated by low levels of DegU~P and inhibited by high levels of DegU~P; the levels of DegU~P that activate and inhibit complex colony architecture respectively.

Activation of a contact-dependent intercellular signalling system by regulated proteolysis

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In response to starvation *Myxococcus xanthus* cells initiate a developmental programme that culminates in the formation of multicellular, spore-filled fruiting bodies. Aggregation of cells into nascent fruiting bodies and sporulation of cells that have accumulated inside the fruiting bodies, are temporally and spatially coordinated by the intercellular C-signal. C-signal transmission occurs by a contact-dependent mechanism. The C-signal is a 17 kDa outer membrane protein (p17), which is synthesized by proteolytic cleavage of an outer membrane precursor protein (p25). p25 accumulates in vegetative as well as in starving cells, however, p17 only accumulates in starving cells To define the mechanism by which *M. xanthus* cells restrict p17 accumulation to starving cells, we used a three-tiered strategy to identify *popC* candidate genes.

Inactivation of *MXAN0206*, which encodes a subtilisin-like protease, results in a mutant with severe developmental defects. Complementation of the *MXAN0206* mutant with the *MXAN0206*$^+$ allele restored all developmental defects. Moreover, an active site mutant of *MXAN0206* displays the same developmental defects as the *MXAN0206* insertion mutant. Accumulation of p17 is neither detected in the *MXAN0206* insertion mutant nor in the active site mutant. Additionally, purified MXAN0206 protein cleaves p25 directly *in vitro*. MXAN0206 accumulates in vegetative and starving cells; however, MXAN0206 is selectively secreted in starving cells. Our data suggest that *MXAN0206* encodes PopC. Moreover, our data suggest that regulated PopC secretion guarantees that p25 and PopC are only present in the same cell compartment during starvation, thus, restricting p17 synthesis to starving cells. To our knowledge the PopC/p25 system represents the first bacterial example in which regulation of proteolysis is based on regulation of secretion.

ecfR – a role in relieving oxidative stress?

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Extracytoplasmic function (ECF) sigma factors often respond to extracellular signals and coordinate the expression of subsets of genes. The pea and vetch symbiont *Rhizobium leguminosarum* has 16 ECF sigma factor genes. One of these, *ecfR*, has been shown to be induced by pea root exudate and is induced in infection threads during infection of vetch. ECF sigma factors are often transcribed by the proteins they encode, and are often negatively regulated by an anti-sigma factor. A strain was isolated harbouring a mutation in the gene adjacent to *ecfR*. An *ecfR'*-GFP fusion was induced in this mutant, confirming that the gene encodes an anti-EcfR factor. The mutant was used for microarray analysis to identify genes transcribed by EcfR. The induced transcripts were mapped by S1 protection assays. The genes regulated by EcfR appear to belong to a group likely to be induced by oxidative stress, and infection threads may be sites of such stress.

Can bacteria differentiate and employ similar strategies as eukaryotic cells?

E. Kondorosi

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Abstract not received

Microarray analysis of *Mycobacterium tuberculosis* gene expression during interaction with the host: persistence and metabolic shifts

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The human pathogen *M. tuberculosis* has the ability to persist *in vivo* in the face of immunity resulting in latent infections in a third of the world's population and creating a major reservoir for reactivation disease. *M. tuberculosis* also persists in the face of chemotherapy during treatment of active disease due to drug tolerance, requiring prolonged antibiotic therapy for 6 months. Whole genome transcriptome analysis of *M. tuberculosis* linked with differential enrichment and mRNA amplification can probe expression patterns associated with bacterial physiological responses to the host environment in vivo. Transcriptomes from pulmonary tuberculosis sputum specimens, macrophage infections and *in vitro* culture models of infection reveal a shift in metabolic phenotype associated with in

vivo persistence from one of rapid replication to a slowed-growth phenotype. This '*in vivo* phenotype' is associated with a change in carbon source from glucose/glycerol to fatty acids and cholesterol and a concomitant switch from aerobic to anaerobic respiration. Definition of such physiological shifts may allow for a more rational approach to the design of new more rapidly sterilising antibiotics that target slow-growing organisms.

Secondary metabolism / carbon flux

The global role of ppGpp in morphological differentiation and antibiotic production in *Streptomyces coelicolor* A3(2)

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Regulation of production of the translational apparatus via the stringent factor ppGpp in response to amino acid starvation is conserved in many bacteria. In addition to this core function, it is clear that ppGpp also exhibits genus-specific regulatory effects. In this study we used Affymetrix genechips to more fully characterise the regulatory influence of ppGpp on the biology of *Streptomyces coelicolor* A3(2), with emphasis on the control of antibiotic biosynthesis and morphological differentiation.

S. coelicolor is a model organism for the streptomycetes, industrially important producers of bioactive secondary metabolites. Our results demonstrate a dramatic switch in cellular physiology following induction of ppGpp synthesis, with activation of transcription of genes involved in stationary phase processes, such as secondary metabolism and alternative ribosomal protein synthesis, and repression of genes with functions important for active growth. ppGpp-mediated effects include changes in transcription of operons and gene clusters that are particularly characteristic of streptomycetes: the repression of conservons, the induction of antibiotic gene clusters, and the expression of the morphogenetic *sapB*, chaplin and rodlin genes.

Artificial symbiosis: isolation, identification and growth of marine sponge bacterial symbionts

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The seas and oceans have been recognised as very potent sources of medicinal products. Marine sponges are the most prolific source of the thousands of bioactive secondary metabolites that have been discovered in the marine environment during the last decades. The variety of sponge-derived products may reflect the diverse and abundant microbial populations that are associated with sponges, often comprising up to 50% of the sponge biomass. For many bioactive molecules that were formerly ascribed to sponges it has now been established that they are produced by sponge-associated microorganisms. The introduction of molecular taxonomy has greatly improved our knowledge on microbial diversity in the marine environment, but it has also demonstrated our inability to cultivate many of them. Entry of bioactive compounds from marine origin into clinical trials is usually curtailed because of our inability to obtain these metabolites in significant quanities.

Cultivation of marine invertebrate-associated bacteria and understanding of the metabolic routes that result in bioactive compounds has been recognised as a viable way to produce these metabolites in the future. We have mimicked microenvironments that occur inside marine sponges, which are significantly different than the surrounding seawater, in order to improve the cultivability of the symbionts. In addition, we have applied cultivation conditions based on the closest cultured relatives of 16S rDNA sequences that were found in the sponge extract. Thus, we have been able to cultivate currently unknown bacterial strains, which allows us to analyse their genetic content in order to discover potential metabolic cooperations between the sponge and its symbionts and predict the role of the symbiont in the sponge-bacterium conglomerate. In addition, the bacterial extracts of the pure cultures can be screened for bioactive metabolites.

Exploring the chemical ecology of uncultivated bacterial symbionts

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Many animals use low-molecular compounds for chemical defense. It has been postulated that a large number of these natural products are produced by symbiotic bacteria. However, due to the general inability to cultivate the suspected producers their study remains challenging. We follow a metagenomic approach to gain insights into the chemical ecology of uncultivated symbionts. Total animal DNA containing all genomes is cloned to generate complex libraries, which are screened for biosynthesis genes of interest. These can then be used to identify the corresponding producer, obtain insights into its biology and create renewable sources of bioactive compounds of pharmaceutical interest. The feasibility of this approach was first demonstrated by using the beetle *Paederus fuscipes* as model, the source of the cytotoxic polyketide-nonribosomal peptide pederin. The entire set of pederin genes was isolated and shown to belong to a symbiotic bacterium with close relationship to *Pseudomonas aeruginosa*, which colonizes the beetle in large numbers [1]. Recently a first draft of the symbiont genome was completed and has been analysed for factors that determine symbiosis or culturability. The metagenomic strategy is also applied in our group to highly complex associations of marine sponges. From *Theonella swinhoei*, a sponge harboring enormous numbers of diverse bacteria, genes involved in the biosynthesis of the onnamides [2] were isolated from a 1 million clone library and found to be of bacterial origin. The talk discusses some of our recent progress to study biosynthetic pathways from symbiotic sources.

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Prize Lectures

Fred Griffith Review Lecture

Bacterial variation, virulence and vaccines

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E. Richard Moxon is Chairman of Paediatrics at the University of Oxford, Head of the Molecular Infectious Diseases Group at the Weatherall Institute of Molecular Medicine and Director of the Oxford Vaccine Group. After graduation from St. John's College Cambridge (1963), and St.Thomas's Hospital, London (1966), he trained as a paediatrician, sub-specialising in infectious diseases and began laboratory research in 1971 at Children's Hospital Medical Center in Boston, USA. His career continued at John's Hopkins University (1974–1984) before returning to the UK to take up the Action Research Professorship of Paediatrics. Using models of infection, classical genetics and whole genome sequences, his laboratory has made contributions to the pathogenesis and prevention of bacterial diseases caused by *Haemophilus influenzae* and *Neisseria meningitidis*.

Peter Wildy Prize Lecture

Ten years in Vietnam

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I currently hold a Chair in Molecular Microbiology at the Royal Holloway University of London where my research interests are primarily with recombinant vaccines. Just before starting my position at the RHUL I made my first trip, as a tourist, to Vietnam in 1995. This was an interlude after spending ten years in the USA including a six year post-doctoral stint with Rich Losick at Harvard working on the genetics of sporulation in *Bacillus subtilis*. Before this I was lucky enough to work with Joel Mandelstam and Jeff Errington (then a post-doc) at Oxford where I obtained my PhD.

This background showed me that microbiology was of historical importance in this war-torn, yet beautiful and beguiling country. Indeed, Vietnam was once home to not one but four Pasteur Institutes. I first visited the Nha Trang Institute where the renowned microbiologist Alexandre Yersin (as in *Yersinia pestis*) lived and worked for 40 years. I have since been back to Vietnam over twenty five times and developed numerous research and teaching collaborations. Inspired by the commitment to learning of many young Vietnamese students I held a training workshop in 1996 teaching molecular biology methods. It was apparent, and surprising to me, that the Vietnamese relished the opportunity for interaction with Western scientists and so began a programme of training workshops held in Ho Chi Minh City (aka Saigon) and Hanoi,. the 12th workshop taking place at the Hanoi University of Science (August 20–23, 2007). Over 60 Western scientists have now attended these workshops giving lectures as well as organising short practical classes and 6 Vietnamese students have now entered labs in the UK and Europe for PhDs. I am of course grateful to the SGM for sponsoring half of these workshops to date. My time in Vietnam has allowed me to work with most of the major academic institutes and this has led to a number of funded research collaborations including a large EU 6FP project on TB and malaria vaccines with Vabiotech (a Hanoi vaccine manufacturer) and the Hanoi University of Science on a British Council funded project (DELPHE). My interests in Vietnam go beyond teaching and research though, and I have started working on a literary project involving an English resident of Vietnam some 100 years ago whose husband was the first doctor to work with Yersin in Vietnam.

Young Microbiologist of the Year Competition

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

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Influenza A virus utilises *cis*-acting packaging signals to package its segmented RNA genome. Large-scale mapping has shown that these signals extend into coding regions, where distinguishing between selection for RNA structure and for the encoded protein complicates detailed mapping. By considering codon conservation, we identified putative *cis*-acting elements within the coding regions, including likely packaging signals. An 8-plasmid reverse genetics system was used to introduce synonymous mutations into conserved codons in segment 7 (M1/M2); mutations were also introduced into non-conserved codons as negative controls. Apart from their changed codon usage these viruses were genetically identical to wild-type. All mutants produced viral protein, vRNA and mRNA at levels comparable to wild-type. However, viruses mutated in conserved codons showed a small-plaque phenotype and replicated to significantly lower titres than wild-type virus, displaying growth deficits of 10 – 1000 fold. Viruses with alterations to non-conserved codons replicated normally. Three debilitated mutants were serially passaged to select fitter variants and all regained high-titre growth and the ability to form normal sized plaques. In all cases, sequencing showed that the original mutations remained, suggesting compensatory second-site mutations. Identification (currently underway) of these mutations will help delineate interactions between individual segments in the process of viral assembly.

Gene regulation and quorum sensing in *Clostridium botulinum*

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

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

The equivalent regions were shown to be present in *C. sporogenes*, and to be highly conserved. Transcriptional linkage assays have shown some of the genes of the *C. sporogenes agr* regions to be co-expressed, and Real Time RT-PCR has been used to determine when these genes are maximally expressed.

Modulation of the expression of the identified *agr* genes is a prerequisite to determining their function, and we have shown that down-regulating some of these *agr* genes has a significant effect on sporulation.

Probiotics as anti-infectives: protecting against *Listeria* infection through bacteriocin production

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Aims The gastrointestinal microbiota presents a significant barrier which must be overcome in order for a pathogen to initiate an infection. Probiotic organisms (live bacteria which have a beneficial effect on the host when consumed in adequate amounts) have been proposed to play roles in improving digestive function, in the reduction of chronic inflammation and in hastening recovery from intestinal disease. Some probiotics have been shown to be capable of producing bacteriocins, including a number which are active against *Listeria monocytogenes*. Bacteriocins are a heterogeneous family of small, heat-stable peptides with potent antimicrobial activity which are produced by many bacterial species, including many probiotic strains.

Methods This study uses a series of genetic manipulations to show that bacteriocin production by the probioitc bacterium, *Lactobacillus salivarius* UCC118 is required for protection against *Listeria* infection in mice.

Results We present evidence that a probiotic strain, *L. salivarius* UCC118, which produces a bacteriocin active against *Listeria*, can protect mice against oral infection. The lack of protection when a bacteriocin negative mutant is used, combined with the ability of an immune strain of *Listeria* to overcome the protection, confirms bacteriocin production as the mechanism responsible for the protective effect.

Conclusions The concept of preventing or ameliorating intestinal infections through dietary interventions designed to manipulate commensal bacteria, or as a means of introducing transiently colonising probiotic strains, has received much attention in recent years. Such strategies could potentially decrease antibiotic use and associated problems of antimicrobial resistance. We show that *L. salivarius* UCC118 significantly protects mice from infection by the potential fatal pathogen, *L. monocytogenes*. Furthermore, this protection is dependent upon production of bacteriocin by the probiotic, resulting in direct anti-*Listeria* activity *in vivo*.

Molecular comparison of microbial consortia demonstrating biodegradative properties towards toxic fractions of oil

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Gas chromatographically unresolvable complex mixtures (UCMs) of hydrocarbons are a permanent toxic feature of weathered crude oil. Their high toxicity and resistance to weathering make them an obvious candidate for potential biodegradation research. Two consortia were obtained from enrichment cultures of soil samples collected from separate sites contaminated with weathered hydrocarbon fractions. These were Whitley Bay, UK, a coal contaminated shoreline and Ons Island, Spain, near the site of the *Prestige* oil tanker crash in 2002. Both consortia have previously been shown to degrade the model

compounds; 6-cyclohexyltetralin and 1-(3'-methylbutyl)-7-cyclohexyltetralin that represent the most toxic fraction of the UCM. Community analysis was performed on each consortium via the creation of 16S rDNA clone libraries that were screened by DGGE analysis. Identification of the genera present was achieved via the comparison of 16S rDNA gene sequences obtained from the clone libraries with NCBI's Blastn database. Sequences were screened for chimeric properties using mallard and the diversity statistics Chao1, ACE and rarefaction were computed using EstimateS. Phylogenetic relationships were revealed via the creation of phylogenetic trees by ClustalX. Monocultures of *Rhodococcus sp.* from the Whitley Bay consortium were isolated in pure culture that show biodegradation in relation to 6-cyclohexyltetralin.

Novel bacterial iron transporters of the MFS and ABC superfamilies

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The biological availability of iron is extremely limited, yet it is a critical element for the growth and metabolism of micro-organisms. The production of siderophores is a common mechanism by which micro-organisms acquire iron. The FhuCDB system of *Escherichia coli* has served as the model for iron acquisition by hydroxamate type siderophores. Hydroxamate siderophore mediated iron acquisition has been found in several species of the rhizobia. *Sinorhizobium meliloti* 2011 is distinguishable in that it does not encode orthologues of FhuCDB and transport of hydroxamate siderophores has been shown to be occurring by novel mechanisms.

A 'Split-ABC' transport system has been identified in *S. meliloti* 2011 where inner membrane components of a haem utilisation system interact with the periplasmic component of a hydroxamate siderophore utilisation system and facilitate transport of hydroxamate siderophores. The genes for these components are encoded distally on the chromosome. Furthermore, a single unit hydroxamate siderophore transporter has also been identified which facilitates transport of the endogenous siderophore rhizobactin 1021 across the inner membrane. This constitutes a novel family of permeases with similarity to MFS type transporters.

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

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Streptococcus pneumoniae is a human pathogen that causes both mucosal and invasive diseases, including pneumonia, bacteremia, otitis media, and meningitis, throughout the world. Currently available vaccines are based on the capsular polysaccharide. As polysaccharide vaccines are poorly immunogenic in those most at risk of disease new protein/polysaccharide vaccines have been developed. These vaccines are expensive to produce, limiting their use in the developing world where the burden of disease is greatest. New strategies are required that provide capsular and serotype independent protection. Here we show that proteins genetically fused to pneumolysin (Ply) become capable of generating an antibody response that is not seen when they are administered as a mix. In this study, pneumococcal surface adhesin A (PsaA) was fused to the N terminal of Ply and administered either intranasally or subcutaneously to mice. Mucosal administration of less than 1µg of fusion protein was sufficient to generate significant systemic titres of serum IgG responding to PsaA and Ply. We also report the protective efficacy of these responses to intranasal challenge with virulent TIGR4 *S. pneumoniae*.

Human antibodies to hepatitis C virus – potential for vaccine design

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The control of hepatitis C infection is associated with both cell-mediated and humoral arms of the adaptive immune response. One important component of this immune response is the production of neutralising antibodies. The HCV envelope glycoproteins are the main target of these neutralising antibodies. Importantly, there is increasing evidence that a strong and broad antibody response to E2 is correlates with resolution of HCV infection.

Using recently established entry and cell culture systems, a number of broadly neutralising monoclonal antibodies (NMAb) have been described. Murine NMAb AP33 targets a conserved linear epitope located immediately downstream of the first hypervariable region (HVR1) of E2. However, the majority of NMAb target conformational epitopes . Knowledge of these neutralising determinants will assist in informing the design of immunogens that preferentially focus the immune response on these important epitopes. This knowledge will also provide important insight into the structure of the E2 protein.

We have compared the reactivity of a number of NMAbs to E1E2 proteins representative of diverse genotypes and to a panel of mutated E1E2s to identify important determinants associated with antibody recognition. These studies demonstrate that the majority of E2-specific broadly neutralising antibodies described to date recognise overlapping epitopes located in regions involved in CD81 binding. Within this immunogenic region, we have identified E2 residues critical to effective humoral responses to HCV.

Tbzfp3 is a positive regulator of ep procyclin mRNA in *Trypanosoma brucei*

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Regulation of gene expression is a key process during the trypanosome life cycle. Although this is largely post-transcriptionally controlled, the trans-acting RNA-binding proteins involved in controlling differentiation events are not well characterised. One group of proteins implicit in the control of several developmental events is the TbZFP family (comprising TbZFP1, 2 and 3). These proteins are characterized by their small size (<150aa) and presence of a CCCH zinc finger RNA-binding domain. Interestingly, the proteins are capable of direct interaction in yeast and co-immunoprecipitate *in vivo*, suggesting association in a complex. Furthermore the TbZFPs show a stage-specific polysomal association in tsetse procyclic, but not bloodstream forms. a-TbZFP3 RNA-immunoprecipitation selected *ep procyclin* mRNA in procyclic cells, suggesting it may be a target for this complex. Reporter constructs with specific deletions within the *ep procyclin* 3'UTR show specific regulatory regions are necessary for this interaction. Additionally, knockdown of ZFP3 protein levels in procyclic cells results in reduced levels of *ep procyclin*. We conclude that TbZFP3 is a positive regulator of *ep procyclin* mRNA.

Using microarray analysis to determine the effect of salt and growth phase on transcription in *Listeria monocytogenes* and characterisation of the specific role played by sigmaB (σ^B).

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The ability of bacteria to survive physiological challenges like osmotic, cold or acid stress depends on how efficiently they can induce and regulate genes with protective functions. The alternative sigma factor, σ^B is partially responsible for the redirection of transcription under stress conditions in *Listeria monocytogenes*. In this study, microarrays were used to determine the effect of salt and growth phase on gene expression in a wildtype strain of *L. monocytogenes* 10403S and also to elucidate fully the role of σ^B in modulating transcription in this strain. The freely available TIGR Tm4 software suite was successfully used to identify differentially expressed genes from experiments conducted using spotted PCR product, glass slide microarrays. Importantly, the analysis successfully identified many known σ^B-dependent virulence genes (*bsh, inlA, inlB*) and stress-related genes (*gad, ltrC clpC*). σ^B was found to regulate genes across the full spectrum of functional categories and does not appear to have a bias towards any particular functional group. A possible role for σ^B in regulating motility was identified. In exponential phase without osmotic stress fewer σ^B-dependent genes were identified. In contrast, σ^B appeared to play a significant role in transcription during stationary phase or when osmotic stress was present. Furthermore, approximately 25% of salt inducible genes were found to be σ^B dependent, confirming the central role for σ^B in regulating the response of *L. monocytogenes* to osmotic stress.

Posters

Cells & Cell Surfaces Group

CCS 01 Structural flagella is not a requirement for *Escherichia coli* O157:H7 persistence in an ovine model of infection

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Escherichia coli O157:H7 is an important food-borne zoonosis and ruminants (Cattle and sheep) are cited as the primary source of infection. Recent studies have suggested that the terminal rectum is the preferred site of colonisation for *E. coli* O157:H7. Furthermore, this phenotype is dependent on fully functional intimin and Tir, and the regulation of flagella. Studies reported from our laboratory have demonstrated that intimin and Tir are a requirement for full persistence in the 6-week-old lamb model.

To investigate whether structural flagella contributed to persistence of a Stx-negative *E. coli* O157:H7 isolate (NCTC12900nal^r) an isogenic aflagellate mutant was constructed. Characterisation of this aflagellate *E. coli* O157 mutant (DMB1) was performed using cell monolayers, ovine *in vitro* organ culture (IVOC) and spiral colon gut loops.

In vivo, groups of 6-week-old lambs were dosed orally with 10^9 CFU/ml of either *E. coli* O157:H7 NCTC12900nal^r or isogenic aflagellate mutant. Two independent ovine models of infection (Study 1 & 2) revealed that the aflagellate mutant was able to persist for at least 21 days longer than the NCTC12900nal^r. Furthermore, Study 2 also demonstrated that a chemotaxis NCTC12900nal^r mutant, but not a non-motile NCTC12900nal^r mutant, was able to persist longer than NCTC12900nal^r. These data suggested that *E. coli* O157:H7 persistence in the 6-week-old lamb model may involve tightly controlled mechanisms that may regulate flagella expression during the course of infection.

CCS 02 The Mcf1 toxin induces apoptosis via the mitochondrial pathway and apoptosis is attenuated by mutation of the BH3-like domain

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Photorhabdus are Gram-negative, nematode-vectored bacteria that produce toxins to kill their insect hosts. The expression of one, makes caterpillars floppy 1 (Mcf1), is sufficient to allow *Escherichia coli* to survive within, and kill, caterpillars. Mcf1 treated caterpillars show rapid loss of body turgor and death is associated with massive apoptosis of both the midgut epithelium and insect phagocytes. Mammalian tissue culture cells treated with Mcf1 also display key features of apoptosis. As Mcf1 carries a single BH3-like domain, we investigate the importance of this domain. A double mutant within the BH3-like domain causes dramatic decline in apoptosis. Wild-type Mcf1 induces key pro-apoptotic mitochondrial events and cells over-expressing Bcl-x_L are resistant to Mcf1-mediated apoptosis, as are cells deficient in Bax. Translocation of Bax to the mitochondrion is observed in response to Mcf1 treatment. Together, these results show that Mcf1 mediates apoptosis via the mitochondrion, and are consistent with the hypothesis that the BH3-like domain in Mcf1 is a functional requirement for pro-apoptotic activity.

CCS 03 Insecticidal toxins of *Photorhabdus luminescens* and *Yersinia*

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Photorhabdus luminescens is a gram-negative bacteria insect pathogen, which lives in the gut of entomothogenic nematodes from the family *Heterohabditidae*. Upon invasion of the insect host, the nematode regurgitates the bacteria, which releases a plethora of virulence factors to aid killing and bioconversion of the insect.

One of the dominant secretion factors are the high molecular weight insecticidal toxin complex (Tc's) proteins, which have been shown to be orally and injectably toxic. Originally isolated from strain W14, these complexes are made from 4 loci; *tca, tcb, tcc* and *tcd*, the genes within these loci labelled according to their order e.g. *tcdA, tcbB, tccC*. Significant homology is observed between the loci and previous work has shown that three components are required for full toxicity, the *tcdA*-like [A], the *tcbB*-like [B] and *tcc*C-like [C] genes.

Interestingly these Tc's are seen in a variety of gram-negative pathogenic bacteria including *Yersinia*, suggesting an evolving function or targets for these proteins directed towards insect and/or mammalian hosts.

We are currently using heterologous expression of the individual polypeptides, from both *Photorhabdus* and *Yersinia*, in a mammalian gut cell culture model system in order to ascribe their function. Here we report the emerging mode of action for the Tc's and their role in pathogenicity.

Posters

Clinical Microbiology Group

CM 01 Incorporation of chitosan in acrylic bone cement: effect on antibiotic release and bacterial biofilm formation

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Gentamicin-loaded bone cement is routinely used for prosthesis fixation at the time of hip replacement surgery, in an attempt to eliminate bacterial infection of the newly implanted device. However, as bacteria which cause these infections can form biofilms on gentamicin-loaded bone cements despite the release of gentamicin, there is a need for the development of alternative antimicrobial strategies.

It has been shown that the natural biopolymer, chitosan, has antibacterial properties. In this study, we added chitosan to unloaded and gentamicin-loaded bone cement and determined the effect this had on staphylococcal biofilm formation and gentamicin release.

The bone cements used in this study were unloaded Palacos R® and Palacos R® with gentamicin. Different quantities (1%, 3% and 5%) of high molecular weight chitosan were added to the polymer powder component of Palacos R® and Palacos R® with gentamicin cements.

Gentamicin release from all bone cements was most rapid during the first 6 h, continuing at a much lower rate thereafter. Addition of chitosan to gentamicin-loaded cement did not increase the efficacy of the bone cement at preventing bacterial biofilm formation.

These results suggest that incorporation of chitosan in unloaded bone cement does not prevent bacterial biofilm formation and that chitosan incorporation in gentamicin-loaded bone cement does not convey any additional antimicrobial benefit.

CM 02 Spore size of *Clostridium difficile* and implication for aerial dissemination

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Clostridium difficile is an increasing cause of nosocomial diarrhoea in the UK. Our group has previously isolated *C. difficile* spores from the air of an Elderly Care ward. Persistence of spores in the air maybe an important factor in their dissemination.

The aim of the current study was to investigate the spore sizes of *C. difficile* strains and relate this to their ability to persist in the air. Three strains representing two clonal lineages of the UK epidemic ribotype 001 were used. Dimensions of dormant spores were determined using a transmission electron microscope and Digital Micrograph™ software. Stoke's Law was applied to the spore length data to calculate the average time for a spore to settle out of the air.

The mean spore size varied from 1.04–1.42µm in length and 0.53–0.67 µm in width. Application of Stoke's Law, revealed that *C. difficile* spores could remain in the air from 4.2 hours up to 7.9 hours when falling from a height of 1 metre (~ hospital bed height) in completely still air.

The data reveals that the time that *C. difficile* spores can reside in the air may vary between strains, which could have implications for how readily they are spread within the hospital environment.

CM 03 Enterotoxigenic *Bacteroides fragilis* in cases of hospital-acquired diarrhoea

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Bacteroides fragilis is the Gram-negative anaerobe isolated most often from human clinical infections. Some strains produce a 20 kDa enterotoxin (BFT). Enterotoxigenic *B. fragilis* (ETBF) have been isolated from various diarrhoeic animal species and a significant association with community-acquired diarrhoea in humans has been noted in epidemiological studies worldwide. In the UK, hospital-acquired diarrhoea is a major health care problem. Although commonly caused by *Clostridium difficile* or noroviruses, in a large proportion of cases, the aetiological agent remains unidentified.

The prevalence of ETBF in 127 cases of nosocomial diarrhoea from a UK District hospital, for which no other pathogen had been identified, was investigated. ETBF gene sequences were detected directly from faecal DNA extracts using a novel multiplex PCR assay. Where ETBF was detected, a second multiplex PCR assay was used to determine which isoform of the toxin gene was present. 14.1% (18/127) of the diarrhoea samples tested positive for *bft*. The predominant isoform in the ETBF positive samples was *bft-1* (72%). This is the first report of ETBF identified in cases of hospital-acquired diarrhoea in the UK.

CM 04 Detection of *erm(X)* and transposon Tn*5432* in propionibacteria isolated from Egyptian acne patients and controls

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Antibiotics remain the mainstay of anti-acne therapy and resistance in *Propionibacterium acnes* is common. This species is unusual in that resistance usually arises from mutation of chromosomal genes. Only one mobile genetic element has been reported in *P. acnes*. Tn*5432* has been found in isolates from Europe, and carries *erm*(X), conferring resistance to erythromycin (Ery) plus clindamycin (Cd). In Egypt antibiotics are available over the counter for acne, but little is known about the resistance mechanisms involved.

Propionibacteria were isolated from the skin of 102 acne patients, controls and dermatology staff in Cairo. Forty-three people (42.2%) carried strains resistant to Ery plus Cd (MIC values >0.5mg/L). 102 isolates with this profile were investigated using PCR assays for *erm*(X) and Tn*5432*. Twenty-nine (28.4%) carried *erm*(X). However, just three of these had Tn*5432*. This is the first report of *erm*(X) being carried by *P. acnes* outside Europe. Whilst the gene is associated with Tn*5432* in some strains, the data suggests other transpositional events have also contributed to the spread of Ery and Cd resistance in Egyptian strains.

CM 05 Development of a rapid microarray for *Staphylococcus aureus* and MRSA

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Staphylococcus aureus is a Gram-positive coccus that has long been recognised as a major human pathogen. Methicillin-resistant *S. aureus* (MRSA) accounts for up to 40% of all *S. aureus* bacteraemia in the UK

and has higher morbidity and mortality rates than Methicillin-sensitive *S. aureus* (MSSA) infection (Brown *et al.* 2005). The current method of diagnosis for MRSA is culture and susceptibility testing, which is slow with turnaround times of up to 24 hours and provides limited information. Molecular techniques such as real time PCR have the potential to reduce turnaround time but are usually limited by the amount of information available. Microarray technology can address this problem as many genes can be detected simultaneously, however this is traditionally a time consuming technique.

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

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

CM 06 Development of rapid, species-specific identification of pathogenic *Candida* yeasts

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Certain yeasts of the genus *Candida* are opportunistic pathogens capable of causing systemic infections (candidaemias) particularly in immunocompromised patients. Candidaemia causes sepsis and can lead to colonisation of one or more major organs with *Candida*, with potentially fatal results. Existing methods of identifying *Candida* in clinical samples are time consuming, require trained personnel to carry out the analysis and are not always specific enough to differentiate between species. The aim of our work is to produce a rapid detection system that can easily be operated at the point of care with minimal training. The work involves developing DNA probes labelled with electrochemically active ferrocene molecules for the detection of *Candida* yeasts in clinical samples with detection being performed on solid state electrodes. The ITS2 region of the rDNA genes was chosen as the target region for the species-specific probes, and the probes have been shown to successfully detect extracted *Candida* DNA and differentiate between *Candida* species. The results demonstrate that the assay can detect DNA from *Candida* yeasts and that species-specific identification is possible.

CM 07 Microarray analysis of the responses of *Clostridium difficile* to stress conditions

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In order to be able to adapt to the intestinal environment, *C. difficile* must react to the many stresses involved with colonisation. To investigate the response of *C. difficile* to various stresses, the *C. difficile* 630 microarray developed by the Bacterial Microarray Group at St. George's, University of London (BμG@S) was utilised. *C. difficile* 630 was grown to logarithmic phase and then subjected to heat shock, oxidative shock, acid pH shock or alkaline pH shock; or was grown in the presence of amoxicillin, clindamycin or metronidazole at sub-inhibitory levels. RNA was extracted and gene expression was analysed using DNA/RNA competitive hybridisations. The data was normalised and then genes with statistically changing expression were identified.

As expected, the classical stress response is observed after heat shock and also after acid shock. Exposure to atmospheric oxygen induces a large number of electron transporters, including components of the alternative oxidative stress protection system. Metronidazole affected very few genes, probably due to the very low concentration necessary to allow *C. difficile* to grow. Heat shock and both clindamycin and amoxicillin exposure resulted in the regulation of many biochemical pathways. All three antibiotics resulted in an increase in the levels of ribosomal protein transcripts.

In this study, regulated genes and potential operons have been identified which are both unique to and common between different stresses. It is hoped that this information will allow us to further understand expression of *C. difficile* genes within the gut.

CM 08 Structure and assembly of the *Clostridium difficile* S-layer

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Clostridium difficile, like many bacteria, possesses a paracrystalline protein surface layer (S-layer). Unusually in *C. difficile* this S-layer is comprised of two proteins derived from a single precursor, SlpA, by post-translational cleavage. Interestingly, purified S-layer proteins (SLPs) can be reassembled *in vitro* but this self-assembly requires calcium. The high molecular weight SLP contains putative cell-wall binding motifs and is highly conserved among *C. difficile* strains; most inter-strain differences involve single amino acid substitutions with occasional small insertions and deletions. In contrast, the low molecular weight SLP shows an extremely high degree of sequence divergence between strains. The function of the S-layer in *C. difficile* is still a subject of some debate but it has been suggested that the layer may act as an adhesin by binding to collagen I, thrombospondin, and vitronectin. Little is known about the structure of the S-layer or the mechanism of its assembly on the cell surface. We have isolated a protein complex containing both the high and low molecular weight SLPs that appears to be the basic subunit of the *C. difficile* S-layer. This complex can be formed *in vitro* using recombinant proteins and, unlike the assembly of an ordered S-layer, is not calcium-dependent. We have also identified a domain within the low molecular weight SLP that is required for the formation of this complex. The presence on this separate interaction domain is consistent with the low-resolution structure as observed by small angle X-ray scattering.

CM 09 Antibody microarray for *Escherichia coli* O serotyping

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E. coli is the predominant facultative anaerobe of the human colonic flora. Nevertheless, many O serotypes can cause diarrhoea, urinary tract infections, meningitis, or systemic disease. Serological typing of *E. coli* O antigens is a well-established clinical method used for differentiation and identification of O serotypes. In this work, we have developed an antibody microarray chip using the IgG fraction of 170 O antisera. Type strains for each serum were used to test the microarry by sandwich immunoassay with fluorescently label antibodies. The aim of this study was to prove that microarrays with inmobilized O-antigen-specific antisera may be used to serotype *E. coli* isolates. Important *E. coli* pathogenic serotypes, such as O157, O111, O113,

O118, O91 and O146, that cause haemorrhagic colitis in humans, were readily identified by sandwich immunoassay by a clear positive and specific fluorescent signal on the array.

CM 10 Characterisation of RecQ helicases from *Bacteroides fragilis* 638R

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B. fragilis infections are treated with metronidazole, an antibiotic causing DNA single and double strand (ds) breaks. The *B. fragilis* genome does not encode the *recBCD* genes required for ds break repair, but contains the genes required for strand gap repair, *recFOR*. This RecFOR pathway, in conjunction with DNA helicases such as RecQ, can facilitate ds break repair. RecQ helicases unwind DNA in a 3'- 5' direction. Prokaryotes generally contain a single RecQ, but bioinformatic analysis of the *B. fragilis* genome revealed three putative RecQ helicases, BF3706, BF3249 and BF3892 (*B. fragilis* NCTC 9343 annotation), showing 38.9%, 43.7% and 39.6% amino acid similarity, respectively, to the *E. coli* RecQ helicase. This study aims to characterise the role of RecQ proteins in metronidazole-induced DNA damage in *B. fragilis* 638R. RecQ gene-specific insertional inactivated mutants of *B. fragilis* 638R were created, and confirmed by PCR and sequencing of the flanking regions. RecQ mutants showed increased sensitivity to metronidazole, compared to the wild type strain, confirming their involvement in the repair of metronidazole-induced DNA damage.

CM 11 Molecular techniques for identification of aerobic and anaerobic bacteria from clinical wound specimens

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Background Molecular techniques offer more accurate and rapid identification of bacteria in clinical specimens than conventional phenotypic testing. We analysed specimens by molecular techniques to establish aerobic and anaerobic bacterial flora of wound infections.

Methods Postoperative and traumatic wound infection and closed soft tissue abscess specimens were incubated for aerobic and anaerobic growth. 800bp region of the 16S rRNA gene was sequenced and analysed using GenBank for each bacterial isolate. Confidence in identification was considered if the sequence match was >99% for species and >97% for genus. Clone libraries targeting 16S rRNA gene were constructed.

Results 393 isolates (215 aerobes and178 anaerobes) were recovered from 125 specimens. Sequencing identified 96.7% to genus level, 85.5% to species level, and 3.6% unidentifiable. *Staphylococcus aureus* was the most common isolate, often in pure culture; *Streptococcus anginosus* group was second. *Bacteroides fragilis* group was the most commonly isolated anaerobe.

Conclusion Sequencing provides rapid and accurate identification but also identifies novel taxa and fastidious organisms overlooked by culture.

CM 12 Experimental infection of rabbits with *Pasteurella multocida* serogroup F

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The role of the *Pasteurella multocida* serogroup F in inducing disease in rabbits was investigated. Three groups of twelve *Pasteurella*-free rabbits were intranasally, subcutaneously, and perorally challenged, respectively, with a *P. multocida* serogroup F rabbit isolate at a dose of $6x10^4$ CFU. Six rabbits of each challenged group were immunosuppressed by dexamethasone. Eight rabbits inoculated intranasally showed symptoms of respiratory distress, resulting in respiratory failure and died or were euthanized 3–6 days post-infection. Fibrinopurulent pleuropneumonia (immunocompetent rabbits) or haemorrhagic pneumonia (immunosuppressed rabbits) was observed. Septicemic syndrome ending with shock occurred in eleven rabbits inoculated subcutaneously which died or were euthanized 2–3 days post-infection. Very extensive cutaneous and subcutaneous haemorrhages and necrosis were found. All of the perorally inoculated rabbits survived the challenge showing no signs of the disease. The observations in this study indicate that besides serogroup A and D of *P. multocida*, serogroup F can be also highly pathogenic for rabbits.

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CM 13 Frequency of Acyclovir-resistance herpes simplex viruses isolated from general immunocompetent population and AIDS patients

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Background Herpes simplex virus (HSV) infections are usually chronically recurrent in normal population and represent a significant cause of morbidity in immunocompromised patients. Acyclovir is an effective antiviral agent against replication of Herpes viruses. It widely use for the treatment and prophylaxis of HSV infections. The emergence of ACV-resistant strains has been frequently reported due to long-term acyclovir therapy. Despite widespread use of acyclovir especially in immunocompromised patients, rate of infection with acyclovir-resistant herpes simplex viruses remains undetermined. The purpose of this study was to evaluate the susceptibility of HSV isolated from normal and AIDS patients to acyclovir.

Methods HSVs were isolated from oro-facial region of normal and AIDS patients. The susceptibility of isolated HSV strains to various concentration of acyclovir was determined by plaque reduction assay. The sensitivity of virus strains are then expressed as IC50 (concentrations of drug reducing viral plaque by 50%).

Results The *in vitro* ACV susceptibilities of 133 HSV clinical isolates recovered from 102 normal and 31 AIDS patients were determined by PRA. Of 102 isolates from normal hosts 97 and 5 isolates were determined as HSV1 and HSV2 respectively. One HSV-1 isolate showed intermediate susceptibility to ACV (IC50 ≥1 to ≤1.5µg/mL), remaining 101 HSV isolates were sensitive to ACV (IC50 ≥0.1 to ≤0.5µg/mL). Twenty-three HSV1 and 8 HSV-2 were recovered from AIDS patients. One strain of HSV-1 and one strain of HSV-2 showed resistance to ACV at ≥2 to ≤3µg/mL concentrations. One HSV-2 isolate was highly resistant to ACV.

Conclusions In our experience, there is no need to routinely test HSV isolates from normal population for susceptibility to acyclovir. Determination of the susceptibilities of strains of HSV should be indicated for viruses isolated from patients who have severe immunologic disturbance and lesions that persist or worsen while they are receiving acyclovir therapy.

CM 14 Prevalence of antibody to human parvovirus B19 in pre-school age/young adult individuals in Shiraz, Iran

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Background Human parvovirus B19 infection causes an acute and self-limiting disease in healthy individuals and associated with some difficulties in anemic patients and pregnant women. Community-based surveys in our region are scarce and information on parvovirus B19 infection in populations is needed. The present study was conducted to determine the prevalence of antibody against the human parvovirus B19 among 5–25 year old individuals in Shiraz, southern Iran.

Methods Serum samples from 908 individuals (505 female and 403 male) were studied. The patients were categorized into seven age groups. The IgG anti- parvovirus B19 was determined by the commercial ELISA kit.

Results Anti-parvovirus B19 IgG was found in 70.09% of the females and 60.04% of the males in this study. The overall gender difference in seroprevalence of parvovirus B19 infection was significant (p= 0.002). In general, 65.63% of the study population had IgG against parvovirus B19.

Conclusion We found that in the majority of cases, infection with parvovirus occurs in the pre-school age period, since 64.8% of 5–7 year-old cases were positive for anti-parvovirus antibody. This result may differ from the findings of many other countries. Despite the high prevalence of parvovirus infection among female individuals, a considerable number of them are still non- infected and are susceptible to this infection in their child-bearing age.

CM 15 Epidemiologic evaluation of virulence genes, *pap/sfa/cnf-1/hly* in *Escherichia coli* strains isolated from children with urinary tract infection in Iran

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Background There is a complex interaction between different uropathogenic *E. coli* virulence factors and the host response that determines the outcome of bacterial exposure.

Materials and methods To evaluate the prevalence of four important virulence genes, *hly*, *cnf-1*, *hly* and *sfa*, in *E. coli* strains isolated from urine samples of children with UTI referred to Motahary hospital, Jahrom, Iran, during Aug 2005- Aug 2006 and their correlation with clinical data, PCR was performed for these four genes.

Results Totally 96 *E. coli* strains were isolated from urine samples of children with UTI aged from 1 month to 14 years (mean 21.8± 26.9 months). Cystitis was diagnosed in 49.2% and pyelonephritis in 50.8% of these patients. Prevalence of genes *pap*, *sfa*, *hly* and *cnf-1* among the strains was 27.1%, 14.6%, 13.5% and 22.9%, respectively. Overall 33.3% of samples were positive for at least one of the genes and 6.3% for all four genes. There was significant correlation between age of patients and presence of genes, as *pap* and *sfa* were more common in ages over 36 months but *hly* was more detected in age under 48 months (P<0.05). Pyelonephritis was more prevalent in cases with positive virulence genes. There was no significant correlation between gender and presence of genes (P>0/05). *cnf-1* gene was significantly more common in samples of the patients with abnormal kidney sonography (P=0.049).

Conclusion This study showed that the prevalence of virulence genes *hly*, *cnf-1*, *pap* and *sfa* in *E. coli* isolates was similar to the results of other studies. Because of higher prevalence of pyelonephritis in presence of these genes, rapid detection of the genes in urine samples may help in more suspicious and rapid management of pyelonephritis.

CM 16 *cnf* and *hly* virulence genes and drug resistance in uropathogenic *Escherichia coli* strains isolated from children

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Introduction and objectives *Escherichia coli* is the most common micro-organism causing urinary tract infections. Recently, some *in vitro* studies have suggested that decreased pathogenicity of *E coli* is associated with the acquisition of resistance to some of antibiotics. This study aimed to investigate two virulence factors of *cnf* and *hly* in resistant in compare with susceptible uropathogenic *E. coli*.

Materials and methods Drug sensitivity of 92 *E. coli* strains isolated from children with urinary tract infection was investigated using standard method of disc diffusion. Polymerase chain reaction was used to analyse prevalence of *cnf* and *hly* virulence genes.

Results Drug sensitivity of the isolates, was 19.8%, 75.5%, 80.4%, 84.6%, 91.3%, 91.4%, and 96.8% to ampicilin, nalidixic acid, cefixime, gentamycin, nitrofurantoin, ciprofloxacin and amikacin, respectively. Resistance to imipenem was less than 1.5%. Multiple resistances to ampicilin, gentamicin, nalidixic acid and cefixime were seen in 2.1 percent of the isolates, but no case of multiple drug resistance to all drugs was seen. Only 12.5% of the strains were susceptible to all tested antibiotics. The remaining strains were resistance to one or more antibiotics. In polymerase chain reaction analysis, 22.9%, 13.5% and 10.4% of patient had positive reaction for *cnf* and *hly* and both genes respectively. Although *cnf* and *hly* positivity was higher in the patients with pyelonephritis (23.3%and 20%, respectively) than that in the patients with cystitis (14.3% and 3.6%, respectively), this was not statistically significant. In all patient, the expression of *cnf* and *hly* were less prevalent in the most antibiotic resistant groups (0-14.3%) than in the susceptible group (13.2–29.6%), but not statistically significantly except for *cnf* and nalidixic acid (4.5% positive in resistance vs 29.6% in susceptible group (P= 0.011).

Conclusion *E. coli* isolates resistant to ampicilin, gentamicin, nitrofurantoin, amikacin, ciprofloxacin and especially nalidixic acid were associated with reductions in virulence traits.

CM 17 Comparison of arbitrarily primed PCR and plasmid profiles typing of *Pseudomonas aeruginosa* strains from burn patients and hospital environment

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Objective The aim of this study was to identify the strengths and weakness of two DNA fingerprint methods for epidemiological typing of *Pseudomonas aeruginosa*.

Methods We investigated usefulness of arbitrary primed PCR (AP-PCR) and plasmid profiles to type seventy four strains of *Pseudomonas aeruginosa* which were isolated from burn patients and hospital environment in south of Iran. The Data was processed with photo Capt *Mw* program and similarity and clustering of strains was assessed with NTSYS-PC version 2.02K software.

Results Based on 50% and 64.7% and 67.5% similarity on the plotted dendrogram, 38 plasmid profiles were classified into, two three and five clusters, respectively. Photo Capt *Mw* program categorized AP-PCR products to 47 different types of 6 to 12 bands between 0.376 to 3.7 kb. Based on dendrogram pattern three levels (62%, 81% &

84.6%) of similarity were selected. Using these criteria two, five and eleven clusters were obtained, respectively.

Conclusion As compared with plasmid profiles AP-PCR analysis differentiated the isolates with higher discrimination power. Furthermore, AP-PCR protocol is rapid and reproducible when compared with plasmid profiles analysis. These results suggest that during admission of patients in burn center a limited number of common strains cross-contaminate burn victims mostly when their wounds were scrubbed in the bathroom. However, transmissions of infection from hospital environment to patients also occur in the minority of the victims. To control cross-contamination of the patient wounds with antibiotics resistant isolates, strong disinfection of patients bathroom after scrubbing of each patient wounds is mandatory.

CM 18 Acute fungal sinusitis in neutropenic patients

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Introduction Fungal sinusitis is a well-documented disease in immunocompromised patients, but recently many reports have indicated an increased prevalence of fungal sinusitis in otherwise healthy individuals. The aim of this study was to review our experience with neutropenic patients with invasive fungal sinusitis (IFS) to determine outcomes and identity factors that may affect patient's survival.

Methods 142 patients who were undergoing chemotherapy were followed by clinical and radiological finding suggestive of fungal sinusitis. Two diagnostic criteria for invasive fungal sinusitis are proposed: sinusitis confirmed by radiological imaging and histopathological evidence of hyphal forms within sinus mucosa, sub mucosa, blood vessels or bone. Patients with fever, headache, facial swelling and radiological finding underwent endoscopic sinus surgery. The biopsy material studied by mycological and histopathological methods.

Results Eleven from 142 patients were identified IFS. Eight of 11 cases dieds.(72.8%) The ethiologic agents were *Aspergillus flavus* (5cases), *Alternaria sp.* (3cases), *Aspergillus fumigatus* (2cases) and *mucor* (1 case).

Conclusions The clinical challenge of diagnosing and treating IFS is evident by the high mortality rates for this diagnosis.

Early diagnosis with aggressive medical and surgical intervention is critical for survival.

Keywords Acute fungal sinusitis, neutropenic patients

CM 19 Prospective screening by panfungal PCR-ELISA in liver transplant recipients for management of invasive fungal infection

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Fungal infections after liver transplantation have resulted in high mortality and potentially fatal complication. The aims of this study were the epidemiology, diagnosis, time of infection before clinical manifestation and management of IFIs by molecular assay in liver transplant recipients.

Forty-eight liver recipients (cadaver donors) were transplanted at the organ transplant unit of Nemazi Hospital in Shiraz, Iran from 2004 to 2005, and were followed for fungal infections for at least 6 months. All clinical samples were cultured and direct microscopic examination was performed. Blood samples were cultured by bedside inoculation to BACTEC. Whole blood specimens were collected prospectively once per week and were analysed for invasive fungal infection by panfungal PCR- ELISA Forty-eight recipient were transplanted between September 2004 and January 2006 (22 females, 28 males, mean age 34.4 years, rang 3 to 57). Forty recipients (83.3%) had *Candida* colonization in different sites of their body pre-liver transplantation.

By PCR assay fungal infections were diagnosed in 10 recipients (20.8%). The mean time interval from transplantation to development of fungal infection was 61.4 days (20–150) and times of infection in blood before clinical signs were 7–70 days with mean of 21.4 days.

The etiologic agents were *Candida albicans* (9 cases) and *A.Fumigatus* (one case).

In conclusion, panfungal PCR and PCR- enzyme link immunosorbent assay performed prospectively once a weak enable to identification of recipients at risk for invasive fungal infections.

Keywords panfungal PCR, PCR – ELISA, liver transplant recipients, invasive fungal infection

CM 20 Preliminary studies on the genomics and proteomics of *Clostridium difficile*

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In order to investigate the microevolution of *C. difficile* over the last 40 years, we are undertaking the sequencing and annotation of the complete genomes of representative strains from 1970s to 2007 while simultaneously data mining the proteome of large numbers of strains for biomarkers using various forms of mass spectrometry. Comparative sequence analysis of a representative strain from 1979 (B, 4.4 MB) and 2006 (A, 4.1 MB) to that of the fully sequenced strain 630 genome revealed that 86.8% and 75% respectively were mapped. Differences in the number of putative ORFs encoding adhesins, proteases and toxin related proteins were identified. While a larger number of motility, surface and phage related proteins were characterised in strain B (2,247) a larger number of hypothetical proteins (3,015) were recorded in strain A. Proteomic analysis to characterise the expressed complement of characteristic chromosomal components of each genome, as well as elucidating the structural configuration on each genome, are currently in progress.

Posters

Environmental Microbiology Group

EM 01 Soil microbial interactions relevant to enteric pathogen survival in sewage sludge-amended agricultural soil

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The aim of this project is to increase understanding of the ecological processes responsible for controlling the decay of enteric micro-organisms in sludge-amended agricultural soil. Soil protozoa have a critical role in soil ecological systems controlling bacterial populations in soil through predation. Protozoa populations and soil microbial biomass carbon (MBC) were measured in a series of field experiments established on two contrasting soil types receiving application of different sludge types. The background MBC content of soil varied significantly between the two field sites and was associated with differences in the organic matter (OM) contents of the soils. In the low OM soil, sludge application markedly increased the soil MBC but had no effect on soil with high OM content. Numbers of protozoa were similar in both soil types. Unamended controls contained 3.5 $\log_{10}$ g^{-1}ds. Sludge application increased the protozoa population by 0.5 $\log_{10}$ g^{-1} ds. The survival of enteric organisms may be potentially a self-limiting process due to the stimulation of soil predator activity in amended soil.

EM 02 Inactivation of enteric pathogens in biosolids amended agricultural soils

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The inactivation of enteric pathogens in soil is an essential component of the multi-barrier approach to protect human health when biosolids are applied to agricultural land as a fertiliser. Ecological processes may have a critical role in eliminating enteric bacteria applied to soil in biosolids and provide an active mechanism of removal. To test this hypothesis and to provide long-term decay information on a variety of enteric pathogens, a series of field experiments have been established on two soils of contrasting organic matter and fertility status, amended with different sludge types. *E. coli* were indigenous to both soils and background populations were highly dynamic fluctuating between 1 and 6 $\log_{10}$ 100 g^{-1} ds. *E. coli, E. coli* O157, *Salmonella enterica* and *Listeria monocytogenes* introduced by spiking sewage sludge showed a 2–3 $\log_{10}$ 100 g^{-1} ds decrease in soil within 40–60 days of application. Results suggest that existing cropping restrictions allow the natural attenuation of enteric pathogens to take place with a significant margin of safety.

EM 03 Rapid detection of pathogens in water and body fluids

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Rapid detection of pathogens in water, food and clinical samples is required for diagnosis, hygiene and safety applications. The purpose of this project was to design and develop new, sensitive, convenient, rapid and specific tests for bacteria. The sensitivity and specificity of the bacterial detection was investigated by a novel tandem technique. An enzymatic step in which products of selected synthetic substrates are formed by a marker enzymes is followed by an immunochemical analysis step in which the product from the enzymatic step was measured by an ELISA specific for the released product. Specific sheep anti-7-hydroxy-4-methylcoumarin (prepared against immunogen made in house) was employed in the construction of the immunochemical ELISA step. The tandem assay was assessed by comparison with the fluorimetric assay alone using commercially available 7-hydroxy-4-methylcoumarin glycoside substrate. The tandem assay gave an increase in the detection limits and the practical convenience of a chromogenic end point given by the ELISA step.

Acknowledgement This work was supported by a grant from the Algerian Government to Linda Amirat.

EM 04 mRNA-based microarray analysis of methanotroph diversity and earthworm-enhanced methanotrophy in a simulated landfill cover soil

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Landfills are an important anthropogenic source of methane (CH_4) and globally account for 13% of total CH_4 emissions. Landfill cover soils are a rich environment sustaining active methanotroph populations above landfills that could potentially oxidize up to 100% of the CH_4 produced in the landfill. In our previous studies we have shown that earthworm-incubated soil has a higher CH_4 oxidation potential than non-incubated soils. This study focussed on comparing the expression of *pmoA* gene (encoding particulate methane monooxygenase enzyme) using mRNA based microarrays in earthworm-incubated and non-incubated landfill cover soils in microcosms simulating *in situ* landfill conditions. *In situ* landfill conditions were simulated in PVC columns with landfill cover soil and by injecting landfill gas (60:40 CH_4:CO_2) from the bottom of the columns. Increased CH_4 oxidation potential was observed in earthworm-incubated soil column. mRNA based *pmoA* microarrays were used to study the difference in expression of *pmoA* gene transcripts between earthworm incubated and non-incubated soils. Bacterial, methanotroph and nitrifier community structure were also characterized over different time periods and soil depths using DGGE and the *pmoA* microarray.

EM 05 Evolution of the streptomycin gene cluster

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S. griseus contains both streptomycin producing and resistance-only *str* clusters. We made a detailed study of *S. griseus* to investigate the extent to which the *str* gene cluster is linked to the species.Seven housekeeping genes from *S griseus* strains, other str-containing species and *S. coelicolor* were sequenced and then subjected to phylogenetic analysis using a concatenated tree of all seven gene sequences and for each gene. In addition Multi-Locus Sequence Typing (MLST) was done. The structure of both the individual and the concatenated trees correlated well but the eBurst diagram failed to uncover any evidence of clonality. Our data demonstrated that the *str* cluster was present in species other than *S. griseus*, indicating that it has been transferred horizontally.

Two strains containing an *str*-producing cluster and two containing a resistance-only cluster *str* cluster were used to make fosmid clone libraries and the str cluster captured on a number of fosmids. From these the strain CR20 *S. platensis* was found to contain a full complement of *str* genes, while AR23 *S. griseus str* cluster contained only the regulator (*strR*) and resistance (*strA*) gene.

EM 06 Community structures of glacial cryoconite holes

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Despite the extreme conditions which are associated with glacial environments, glacier ice has the ability to support relatively diverse microbial (prokaryotic and picoeukaryotic) communities. In particular, small water-filled holes (1cm to 2m diameter), named cryoconite holes, found on the surface of permanent ice structures, provide valuable, safe niches for multi-trophic communities to survive.

In this study molecular approaches have been used to investigate the biotic component of cryoconite holes in Arctic and Antarctic ecosystems. Sequence analysis using 16S rRNA genes amplified from DNA extracted from cryoconite samples has revealed a wide range of organisms inhabiting the holes including cyanobacteria, β-proteobacteria and actinobacteria. Analysis of genes encoding key photosynthetic functions such as *cbbL* and *rbcL*, has enabled investigation of the photoautotrophic communities. Comparison of these genes in Arctic and Antarctic cryoconite samples suggests differences between the taxonomic compositions of the phototrophic micro-organisms in these communities.

EM 07 *Citrobacter rodentium* phage: characterization and screening for phage therapy applications

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Phages are viruses that specifically infect bacteria. They play important roles in ecology, bacterial evolution and biotechnological applications such as phage therapy: the use of phage as antibiotics. *C. rodentium* represents a potential candidate for phage therapy due to the non-invasive, lumen-restricted characteristics of the infection.

C. rodentium lytic phages were isolated from the environment and characterized by host range, restriction profile, genome size, electron microscopy, structure protein profile and DNA sequencing. They constitute a heterogeneous group both in morphology and genome size which ranges from 30kb to up 290kb. All the isolated phages belong to the *Myo-* and *Podoviridae* families.

The *in vivo* studies showed that a cocktail of phage is more effective in the resolution of the infection than individual phage.

This work contributes to our understanding of the design of future therapy trials and the diversity of the phage at the genome level to maximise its potential.

EM 08 The lytic bacteriophage of *Salmonella typhi* – characterisation of the Vi capsule targetting phage, E1

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The most studied lytic bacteriophage of *Salmonella typhi* target the Vi exopolysaccharide capsule. First described in the 1930's and used for typing and occasionally for phage therapy (until the advent of antibiotics curtailed the limited clinical trials using it), the Vi typing phage have not been characterised in detail. As a first step, we have undertaken an analysis of a representative of the type II Vi phage called E1. The type II phage form the platform of the *S. typhi* typing scheme that has been used since the 1930's and has proved its worth in tracking *S. typhi* outbreaks throughout the world. The E1 type II phage was shown to have an icoshedral head and a long non-contractile tail structure and a genome of 45,362 bp. A number of regions, especially the capsid and some tail genes, were found to be highly homologous to regions of the *S. enterica* transducing phage ES18. Mass spectrometry demonstrated that a number of phage proteins identified had been modified post-translationally. The genome of the Vi E1 phage was shown to be highly related to another bacteriohage, D1, belonging to the same *S. Typhi* typing phage set.

EM 09 Planktonic ammonia-oxidizing bacteria in the NW Black Sea coast

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Knowledge about the structure and activity of ammonia-oxidizers community, an essential component of marine nitrogen cycle, is continuously increasing, new perspectives on the nature of ammonia-oxidizers communities in the sea have been obtained during the last decades. For the NW Black Sea, a dynamic region largely influenced by Danube River, few data on taxonomic diversity and ecological roles of ammonia-oxidizers community are available.

In this study, quantitative PCR and catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) with specific HRP-oligonucleotide probes were used to determine the presence and the relative abundance of the main prokaryotic taxa for Bacteria and ammonia-oxidizing bacteria (AOB) in the Danube-Black Sea mixing zone during 2004 and 2007.

Total bacterial assemblage was dominated by members of the beta-Proteobacteria. High abundance of AOB was detected in the less saline and nutrient-rich waters of the NW Black Sea. We found that beta-proteobacterial AOB formed a considerably larger component in the total microbial community and dominated over gamma-proteobacterial AOB.

Our data indicated that planktonic AOB may constitute significant players in nitrification within the NW Black Sea coastal system.

EM 10 Integrases of Stx phages and characterisation of multiple lysogeny in *Escherichia coli*

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The key virulence factor in STEC is the expression of Shiga toxin encoded in the late gene region of temperate lambdoid phages. It had been assumed that Stx phages would behave similarly to λ phage. However the use of differentially labelled isogenic recombinant Stx phage ($\Phi 24_B$) demonstrated the production of true multiple lysogens in *E. coli*. The lambdoid immunity model precludes the integration of homo-immune bacteriophage by the production of a repressor molecule, cI, which binds to phage operator regions effectively shutting down gene expression. We have identified the $\Phi 24_B$ integrase gene and the preferred site of insertion in the *E. coli* genome. The integrase encoded by $\Phi 24_B$ differs significantly from the integrases of all other previously characterised phage. Furthermore an additional 3 integration sites in the *E. coli* genome have now been elucidated. The complete genome sequence of $\Phi 24_B$ has revealed the presence of a gene homologous to the putative anti-repressor encoding genes of VT2-Sa and Lahn1 and similarity to the well-characterised P22 *ant*

gene. Reverse transcriptase PCR has shown that the gene is transcribed in a lysogenised *E. coli* host. Expression of an anti-repressor may go some way to explaining the lack of superinfection immunity in this phage. In addition, an existing bank of 11 integrase primer sets were supplemented with specific $\Phi 24_B$ integrase primers, and applied in the identification of integrase types from 113 wild-type Stx phage isolates with the aim of examining integrase diversity within these populations of phage induced from a collection of STEC isolates. Demonstration of the ability of Φ24B to form multiple lysogens, as well as the presence of different integrases within a population of Stx phages, has a potentially serious impact on the evolution of pathogens as novel Stx phages can emerge as a result of intracellular recombination events. Multiple lysogens will also produce more Stx toxin and thus have increased virulence.

EM 11 Multilocus PCR typing of Shigatoxin – encoding bacteriophages

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Shigatoxigenic *Escherichia coli* (STEC) e.g. O157:H7 cause diarrhoea with potentially fatal complications such as haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). STEC are both diverse and widespread in the environment, and are a good example of a zoonotic pathogen that has emerged relatively recently due to the acquisition of a major virulence determinant, Stx-toxin, which is encoded by lambdoid Stx-phages. In certain animal species, notably livestock, STEC can asymptomatically colonise the gastrointestinal tract and serve as a source for human infection. To date, over 500 serotypes of *E. coli* have been found to produce Stx as well as other Gram-negative bacteria including strains of *Vibrio cholerae, Citrobacter freundii, Enterobacter cloacae* and *Aeromonas spp*. Little or no attention has been paid to the typing/characterisation of Stx phages. A typing scheme would enable the generation of epidemiological information that could be interpreted alongside the large volume of data on STEC occurrence and distribution. While Stx phages are known to be heterogeneous, there is a conserved gene organisation that dictates infection of the bacterial host, integration into the chromosome and regulation / timing of propagation and release of phage into the environment. We have designed a bank of oligonucleotide PCR primers for many of these conserved genes using previously sequenced genes associated with STEC and Stx-phages. The system was applied to a collection of 70 Stx phage preparations induced from STEC strains isolated from several livestock farms. All 12 genes were amplified in each case, and the majority of preparations contained short-tailed Stx bacteriophages similar to the archetypal sequenced Stx phage, 933W. In general, we encountered less heterogeneity than expected, but we have been able to identify genes in infective phage that previously have been associated with remnant phages in the *E. coli* genome only.

EM 12 Environmental transmission of free Shiga toxin-encoding bacteriophages on a beef cattle farm

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Ruminants are established as a primary reservoir of Shiga-toxin producing *Escherichia coli* (STEC), and it is likely that most cattle carry STEC in their gastrointestinal tract at some point in their lifetime. STEC are foodborne pathogens of global significance causing potentially fatal disease; *E. coli* 0157:H7 is the most notorious pathogenic strain. The characteristic virulence determinant of STEC is the production of Shiga toxin (Stx), encoded by a gene (*stx*) that is carried by bacteriophages (Stx-phage). These are lambdoid phages sharing a distinct genome organisation of core functional genes and a complex genetic switch that enables them to enter one of two different life cycles in a manner defined by the archetypal phage, λ. Although there are data on the distribution of *E. coli* Stx-phage lysogens in the natural environment, the occurrence and distribution of Stx-phage as free particles has received little attention. The aim of this study is to assess the epidemiological significance of free Stx-phages in farming environment, both their role as transmission agents and as survival capsules for the *stx* genes. The viruses recovered directly from the farm environment will be further characterised by a multi-loci typing scheme to determine the relative predominance of phage types.

EM 13 Primary degradation of crystalline cellulose by a *Micromonospora* community in freshwater lakes

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Cellulose is a major carbon source only made available through the action of cellulolytic microorganisms, but the ecology of its degradation in lakes has been little studied. Micromonosporas are a ubiquitous group of actinomycetes, primarily isolated from soil but potentially well adapted for growth in freshwater. Micromonosporas were isolated from cotton colonised at different depths and in the sediments of two freshwater lakes. Evidence was produced for the presence of growing hyphae in the lakes, and for the ability of isolates to grow in lakewater and degrade crystalline cellulose *in situ*. The DNA gyrase genes (*gyrB*) of 67 isolates were amplified by PCR and grouped into RFLP types, a large number of representatives of which were cloned and sequenced. While some novel clusters of strains were identified, the best cellulose-degraders grouped with *M. chalcea* and it is concluded that this species at least is likely to have an indigenous role in the recycling of cellulose in aquatic environments.

EM 14 Dissemination of Stx genes by bacteriophages that recognise an essential outer membrane protein in *Escherichia coli*

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Shigatoxigenic *Escherichia coli* (STEC) e.g. O157:H7 are a global health concern and infection results in acute diarrhoea and can lead to severe downstream disease, including hemorrhagic colitis (HC), haemolytic ureamic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). The major virulence determinant is Stx toxin, which is disseminated by lambdoid bacteriophages (Stx-phages) that convert recipient bacteria into toxigenic lysogens. Most Stx phages are short-tailed viruses of the podoviridae, and we have identified an outer membrane protein, YaeT, that is responsible for phage adsorption to the host bacterial cell. YaeT is an essential protein that is conserved throughout Gram negative bacteria, and it is intimately involved in the biogenesis of the bacterial outer membrane. Through the exploitation of differences in YaeT sequence amongst members of the *Enterobacteriacae* and variances in tail spike proteins from wild-type Stx-phages , we have been able to identify those regions of YaeT that are involved in phage binding. This was made possible by the development of phage adsorption assays in combination with specific antibodies to purified recombinant YaeT. Consequently, we can hypothesise that of the 6 extracellular loops of YaeT exposed on the

bacterial surface, extracellular loops II, III and V are sufficient to support the adsorption of short tailed Stx phages, the first step in the infection process. This degree of conservation in the phage and bacterial elements responsible for adsorption may explain the widespread occurence of *stx* amongst different *E. coli* serotypes and their relatives promoting the rapid phage-mediated evolution of bacterial genomes that can result in increased virulence.

EM 15 Quantification of herbicide degradation gene expression in *Burkholderia* sp

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Phenoxyalkanoic acids are widely used as broad-leaf herbicides in agriculture. Considerable amounts of these ecotoxic herbicides remain in the soil, where they are slowly degraded by micro-organisms. The degradation pathway for phenoxyalkanoic acids has been well characterised; the genes usually lie in transposons harbouring all the degradative genes in addition to genes with regulatory and uptake functions. In *Beta-* and *Gammaproteobacteria* the *tfdA* gene codes for the first degradation step. Recently, the existence of stereospecific enzymes (*rdpA* and *sdpA*) facilitating the cleavage of either the *R-* or *S*-enantiomer of chiral herbicides has been reported. The *tfdK* gene product is involved in the uptake of the herbicide.

The aim of this work is to quantify the expression of herbicide degradative and uptake genes in *Burkholderia* sp.

Burkholderia sp. EX1156, isolated from soil, is able to degrade several herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxy propionic acid (mecoprop). The strain was grown on minimal salts medium supplemented with 20 mM glucose and 20 µg/ml mercury chloride to the early log phase, then challenged with 2 mM 2,4-D, racemic or *R*-mecoprop. Samples were taken at several time points up to 72 h and frozen for RNA extraction, the herbicide degradation was monitored by chloride ion measurement and cfu counts were determined. Gene expression was monitored by RT-QPCR.

EM 16 Presence and diversity of viruses in granitic groundwater from 69 to 450 m depth

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The presence and morphology of bacteriophages in deep granitic groundwater from Äspö hard rock laboratory (HRL) tunnel was investigated and the number of phages on the endemic species *Desulfovibrio aespoeensis* was analysed. Ten boreholes ranging from 69 to 450 meters depth in the tunnel were included. The total numbers of viral like particles (VLP) and microbial cells (TNC) were counted with fluorescence microscopy on 0.02 µm filters, after staining with SYBR Gold. A large diversity of present virus was confirmed with transmission electron microscopy (TEM). The number of phages infecting *D. aespoeensis* was estimated with an anaerobic most probable number technique by infecting bacterial batch cultures under mid logarithmic growth. The number of VLP ranged from 7.3 x 10^4 to 1670 x 10^4 ml^{-1} and the TNC ranged from 1.6 x 10^4 to 92 x 10^4 ml^{-1}. The ratios of VPL/TNC were between 1.1 and 19.1. Four boreholes had phages in numbers between 0.2 to 80 phages ml^{-1} that infected *D. aespoeensis*. It is concluded that a significant diversity of viruses are present, active and widely distributed in the deep groundwater of Äspö HRL, indicative of active predator-pray ecosystems.

EM 17 Formate dehydrogenase diversity in termite hindgut microbial communities suggests the adaptation of homoacetogenic spirochetes to a changing selenium environment

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CO_2-reductive acetogenesis is a bacterial activity having a great impact on the nutritional mutualism that occurs between wood-feeding termites and their hindgut microbiota. The termite gut homoacetogenic spirochete, *Treponema primitia*, is only distantly related to other homoacetogenic bacteria, leading to studies on the nature and origins of the Wood-Ljungdahl pathway in spirochetes. In a companion poster, we present results showing that *T. primitia* regulates cysteine and selenocysteine variants of the gene for formate dehydrogenase, a key acetogenesis enzyme. These genes were employed in designing degenerate PCR primers that amplify a diversity of *fdhF*-type formate dehydrogenase genes from both pure cultures and hindgut communities of termites. Phylogenetic analyses revealed ca. 50 novel and diverse *fdhF* variants, with similar frequency of recovery for selenium and cysteine variants in termites representing 3 of 6 major lineages. These results suggest that homoacetogenic spirochetes have adapted to meet the challenge of changing selenium availability in termite hindgut microbial communities.

EM 18 Selenium controls transcription of *sec* and *cys* variants of formate dehydrogenase in a homoacetogenic termite gut spirochete

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CO_2-reductive acetogenesis is of great importance to the nutritional mutualism occurring between wood-feeding termites and their hindgut microbiota. An acetogenic symbiont was previously isolated from a termite gut and characterized as a novel spirochete species, *Treponema primitia*. Here, we describe *T. primitia* genes for CO_2-reductive acetogenesis. A 52 kb region of the *T. primitia* genome was sequenced, revealing 43 putative open reading frames that included methyl-branch genes encoding tetrahydrofolate-dependent functions of the acetogenesis pathway and two genes for formate dehydrogenase. The formate dehydrogenases cluster phylogenetically with hydrogenase-linked (*fdhF*-type) homologs found in enteric *Gammaproteobacteria* and differ from each other in that one encodes cysteine at amino acid 145 and the other encodes selenocysteine at the corresponding position. Real-time reverse transcriptase PCR revealed that both genes are regulated in response to selenium availability. This finding provides the first evidence that selenium dynamics in the guts of termites might influence the evolution and function of their symbiotic CO_2-reductive acetogens.

EM 19 Role of dsRNAs and microbe (pathogen) associated molecules with the mysterious mushroom virus X (MVX) patch disease

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The emergence of an unusual *Agaricus bisporus* mushroom 'patch disease' first reported in 1996, later termed as 'mushroom virus X' (MVX), exhibited a wide range of symptoms (e.g. barren patches beside healthy looking mushrooms, arrested pins, premature opening, brown, off-colour and distortions in shape). A variable compendium of novel

26 (dsRNA) elements, ranging in sizes between 20.2Kb to 0.64kb, several of them (~17/26) in non-encapsidated form have been shown to occur in the diseased mushroom fruiting bodies and are thought to comprise multiple viruses. Ten years on, this devastating disease is now more widespread and prevalent in a number of European countries (e.g. The Netherlands, Ireland) ranging from occasional to severe outbreaks leading to crop losses. We have recently identified a browning factor lipodepsipeptide (tolaasin), among other peptides produced by *Pseudomonas* tolaasii and other *P syringae* strains co-colonising on the skin of mushrooms. To date, the results reviewed (Rao *et al.*, *Curr Iss Mol Biol* 9, 103–122, 2007) suggest that with the exception of 4 low molecular weight dsRNA bands (sizes 2.0. 1.8.0.8 an 0.6 kb) found synchronous to mushroom off-colour/browning symptoms, other individual MVX dsRNAs or their banding patterns clearly lack credible relationship with other symptoms of the MVX disease complex. In this study, we present our findings on the role of dsRNAs and the stress induced by translational inhibitors and microbe (pathogen) associated molecules (MAMP) found in the vicinal environs of host mushroom cells and the host's 'ribotoxic stress responses' during the developmental stages of the mushroom growth and the molecular interactions of 'immune signalling pathways' out with dsRNAs in virus infected mushrooms.

EM 20 Activation of post-transcriptional RNA mechanisms after perceiving *Pseudomonas* pathogen associated molecular patterns in browning mushrooms infected with virus X patch disease

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Plants and animals activate defences after perceiving pathogen-associated molecular patterns (PAMPs). Recent reports (e.g. *Science*, 2006 312, 436–439) showed that plant microRNAs (miRNAs) down regulates messenger RNAs in *Arabidopsis* for auxin receptors contributing to antibacterial resistance in response to *Pseudomonas syringae* flagellin-derived protein and suggested that such mechanisms may apply for other stress induced miRNAs in other eukaryotic cells. In our study, we have been examining an infectious Mushroom Virus X (MVX) patch disease on cultivated mushrooms (*Agaricus bisporus*) The diseased mushrooms carried at least 26 characteristic (MVX) dsRNA bands; four low molecular weight dsRNAs (2.0–0.6 kb) are diagnostic MVX bands associated with mushroom browning. The aetiology of these unusual dsRNAs is yet unknown. We have recently identified a) the occurrence of a unique dsRNA carrying *Pseudomonas* bacteriophage (phi 6) in the skin brown/discoloured infected mushrooms; the phage phi-6 dsRNAs exhibited high homology to the low molecular weight dsRNAs found in MVX infected mushrooms and b) a browning factor lipodepsipeptide (tolaasin), among other peptides produced by *Pseudomonas tolaasii* and other *P syringae* strains co-colonising on the skin of mushrooms. In this study, we present our findings on the posttranscriptional RNAs produced in response to the stress induced by the pathogenic *Pseudomonas* spp and their peptides synthesised in developmental stages of the mushroom growth and their molecular interactions with dsRNAs in diseased mushrooms.

EM 21 Environmental transcriptome analysis of the iron oxidizing bacterium *Leptospirillum ferrooxidans*

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We used total amplified environmental RNA (meta-transcriptome) from extremely acidic and metal rich waters (20g l^{-1} of iron) for global gene expression by DNA microarrays of the acidophile, strict iron oxidizing bacterium *L. ferrooxidans* (1). Here, we study the differential gene expression between two natural life styles, the plankton-like versus the sessile life style in a biofilm. The bacterial diversity in both places was determined by fluorescence in situ hybridization (FISH) and by oligonucleotide microarrays hybridization using the same total amplified RNA as for gene expression studies. The use of total environmental RNA for biodiversity assessment by oligonucleotide microarrays allows checking specific probes for 16S and 23S rRNA as well as any other RNA or functional genes. Our transcriptome analysis allowed us to select spots of the *L. ferrooxidans* microarray showing significant induction ratio under both the plankton-like and the biofilm life styles. Some of the identified genes are related to biofilm formation or maintenance.

Reference Parro *et al.* (2007). *Environ Microbiol* **9**, 453.

EM 22 *Penicillium* strains as dominant degraders for cellulose/pectin-rich agrowastes

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It is known that cellulose/pectin-rich agrowastes are mainly assimilated by insects and herbivores and the real contributors to the degradation are their rumen microflorae. They are however strictly anaerobic, and hence are not major cellulose/pectin degraders in aerobic environments. Therefore, aerobic degraders for cellulose/pectin-rich agrowastes were screened, isolated, and characterized. Environmental samples were collected and cultured in a medium containing satsuma peel or coffee residue as a carbon source. Cultures showing a strong degrading activity were streaked on agar plates to isolate degraders. Two satsuma peel-degraders and six coffee residue-degraders were obtained. Pure cultures of satsuma peel-degraders showed both cellulolytic and pectinolytic activity, while pure cultures of coffee residue-degraders showed cellulolytic, pectinolytic, and mannolytic activity. It was therefore implied that polysaccharides in agrowastes are not degraded independently by different microbes, but degraded simultaneously by strains with "multi-polysaccharolytic" activity. DNA analysis suggested that most of the degraders belong to the genus *Penicillium*. Although it is generally thought that wood-rotting fungi contribute largely to aerobic degradation of lignocellulosic biomass, our data suggested that *Penicillium* spp. overwhelm wood-rotting fungi in degradation of cellulose/pectin-rich agrowastes.

EM 23 Enhanced biosorption of mercury by cold-induced hydrophobic exobiopolymer of *Pseudomonas fluorescens* bm07

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Pseudomonas fluorescens BM07 was found to secrete exobiopolymer, composed of polypeptide(s) (~50% hydrophobic amino acids), constituting spherical shell-type envelopes when grown on limited M1 medium with fructose at low temperatures (0–10°C) but little secretion at high temperature (30°C). Biosorption of mercury (Hg^{2+}) and cadmium (Cd^{2+}) ions by non-living BM07 cells (10 and 30°C) were investigated. Biomass of 10°C cell exhibited higher adsorption of Hg^{2+} than 30°C. Equilibrium adsorption data for Hg^{2+} were nicely fitted into Langmuir adsorption model and maximum adsorptions were 194.3 and 111.3 mg Hg^{2+} and 34.1 and 21.4 mg Cd^{2+} per g dry biomass for 10 and 30°C cells, respectively, showing a strong interaction between Hg^{2+}

and exobiopolymer, also demonstrated by FTIR spectroscopy and Energy dispersive X-ray analysis. In conclusion, BM07 strain can be considered to adsorb Hg^{2+} efficiently, especially in temperate region as it secretes Hg^{2+} adsorbing exobiopolymer in low temperature.

EM 24 Molecular studies on the diversity of marine *Prochlorococcus* strains and their viruses

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The aim of this project is to study the diversity of Prochlorococcus and its co-occurring viruses in the Atlantic Ocean and to use multivariate statistical analyses of the obtained data to elucidate the impact of the viruses on their host. The project therefore involved the development of appropriate molecular methods followed by their application to environmental samples collected during an Atlantic Meridonial Transect cruise (AMT-15). To elucidate the degree of microdiversity within the genus Prochlorococcus novel Prochlorococcus-specific PCR primers were developed for the RNA polymerase core subunit gene rpoC1. The size of the PCR fragment (925-bp nucleotides) coupled with high sequence variation within the rpoC1 fragments (70–99% sequence similarity, 16S rRNA sequences show >97% sequence similarity) meant that it was possible to distinguish Prochlorococcus strains by restriction fragment length polymorphism (RFLP) analysis. Clone libraries were constructed from environmental DNA samples from the Atlantic Ocean. RFLP and subsequent phylogenetic analysis of the nucleotide sequences of the Prochlorococcus RFLP-types, showed that the Atlantic seawater samples analysed contained representatives of each of the currently recognised Prochlorococcus clades (based on the ITS region as molecular marker). Analysis also showed that the Prochlorococcus populations from different latitudes were dominated by genetically different clones. Overall, these analyses led to the discovery of a previously unseen genetic microdiversity. Moreover, the high genetic resolution approach also revealed that there are two putative novel lineages within the Prochlorococcus HL I clade.

Myovirus specific primers have previously been developed for the capsid protein gene g20 which amplifies both Prochlorococcus and Synechococcus myoviruses. Analysis of Atlantic Ocean samples with the g20 primer set revealed that the majority of clones from gyre sites represent previously unseen diversity. Recent nucleotide sequence data has become available for photosynthesis genes found in Prochlorococcus and their viruses. These sequence data were the basis for the development of Prochlorococcus-podovirus specific PCR primers to analyse their genetic diversity. Attempts to isolate Prochlorococcus-infecting viruses have so far resulted in a podovirus infecting Prochlorococcus strain MIT9312.

EM 25 Sequence analysis of genes encoding cellulase and xylanase from environmental libraries of soil DNA

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To isolated novel functional enzymes, metagenomic libraries were constructed from soil sample (Upo wetland, Mujechi bog, and Daebudo sandbar in Korea) by directed DNA extraction and purification. This gene banks and shotgun libraries were screened for clones expressing cellulolytic activity by using a Congo red overlay method. One clone which had appeared to form clear zone on a medium containing carboxymethyl cellulose as carbon source was isolated. This clone, pCM2, was sequenced using a shotgun approach. Sequence analysis of the shotgun clone of pUCM2-1 showed two open reading frames (*xynM2* and *celM2*) predicted to encode 227 and 663 amino acid proteins, respectively. The amino acid sequence of *xynM2* showed 66% identity with endo-1,4-β-xylanase A from *Geobacilus stearothermophilus*. The amino acid sequence of *celM2* showed 36% identity with endoglucanase from *Synechococus* sp.

EM 26 The characteristics of porphyrin photosensitizers and eukaryotic cell interaction

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Recently photosensitizer (in particular porphyrins) applications grow. For microscopic fungi, these substances are an alternative for expensive and rather toxic antibiotics.

The aim of the work is to characterize the *Candida albicans* interaction with 5,10,15,20-(N-methyl-pyridyl)porphyrin (TPP), 5-hexadecyl-10,15,20-N-methyl-pyridylporphyrin (HPP) and zinc complexes – Zn-TPP and Zn-HPP. The photo- and dark activities were detected used *C. albicans* growth with 0.01–10.0 μM derivatives during 24 and 48 hrs after light exposure or its absence. The cell characteristics were detected by flow cytometry. The annexin V-FITC (AV-FITC) and propidium iodide were used as fluorochromes.

After 24-hour incubation, the most active was Zn-TPP caused 80% growth decreasing. The other derivatives made 40–60% *C. albicans* suppression. The extension for 48 hrs did not induce the action changes of substances. In the light absence during 24 and 48 hrs, the culture growth correlated with the control one. These features were evidence of nontoxic compound properties.

After light exposure, the derivatives, except for TPP, reliably increased apoptotic cell compared to the control and 6–10-fold – compared to the dark probes.

Thus, all studied derivatives are characterized by high photosensitizing level with respect to *Candida albicans*. There is no activity in the light absence. The death form of yeasts developed because of the compound corresponds to the apoptosis. The process intensity depended on the conditions, in particular light exposure.

EM 27 *Not being presented*

EM 28 *Not being presented*

EM 29 *Not being presented*

Posters

Eukaryotic Microbiology Group

EukM 01 Identification and quantification of *Candida* species by using real-time PCR

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Candida are generally classified as opportunistic pathogens, they can cause infection of the skin, the mucous membranes of the mouth or vagina and are also involved in human allergic disorders. The occurrence of yeasts in soil has been studied in various parts of the world, as well as the occurrence of yeasts in food. QPCR or real-time PCR is an improvement of the established PCR technique which increase specificity, sensitivity, and shorter detection times for both identification and quantification of *Candida* species. The aim of this study is to identify and quantify *Candida* from soil and food by using real-time PCR. A total of 32 reference strains of *Candida*, 19 yeasts isolated from soil, and 23 yeasts isolated from food were tested by SYBR Green and fluorescent probe method. Melting temperature (Tm) values of *Candida* were at 78–79°C and showed sharp peak, whereas *Saccharomyces* have Tm values at 75°C but no peak. *Pichia angusta*, a teleomorph of *Candida*, also showed the same Tm peak as *Candida*. Twenty-seven of the unknown yeasts showed Tm peak at 78–79°C. Real-time PCR using the WANFOR and WANREV1 primer pair, targeting amplification of the 18S rDNA, allowed for the differentiation of *Candida* and *Saccharomyces*, based on the Tm peak of the resultant products whilst the sensitivity of real-time PCR was 500 femtogram of DNA.

EukM 02 Endosymbionts of the chicken red mite (*Dermanyssus gallinae*)

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The poultry red mite is considered as an important pest of poultry. The importance of the poultry red mite as a direct parasite is well documented. Its role as a vector may also be important but it is not well understood. Nevertheless, it has been associated with several pathogens such as *Salmonella* spp., *Mycobacterium* spp., Newcastle disease, and Equine Encephalitis just to mention a few. Furthermore, the presence of endosymbionts inside the poultry red mite has yet to be proven. Endosymbiont micro-organisms play an important role on the ecology, evolution, and survival of their arthropods hosts. Endosymbionts have been found distributed widely inside arthropods. The *Bacteroidetes* group, which contains the relatively recently described bacterium *Cardinium* spp., have been found responsible for causing reproductive anomalies such as cytoplasmic incompatibility in some arthropod species and has not been previously detected in Dermanyssoid Acari. We have successfully amplified partially the 16S ribosomal gene similar to those of *Cardinium* from DNA extracted from poultry red mites. The presence of bacteria from the group *Bacteroidetes* inside the poultry red mite is likely to have an influence on its biology, ecology and evolution. It could also open a new perspective of control for this ectoparasite by targeting their endosymbiont population.

EukM 03 Rapid and accurate diagnosis of clinically and veterinary relevant fungi

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Fungi are a major agricultural, veterinary and medicinal problem across the world; Candid species are opportunistic infecting immunocompromised patients, ie those with AIDS, undergoing chemotherapy or transplant procedures. Aspergillus species affect human, animals and crops. Presently, lengthy culture methods are used clinically for microbe identification. Rapid diagnosis using molecular sequence recognition methods can be used to enable timely prescription of effective antimicrobial chemotherapy. Molecular methods while faster and more accurate are yet to have made an impact within the clinical setting, due to problems of specificity, complexity of process and insufficient reduction in time of screening and analysis. For molecular detection to work it must be specific, rapid and simple to use, in order to overcome the dominance of the present techniques.

Presented here is a rapid, nanolitre sampling multiple probe approach developed for fungi Candida and Aspergillus species determination. The 18S ribosomal RNA small subunit sequence is present in high copy numbers per cell, allowing identification without fragment amplification. The variation within the molecule is limited therefore a bioinformatic analysis has been used to develop a multiple probe system to take advantage of conserved and variable regions. Samples are extracted rapidly by microwave, then passed through a micro channel device where by the sample is interrogated by multiple probes pattern on a surface, initially a reverse line blot membrane but now reduced to a microscope slide. The result is a method that provides rapid and accurate diagnosis of fungal pathogens.

EukM 04 The flexible cell wall proteome of *Candida albicans*

Carol Munro[1], **Kerstin Nather**[1], Steve Bates[2], Piet de Groot[3], Frank Klis[3] & Neil Gow[1]

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The surface of *Candida albicans* is rich in highly glycosylated mannoproteins that make intimate contact with the host and play an important role in adherence, antigenicity, and the modulation of the host immune response. The fungal cell wall is a dynamic structure responding to the environment and perturbations in cell wall homeostasis. Analysis of the genome wide response of *C. albicans* to glycosylation defects and cell wall perturbing agents by transcript profiling has highlighted alterations in cell wall protein (CWP) expression. Mutants with defects in *N*-glycan outer branch elaboration (*och1*Δ and *pmr1*Δ had transcriptomes indicative of activation of the unfolded protein response and cell wall salvage pathways. The genes activated in *och1*Δ were enriched in the class of CWPs tethered to the wall via a GPI-anchor remnant. Proteomic analysis showed that novel GPI-CWPs were present on the *och1*Δ cell surface. Together these findings suggest that *C. albicans* can alter its cell wall proteome in

response to different conditions. The regulation and function of cell wall stress responsive-CWPs is under investigation.

EukM 05 Caspofungin treatment activates chitin synthesis via a cell wall salvage response in *Candida albicans*

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Echinocandins are a new class of antifungal agent which target the fungal cell wall by inhibiting β(1,3) glucan synthesis. Caspofungin is the first echinocandin to be approved for clinical use. We have shown that treatment with caspofungin results in a compensatory increase in *CHS* gene expression, chitin synthase activity and cell wall chitin content through activation of the PKC, Ca^{2+}/calcineurin and HOG signalling pathways. Pre-treating wild-type cells with $CaCl_2$ and Calcofluor white to increase chitin content, lead to reduced susceptibility to caspofungin. Pre-treatment stimulated production of salvage chitin in *chs* deletion mutants and resulted in reduced susceptibility to caspofungin. All four chitin synthase genes were involved in the synthesis of salvage chitin. In *C. albicans* septum formation is dependent on two chitin synthase genes, Chs3 and Chs1 which is an essential gene. In the absence of both Chs1 and Chs3 pre-treatment stimulated production of a novel salvage septum and restored viability. These results indicate a possible alternative mechanism of resistance to caspofungin.

EukM 06 *In vitro* hemolytic activity of *Candida* species

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Candida species secrete various hydrolytic enzymes which are involved in infection pathogenesis. There are few studies on the hemolytic activities of the *Candida* species. In this study, investigation of the hemolytic activities of the *Candida* species was aimed. A total of 70 *Candida* strains were investigated for in vitro hemolytic activity in Sabouraud Dextrose Agar medium containing sheep blood, supplied with 3% glucose. While 53 *C. albicans* and 2 *C. guilliermondii* strains exhibited alpha-beta hemolysis, and 4 *C. albicans* strains revealed alpha hemolysis, no hemolytic activity was observed in 6 *C. albicans* , 3 *C. kefyr*, and 2 *C.tropicalis* strains. As a result, it was observed that Candida species exhibited variable hemolytic activities.

Posters

Food & Beverages Group

FdBev 01 The use of DHPLC (wave) system) to detect *Lactobacillus plantarum*

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The aim of this investigation was to determine if DHPLC could be used to detect a 500bp 16s rDNA fragment of *Lactobacillus plantarum*, and if this procedure could be extended to look for the presence of other organisms. Conditions considered in the preparation of the DNA fragment were type of extraction method, primer dilution, and annealing temperature during PCR (polymerase chain reaction). DHPLC conditions optimised were oven temperature, buffer composition and flow rate.

Different extraction methods gave differing DHPLC results. Optimum PCR conditions were found to be a 1/10 primer dilution, and an annealing temperature of 63.5°C. For the WAVE system, the optimum flow rate was found to be 0.35ml min^{-1}, at 61.2.°C and optimised buffer range was 54.6–72% Buffer B.

Each of the variables studied affected the results, suggesting that to be used as a detection method for mixtures of organisms conditions should be optimised for each organism tested.

FdBev 02 Development of a novel quantitative reverse-transcriptase real-time PCR for total viable counts on fresh meat

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The total viable count (TVC) is used to determine the microbiological quality of foods. A TVC can be performed by a 3 day cultural method or by an alternative rapid method if shown to be equivalent (ISO 16140:2003). The aim of this research was to develop a reverse transcriptase real-time PCR assay for the determination of TVCs on fresh meat ensuring only viable organisms are detected. A novel target gene was identified and two sets of primers were designed. One of these assays targets all Gram positive bacteria and the other all Gram negative bacteria. Both methods have been shown to be sensitive and selective for each group. Studies using typical meat micro-flora have shown a high correlation ($r^2 = 0.98$) between the quantitative results obtained using the developed PCR methods and a traditional plate count method.

FdBev 03 A molecular study of *Listeria* species isolated from food of animal origin in Ireland

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Listeria monocytogenes is an opportunistic food-borne pathogen that causes listeriosis in humans. In Ireland between 2000 and 2003 there was an average of 6.5 cases of human listeriosis per annum. A collection of four hundred samples is currently being compiled at the Central Veterinary Research Laboratory (CVRL) at the Department of Agriculture and Food (DAF) from industry and private laboratories. Preliminary analysis of this collection indicated that forty percent of the strains possibly were *Listeria monocytogenes* and the CVRL is currently conducting a survey of food processing environments at ready to eat (RTE) food suppliers regulated by DAF. Rapid identification of *Listeria* spp. is been carried out through species-specific markers on conventional polymerase chain reaction (PCR). Work is ongoing on the Real-time PCR to develop a multiplex assay that can discriminate individual strains of *Listeria* along with an in-house positive control at Waterford Institute of Technology. As *L. monocytogenes* and listeriosis has grave implications for the consumer, food manufacturer and the food industry, rapid but accurate molecular techniques hold the key to preventing the entry of this hazardous pathogen into the food chain.

FdBev 04 Rapid detection and identification of *Salmonella enterica* serovar Choleraesuis and Paratyphi C isolates by mPCR

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A multiplex PCR (mPCR) assay has been developed for the rapid identification of two globally pathological serovars of *Salmonella enterica*, Choleraesuis and Paratyphi C. Recent emergence of Multidrug Resistant (MDR) strains of both serovars highlights the need for rapid detection of these pathogens. This is vital so that treatments and prophylactic interventions can be put in place. A comparative genomic approach was employed to generate serovar-specific primer sets. The optimized mPCR was screened against a large collection of strains to validate the specificity of the assay. Sensitivity was investigated using food matrices spiked with several contaminating food-borne pathogens. We are currently converting this assay for Real-Time mPCR rapid detection of *Salmonella* serovars, eliminating the current lengthy procedures for *Salmonella* confirmation and serotyping.

FdBev 05 Antibiotic resistance and PFGE Profiles of *Salmonella* isolated from pork in Northern Ireland

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Pig herds have been identified as one of the main reservoirs of *Salmonella* in the food chain. Throughout Europe *Salmonella* type *S. typhimurium* DT 104 has been noted as the most commonly isolated *Salmonella* serotype. This is cause for concern as *S. typhimurium* DT 104 has been documented for its resistance to five major antibiotics: ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT). For the reasons stated it is important to monitor *Salmonella* prevalence within pork products and the potential risk of antibiotic resistance. In this study pork samples were collected from boning halls in two different pig abattoirs based in Northern Ireland over a six-month period. From these pork samples 38 *Salmonella* were isolated. The antimicrobial resistance profiles of all *Salmonella* isolates in this study were determined by disk diffusion tests. A panel of 12 antibiotics were utilized throughout the profiling procedure. Pulsed

Field Gel Electrophoresis (PFGE), which is regarded as the "Gold Standard" for the typing and strain identification of *Salmonella* isolates, was used to determine the genetic diversity of the *Salmonella* isolates using the restriction enzyme *Xba*I. The *Xba*I restriction fragments were separated by PFGE in a Chef DR II system enabling the comparison of restriction profiles of the *Salmonella* isolated in this study.

FdBev 06 Evaluation of the extraction methods for aflatoxin M1 (AFM1) in yoghurt for HPLC technique

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The mould, *Aspergillus parasiticus* produce 4 toxic metabolites, AFB_1, AFB_2, AFG_1 and AFG_2. AFB_1 which may be present in animal feedstuff appears as aflatoxin M_1 (AFM_1), a genotoxic carcinogen, in milk and milk products. This paper presents an evaluation of AFM_1 extraction methods from yoghurt prior to immunoaffinity column procedure. Three AFM_1 extraction methods were employed and low fat natural yoghurt was used for the study. In the first method, yoghurt was contaminated with 0ng/ml, 5ng/ml, 10ng/ml and 12ng/ml and AFM_1 was purified using methanol:water (60:40 v/v). In the second method, concentrations of AFM_1 were 0ng/ml, 5ng/ml and 10ng/ml. AFM_1 was extracted using dichloromethane and diatomacious earth following washing with *n*-hexane. Concentrations of AFM_1 used were 0ng/ml, 5ng/ml, 10ng/ml and 20ng/ml in the third method. Methanol:water (80:20 v/v) was mixed with AFM_1 contaminated yoghurt and centrifuged to obtain the supernatant. The extractions were analysed using High Performance Liquid Chromatography (HPLC). Recovery rates obtained by the first method ranged approximately between 28% and 38% while with the second method recoveries were between 10% and 26%. Recoveries using the third method were between 75% and 93%, and therefore, the third method is recommended for extraction of AFM_1 from yoghurt.

FdBev 07 New laboratory tools for investigating reservoirs and routes of transmission of human infection with *Cryptosporidium*

Rachel Chalmers[1], Stephen Hadfield[1], Kristin Elwin[1] & Paul Hunter[2]

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Cryptosporidium transmission routes cannot be fully elucidated using routine diagnostics and traditionally relied on epidemiological data. Supplementation by genotyping has identified anthroponotic transmission of *C. hominis* and zoonotic transmission of *C. parvum*. By subtyping isolates, the distribution of variants indicates more specific hosts and routes that may influence local or global transmission.

Genotypes were identified by PCR-RFLP of COWP and ssu rRNA genes in sporadic and outbreak cases, followed by multilocus fragment size analysis at three microsatellite loci (ML1, ML2 and GP60) and sequence analysis of the GP60 gene. Over 90% of *C. hominis* isolates were indistinguishable (fragment type H1 and GP60 subtype IbA10G2) but minor types were significantly linked with foreign travel outside Europe. *C. parvum* was more diverse and variants linked to risk factors, separating zoonotic and anthroponotic transmission. SSCP of the GP60 gene showed good accordance, and development of rapid methods or targeting of predictive loci will reduce resources and broaden testing capacity.

FdBev 08 Detection and quantification of *Aspergillus westerdijkiae* in coffee beans based on selective amplification of β-tubulin gene by using Real-Time PCR

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Aspergillus westerdijkiae is a new species of fungus that was recently dismembered from *Aspergillus ochraceus* taxon. Most isolates of *A. westerdijkiae* are able to produce large amounts of a mycotoxin named ochratoxin A (OA), which has been found in coffee. *A. westerdijkiae* is very similar to *A. ochraceus*, and several isolates previously identified as *A. ochraceus* are now identified as *A. westerdijkiae*. In fact, most (84%) were identified as *A. westerdijkiae*. Since this species consistently produces large amounts of OA, we developed a specific primer-pair for detecting and quantifying it in coffee beans by using Real-Time PCR. The primers Bt2Aw-F 5'TGATACCTTGGCGCTTGTGACG and Bt2Aw-R 5'CGGAAGCCTAAAAAATGAAGAG provided an amplicon in all *A. westerdijkiae* isolates, and no cross-reaction was observed using DNA from *A. ochraceus*. The sensitivity of real-time PCR was more than 100 times higher than the cfu technique.

FdBev 09 Genetic relationships among strains of the *Aspergillus niger* aggregate collected from dried fruit samples of worldwide origin

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The presence of ochratoxin A in dried fruits is possible due to contamination by strains of the *Aspergillus niger* species aggregate. We analysed by RAPD and β-tubulin sequences the genetic relationships among 51 isolates from the *A. niger* aggregate collected from dried fruit samples e.g. black sultana, dates, figs and plums in different countries. *A. niger sensu stricto*, *A. foetidus* and *A. tubingensis* were found, but *A. niger sensu stricto* was the most prevalent species that was found in all fruit substrates of all geographical origins. *A. tubingensis* isolates, which were the second most frequent, were subdivided into two subgroups IA and IB. RAPD profiles, three single nucleotide polymorphisms in the region β-tub2 of the β-tubulin gene and other three in the region β-tub1 clearly discriminated the two groups of this species. Although it is still premature to assign a new taxonomic rank to these groups, our result may explain the incongruence in the literature about the capability of *A. tubingensis* to produce ochratoxin A.

FdBev 10 Comparison of two molecular methods rep-PCR for identification and typing of *Bacillus cereus*

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We have monitored sporeforming bacteria *B. cereus* in raw milk, pasteurized milk and final products (cheese, yoghurt and UHT milk) during milk processing. For differentiations of isolated *B. cereus* strains two molecular based fingerprint methods were used: rep-PCR with $(GTG)_5$ and BOXA1R primers. *B. cereus* CCM 2010^T and *B. licheniformis* CCM 2145^T were used as reference strains. Band-pattern and cluster analysis were carried out using GelCompar II software. From our results it is interesting that *Bacillus* strains are

clustered according to their origin. The strains orginated from UHT processed milk introduce a very homogenous group (except strain M20). All samples from yoghurt making procedure were clustered into one (BOX fingerprint) and two groups ($(GTG)_5$). Two strains, M2 and M3, from cheese production did not form homogenous group. These strains were dissimilar because they were collected from quite different places. These molecular methods are useful for determination of bacteria origin. However, by our results is *rep*-PCR and primer $(GTG)_5$ seem to be more suitable for our purpose and for differentiation of isolated *B. cereus* strains.

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FdBev 11 A study demonstrating the use of MALDI-TOF mass spectrometry for rapid identification and comparison of probiotic bacteria

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Background: Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. One of the most recognised Genera of probiotic organisms are *Lactobacillus spp*. This study explores whether MALDI-TOF mass spectrometry is able to rapidly identify and compare these bacteria.

Method: MALDI-TOF mass spectrometric analysis of intact bacterial cells for two proprietary strains of *Lactobacillus casei* and 20 clinical isolates of Lactobacilli were performed on the 2nd and 3rd sub-culture of the organisms under two different culture conditions. These spectral fingerprints were identified in minutes against a database of 4851 entries containing three *Lactobacillus* strains.

The two proprietary strains were analysed on three further occasions and the 4 datasets used to examine the reproducibility of the spectral fingerprints and to ascertain any differences. A control was also used to check the performance of the instrumentation and protocols.

Results: Both proprietary strains were identified as *Lactobacillus casei* on each of the 16 occasions tested, with the exception of one result for subculture 3. Eighteen of the 20 clinical isolates were also successful identified as *Lactobacillus* sp. 17 as *Lactobacillus casei*, for the CBA culture from sub-culture 3. Results for sub-culture 2 (CBA culture) and sub-cultures 2 and 3 (CHOC culture) were less conclusive, with only 8, 6 and 6 of the 20 isolates correctly identified respectively, suggesting the isolates had not fully recovered from storage and transportation. The control identified to strain level for all 16 test datasets.

Comparison of the numerous fingerprint patterns for the two proprietary strains suggested that these are indistinguishable under these culture conditions.

Conclusion: The results demonstrate that MALDI-TOF mass spectrometry can be used for the identification of *Lactobacillus casei* although comparison of two proprietary *Lactobacillus casei* strains cultured on CBA and CHOC agar suggest they are indistinguishable. Further work on exploring environmental factors may demonstrate spectral differences between these strains.

Posters

Hot topic

HT 01 Use of ^{35}S-methionine-labelling and proteomics to identify proteins synthesized during different phases of a bioreactor run for enhanced biological phosphorus removal

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Our research aims to understand further the biochemistry of enhanced biological phosphorus removal (EBPR). In EBPR systems, activated sludge cycles through anaerobic and aerobic phases, resulting in microbial intracellular accumulation of phosphorus, and its removal from effluent wastewater. Metabolic details of biotransformations that occur during these alternating phases are not well understood. We used ^{35}S-methionine to label proteins that were newly synthesised in either the anaerobic or aerobic phases by a sludge operating for high performance EBPR. Two-dimensional gel electrophoresis, phosphor-imaging of radiolabelled proteins and mass spectrometry enabled identification of proteins expressed differentially in the phases. Most highly expressed proteins matched closest to those from '*Candidatus* Accumulibacter phosphatis', an uncultured polyphosphate accumulating organism associated with EBPR. Differentially expressed proteins included TCA and glyoxylate cycle enzymes. Proteins expressed highly in both phases included glycolytic enzymes, stress-response proteins and enzymes for polyhydroxyalkanoate synthesis. Results will be discussed in relation to current EBPR models.

HT 02 Effects of veterinary antibiotics on soil microbial community structure and activity

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One of the recommended practices in organic agriculture is the spreading of animal manure to add nutrients to the soil. However, manure often comes from intensive farming where antibiotics are used for disease control and enhanced growth. Antibiotics are excreted with the manure because they are only partially assimilated by the animals. When manure is contaminated with antibiotics and applied to the land, these substances may affect soil microdiversity. This study determined the effects of sulfonamides on the soil microbial communities after a 200-day exposure, using radiorespirometry with ^{14}C-sufamethazine, culture methods, molecular analysis by Terminal Restriction Fragment Length Polymorphism (TRFLP), and the generation of a clone library to refine phylogenetic identification. Results indicated that most of the micro-organisms found in the exposed soil belong to the α, β, γ and δ subclasses of Proteobacteria. Biodegradation of sulfonamides by a sulfonamide-resistant consortium was also identified. Contamination of soil with sulfonamides had an effect on the biomass, diversity and function of the microbial communities.

HT 03 Systems-level and molecular analysis of the Winogradsky column

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Modern microbial ecology employs a host of advanced molecular techniques to study microbial ecosystems such as biofilms and bioreactors. These are open systems driven by significant chemical input fluxes, but the biosphere as a whole is a closed system driven by solar radiation. Here we describe analysis of the closest laboratory analogue of such a closed system, the Winogradsky column. Powerful modern techniques such as fingerprinting by denaturing gradient gel electrophoresis and species determination by 16S rRNA sequencing have been applied to this system for the first time to yield a description of its changing species composition on a timescale of weeks to months. We have also applied computer simulations to analysis of the inter-species interactions within such a column, and describe how this modelling approach facilitates greater understanding of the species-level variations in microbial composition. Together with systems-level experimental observations on developing columns, these approaches allow the reproducible spatio-temporal evolution of a closed, light-driven microbial ecosystem to be understood at multiple levels. The results obtained have relevance to the experimental testing of theories of ecological goal functions and system stability at the ecosystem and biosphere scale.

HT 04 Accessing marine microbial diversity changes in an increasingly acidic environment

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The current average pH of seawater is around 8.2, if atmospheric levels of CO_2 continue to increase then it is expected to decrease to pH 7.9 within a century. As part of the NERC funded consortium (investigating the metagenome of marine microbial communities) the Bergen mesocosm was set up to investigate how ocean acidification will affect marine microbial communities. Techniques such as tRFLP, SIP (stable isotope probing) and sequencing of clone libraries (DNA and cDNA) have been used to investigate community structure and diversity throughout the Bergen experiment. The clone library and tRFLP data demonstrated that the *Proteobacteria* are the most dominant members of the community. Furthermore, the communities do appear to respond to the different environmental conditions. Notably, the acid bag community becomes dominated by one or two key organisms whereas the background bag appears to maintain a more heterogeneous community throughout. By combining the tRFLP data with the clone library sequences it was possible to allocate taxic itentification to specific peaks. As expected *Alpha-* and *Gammaproteobacteria* dominated the species identified. However, although taxic richness was fairly constant at higher phylogenetic levels throughout the study population dynamic changes were fairly dramatic.

HT 05 Functional genomic approaches to elucidate novel transcriptional regulators with a role in *Pseudomonas*–plant interactions

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There is increasing pressure to reduce the use of chemical inputs in agriculture due to growing concerns for environmental protection and human health. One alternative is the use of micro-organisms such as *Pseudomonas* that can stimulate plant growth and are antagonistic to

plant pathogens. However, complex interactions in the rhizosphere can limit development of such alternative strategies. Molecular signalling between microbes and their eukaryotic hosts can influence both beneficial and pathogenic interactions in the rhizosphere, however, little is known about the influence of plant-derived signals on bacterial gene expression and function. To address this, a transcriptome profiling study was conducted to investigate the effects of plant root exudates on global gene expression in *Pseudomonas aeruginosa* PA01. This identified subsets of genes that were similarly and differentially regulated in response to the exudates.

One set of differentially regulated genes that were identified encode for probable transcriptional regulators. These genes have been analysed for roles in rhizosphere colonisation and a number of associated phenotypes. We have identified a gene which influences the degree of host preference during competitive colonisation. Phenotypic and proteomic analysis have indicated that this gene plays a role in modulating a response to oxidative stress in *Pseudomonas aeruginosa*.

HT 06 Analysis of multiple genes of human and environmental isolates

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16S rDNA sequence analysis has facilitated microbial community analysis across a diverse range of habitats. Consequently, it is now possible to begin tracing the transition of otherwise quiescent species into the human ecosystem and ascertain the factors the mediate progression. To gain insight into this complex process, we have utilised this method to circumscribe over 3,600 species that span both human and non-human sites. As the range of organisms analysed continued to expand, it was evident that many clusters of species could not be delineated solely by this method and additional genes (eg *rpo*B, *gyr*B, *rec*A etc) were sequentially investigated. Many taxa identified such as *Bacillus, Coryneacterium, Tsukamurella, Rhodococcus, Exiguobacterium* species etc are typically environmental but were found in blood or tissue cultures. Many taxa such as *Burkholderia, Legionella* etc are now well documented but it is still uncertain what selection pressures operate to retain or release a particular taxon from its normal habitat. We envisage that as more analyses are undertaken, common processes will begin to emerge that can be investigated further through post-genomic analysis.

HT 07 Comparative genomics to identify genetic variability associated with *Salmonella* epidemicity

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Salmonella are common zoonotic organisms. The deleterious effects of *salmonellosis* to public health and the socioeconomic costs are significant. Factors that help certain *Salmonella* to be dominant are yet to be discovered. In this study we hypothesise that there are differences at the genetic level between frequent or "epidemic" and sporadic or "non-epidemic" groups. A total of 24 epidemic and non-epidemic strains of *S.* Enteritidis from six phage types PT4, PT6, PT8, PT 9, PT11 and PT13a, were selected to investigate *Salmonella* genome variations using Comparative Genomic Hybridisation (CGH) DNA microarrays. The microarray contains over 6,000 genes representing the genomes of 7 strains from serovars *S.* Typhi, Typhimurium, Gallinarum, Enteritidis and *S. bongori*. Analysis of CGH microarray data revealed variations in nearly 400 genes of which only 40 were hypothesised to be related to epidemicity. These included *Salmonella* plasmid genes *pef* genes, a *sdi*A-regulated gene and a group of ABC transporter genes. Investigation into variations between phage types illustrated close relationships between PT4 and PT6, as well as PT8 and PT13a. Interestingly, the non-epidemic phage type PT9 displayed its gene content to be highly similar to epidemic phage types PT4 and PT6. The genetic variations and diversities found in strains included in our study have revealed information about strains associated with epidemics and what may be unique to these strains. The identification of novel epidemic markers will assist in the development of new approaches enabling early intervention strategies.

HT 08 Combing of DNA-based stable isotope probing, whole genome amplification and metagenomic analysis of uncultivated methanotrophs in peatlands

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Northern peatlands represent one of the largest sources of atmospheric methane. Peatlands harbour diverse groups of acidophilic or acid-tolerant methanotrophs, including *Methylocella*, *Methylocapsa*, and peat-associated *Methylocystis* which have not been cultivated so far. In order to analyse metabolically active methanotrophs in peatlands, DNA-stable isotope probing (SIP) experiments were carried out using $^{13}CH_4$ with a heather dominant peatland soil. Only minimum labeling was achieved in order to minimize potential cross-feeding. The ^{13}C-labelled DNA was isolated and purified from the SIP gradient and used as template for whole genome amplification (WGA) to generate sufficient DNA for the construction of a fosmid library. A serial dilution of template DNA was used for WGA and the potential bias was analysed by DGGE fingerprinting of 16S rRNA gene fragments using primers targeting bacterial 16S rRNA genes. The metagenomic library was screened for methane monooxygenase and 16S rRNA of methanotrophs by the polymerase chain reaction. After screening 1000 clones, a 20kb clone containing 16S rRNA gene from uncultivated *Methylocystis* has been identified.

HT 09 The microbial ecology of a phenol polluted aquifer

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The sandstone aquifer at Four Ashes (Wolverhampton) is contaminated with high concentrations of organic pollutants. This study investigates how microbial diversity and function of planktonic and attached communities differ in polluted and unpolluted regions of the aquifer. Multilevel boreholes allowed sampling of the planktonic and attached communities across the contaminant plume. Community diversity was profiled using molecular techniques such as DGGE and cloning. These revealed that community composition differed markedly between planktonic and attached forms and was strongly influenced by the concentration of pollutants. Some bacteria isolated from the borehole could use phenol as a carbon source and formed biofilms.

HT 10 Temporal dynamics of microbial colonisation of forage in the rumen

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Microbial colonisation is central to ruminal degradation of dietary material, yet little is known about the temporal dynamics of this process. The aim of this study was to characterise the initial dynamics of microbial colonisation of forage in the rumen ecosystem, using molecular analysis of rumen *in sacco* incubated fresh perennial ryegrass (PRG). Colonised bacteria and anaerobic fungi (*Neocallimastigales*) were profiled (DGGE and ARISA) and quantified (QPCR) over a 30 min rumen incubation period. A diverse and consistent bacterial and *Neocallimastigales* population rapidly colonised the PRG, although the fungal population was less diverse. Bacterial and *Neocallimastigales* population sizes were both significantly affected by time (P <0.001 and P = 0.01 respectively), however the dynamics of the microbial population sizes differed. Bacteria rapidly colonised within 5 min, stabilising after 15 min. *Neocallimastigales* had a 5 min lag and continued to increase over the incubation period. This suggests that fundamental differences in the biology and/or attachment mechanisms between these taxa may enable initial colonisation to occur non-competitively.

HT 11 Molecular ecology of anaerobic cellulose-degrading microbial communities in landfill sites

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Cellulose is the most abundant biodegradable polymer in landfill sites, and therefore, its hydrolysis under anaerobic conditions provides the primary carbon source for the indigenous microbial population. However, the true diversity and abundance of cellulose-degrading micro-organisms in such environments is unknown. Members of the genus *Clostridium* are thought to be the principal cellulose degraders in landfill, although there are other known cellulolytic bacteria which have not been detected. Here, genus-specific 16S rRNA gene PCR primers were designed for *Fibrobacter* spp., a group of important cellulolytic gut bacteria which have never been detected outside the gut ecosystem. Sequencing of PCR products from community DNA extracted from landfill leachate demonstrated the presence of novel lineages related to the genus *Fibrobacter* in a number of landfill sites. We are applying RT-QPCR to provide quantitative data on the relative abundances of cellulose degrading taxa and SIP of landfill leachate samples using C^{13}-labelled cellulose to implicate species and ascertain their relative importance to the process.

HT 12 Identification of contaminating bacteria in Cachaça ferments of an artisanal still from the sequence of the 16S rDNA gene

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Our objective was to study the bacterial contamination in ferments used in the production of Cachaça, a typical Brazilian spirit, in an artisanal still. It was collected three samples during a month and other one year previous (control sample). It was obtained from the sequence of the 16S rDNA bacterial gene, 587 sequences. DOTUR program revealed 170 operational taxonomic units: 43 in the control sample, 38 in the first, 57 in the second and 38 in the third sample. Many bacterias not described in ferments of Cachaça were founded, such as *Weissella cibaria*, *Leuconostoc citreum* and some *Lactobacillus* species, moreover bacterias uncultured. The results of statistical analysis demonstrated significant differences among them, providing a large dynamic during the fermentative process. The results revealed that the communit participating in the fermentative process and influencing in the beverage quality is more complex that it knew. This is the first study using this techinique to determine contaminate bacterias in the production of Cachaça.

HT 13 Monitoring on antimicrobial resistance from livestock farming without antibiotic usage

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Introduction We monitored about the antimicrobial resistant bacteria from livestock environment without antimicrobial usage.

Methods 786 strains of *E. coli, E. faecalis* and *E. faecium* were isolated from fecal and feed samples of cattle, chicken and pig that without feeding and treatment of any antimicrobials at the livestock farm, and tested for the tendency of antimicrobial resistance.

Results The results showed that, in the case of cattle, 18.9% of *E. coli* from fecal samples were resistant to tetracycline, 23.6% of them were resistant to cephalothin, 60% of *E. aeacalis* and 75% of *E. faecium* were resistant to tetracycline. In the case of chicken, 89.2% of *E. coli* was resistant to tetracycline and 67.6% of them were resistant to ampicillin, 81.5% of *E. faecalis* and 66.7% of *E. faecium* were resistant to tetracycline. In the case of pork, 80.1% of *E. coli* was resistant to tetracycline and 25.0% of them were resistant to ampicillin, 44.8% of *E. aeacalis* and 60.7% of *E. faeacium* were resistant to tetracycline. 92 strains of *E. faecium* that were isolated from fecal and feed samples were carried PFGE for identification of genetic correlation, and the genetic correlation were identified.

Conclusions The rate of antimicrobial resistance of *E .coli* isolated from livestock without antimicrobial usage were low in cattle, pig and chicken and it was also relatively low for the case of *E. faecalis* and *E. faecium* when it compared to livestock using antimicrobials.

HT 14 The prevalence of CRISPR-associated (*cas*) genes in archaeal and bacterial genomes

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Repeat regions of bacterial genomic DNA known as Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) regions are generally found in close proximity to a number of CRISPR-associated (*cas*) genes. These *cas* genes were originally believed to comprise a novel, thermophile-specific DNA repair system; however, they are not exclusive to archaeal species or eubacterial extremophiles, including thermophiles, but are in fact widely distributed throughout sequenced archaeal and eubacterial genomes. Recently, it has been hypothesised that these genes comprise a bacterial RNAi system which plays a functional role in defense against invading phage and plasmids. This contention is both exciting and controversial, although the importance of these genes remains unclear at a functional level.

The broad aim of the current research, i.e. investigation of gene ontology and synteny of some 25 RAMP/*cas* genes in sequenced microbial genomes, has allowed us to gain insight into the evolutionary relationships between *cas* genes and clusters. Analysis of 66 microbial genomes has allowed construction of *cas* gene clusters from a range of thermophiles, mesophiles and microbes of significance in human health, such as *C. difficile* and *M. tuberculosis*. Genomic context analysis of *cas* gene clusters provides some hints as to their evolutionary and functional importance however the next stage of work will deploy functional genomics as a route to determine *cas* gene function in selected bacteria.

Posters

Microbial Infection Group

MI 01 High-pressure resistance of *Listeria monocytogenes*

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Listeria monocytogenes is a Gram positive intracellular food-borne pathogen that is ubiquitous in the environment. Listerial infection can cause illnesses such as spontaneous abortion in pregnant women. High pressure (HP) processing is a non-thermal process that can inactivate micro-organisms thereby producing high quality foods that display characteristics of fresh products with the advantage of an extended shelf-life.

The aim of this study was to determine the molecular basis of how *Listeria monocytogenes* copes with HP. Pressure-resistant listerial genes were screened for subjecting a library of listerial genes in an *E. coli* background to pre-determined sub-lethal high pressures. Genes involved in motility and cell division were identified as being HP resistant, with an increase in survival of 1–2 logs compared to the wild-type observed. Further analysis is ongoing to determine if the motility and cell division genes confer resistance to other stresses.

These results demonstrate a role for motility and cell division genes in increasing cell survival post HP and may reveal a molecular basis for listerial survival under pressure.

MI 02 The role of Zur in regulating environmental stress resistance and virulence potential in Listeria monocytogenes

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Background *Listeria monocytogenes* is a Gram positive pathogen that is ubiquitous in the environment. The facultative anaerobic rod causes the serious infection, listeriosis and can have lethal effects for susceptible individuals including pregnant women, neonates and those suffering from immunosuppressive disorders. Listerial infection occurs via the oral route and once within the gastrointestinal tract the pathogen encounters a variety of sub-optimal conditions. In particular the pathogen must sequester metal ions from the environment as they act as vital biocatalysts in cellular processes.

Methods and results The zinc uptake regulator (Zur) is predicted to coordinate uptake of zinc from the external environment. An in-frame deletion of the Zur gene was created using the SOEing PCR procedure. We demonstrate that the mutant is compromised under a number of stress conditions encountered during infection including exposure to hydrogen peroxide. Using a bioinformatic approach we identified a number of Zur-regulated genes in the genome of *Listeria monocytogenes*. RT-PCR was used to determine that these loci are deregulated in the Zur mutant background indicating regulation by Zur. A number of these genes have been mutated using the pORI19 mutagenesis strategy and the physiology of these mutants has been investigated in our laboratory.

Conclusions We suggest that Zur plays a role in the ability of *Listeria monocytogenes* to cope with environmental stresses encountered during environmental growth and infection.

MI 03 Iron acquisition, an essential feature of *Listeria monocytogenes* infection

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Background *Listeria monocytogenes* is a Gram positive intracellular pathogen responsible for the disease listeriosis. In order to cause infection the pathogen must sequester iron within the host. The availability of iron is extremely restricted in mammalian hosts, so the ability to attain iron *in vivo* proves essential for systemic *L. monocytogenes* infection. In many pathogens this process is co-ordinated by the regulatory protein Fur (ferric uptake regulator).

Methods and results Utilizing a bioinformatics approach, we predicted several Fur-regulated genes, one of which is *lmo0365*. This locus was confirmed to be Fur-regulated by reverse transcription PCR. *lmo0365* was mutated by insertional plasmid mutagenesis (pORI19) and also by gene deletion. Both mutants were compromised in *in vitro* growth in defined media under iron-limitation conditions. Furthermore, the lmo0365 mutants were significantly impaired in virulence in the murine model of infection.

Conclusion lmo0365 is a Fur-regulated gene in *L. monocytogenes* that is essential for growth under iron-limiting conditions and during infection.

MI 04 The role of *tolA* in *Salmonella* biology and virulence

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The Gram-negative Tol-Pal system of envelope proteins plays a key role in outer membrane integrity and contributes to the virulence of several pathogens. We are investigating the role of one of these proteins, TolA, in the biology of *Salmonella enterica* Serovar Typhimurium. Following intravenous infection of mice an isogenic *tolA* mutant is severely attenuated compared to wild type SL1344 as measured by bacterial loads in the liver and spleen. *tolA* is therefore important for full virulence in *Salmonella typhimurium*. We are currently characterising the basis for this attenuation.

Prior infection with the *tolA* mutant conferred protection against subsequent challenge with the fully virulent parent strain. The *tolA* mutant may therefore be useful in the development of novel live attenuated vaccine strains.

MI 05 Characterisation of ATPase in *Salmonella enterica* serovar Typhimurium

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Salmonella enterica serovar Typhimurium is a facultative intracellular pathogen used in the mouse to study human typhoid fever.

The F_0F_1 ATPase is an important enzyme complex, located in the bacterial membrane which, under aerobic conditions produces ATP and generates a proton motive force. Production of ATP and the proton motive force in the bacterial cell is a fundamental event in providing

energy to the cell and in the proper functioning of numerous cellular functions.

We have generated isogenic ATPase mutants lacking either the F_0 or F_1 components or the entire enzyme complex in the SL1344 background of *Salmonella typhimurium*. These mutants are being examined in our murine infection model to assess their virulence and potential as live attenuated vaccine strains. Data indicates that the *Salmonella typhimurium* ATPase is a key virulence factor and that mutants in this enzyme complex have potential utility as vaccine strains.

MI 06 Role of *trxA* in inflammation and pathology induced by *Salmonella enterica* serovar Typhimurium in mice

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The important pathogen *Salmonella enterica* serovar Typhimurium expresses a thioredoxin protein, encoded by *trxA*, which contributes to virulence and survival against oxidative stress. Here, we have examined the contribution of *trxA* to *Salmonella*-induced inflammation and pathology using our murine infection model. Histopathological examination showed that, upon injection into mice, mutants in which *trxA* had been deleted resulted in reductions in both splenomegaly and hepatic lesions in comparison to the well-characterised attenuated strain SL3261, and that this was independent of the bacterial loads in the organs. We conclude that the TrxA protein contributes to the ability of *Salmonella typhimurium* to induce pathology in the liver and spleen and we are characterising this phenomenon and its mechanism. This work has implications for understanding the basis of *Salmonella* pathogenesis and in the development of live attenuated vaccine strains that have reduced pathology.

MI 07 Novel methods for analysis and visualisation of Transposon-Mediated Differential Hybridisation (TMDH) data

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Transposon-mediated differential hybridisation (TMDH) is a "tag-array" method of screening libraries of mutants to identify genes essential for survival or virulence. Mutants are generated using a custom transposon with outward-facing promoters, from which labelled RNA run-offs are produced. These are hybridised to a microarray to determine the location of the insert. We have developed the "Eagle viewer", an online tool that allows visualisation of TMDH microarray data and enables unambiguous identification of the sites of transpon insertion. Automated approaches to identify and score the positions of insertions have been developed and applied to *in vitro* and *in vivo* TMDH data from *Salmonella enterica* and *Staphylococcus aureus*.

MI 08 Microbial ecology of the intensive care unit

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Hospital-acquired infections (HAIs), such as those due to methicillin resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*, have attracted public concern and much coverage in the news media. Although all hospitalised patients are at risk of HAIs, those in intensive care unit (ICUs) are often immunocompromised and highly susceptible. Antibiotic resistance is a particular threat to ICU patients, who are regularly treated with antimicrobials to control sepsis, which is a frequent and costly problem. Here, we are studying the microbial ecology of the ICU environment and analysing environmental samples for the presence of antibiotic resistance genes. In parallel, clinical isolates from ICU patients have been screened using PCR for extended-spectrum beta-lactamase (ESBL) genes, $bla_{TEM,SHV,CTX-M}$. To date all bacterial isolates from ICU patients have been negative for ESBLs, although ESBLs were isolated from patients elsewhere in the hospital. Since ICU patients have not become infected with ESBL strains, despite their presence elsewhere in the hospital, it is clear that the infection control measures in the ICU are largely effective. By analysis of the microbial population of the ICU environment we propose to assess the risk from environmental organisms and evaluate whether review of the infection control measures could minimise the threat they pose.

MI 09 Fibrinogenolytic activity of *Bacteroides fragilis*

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Bacteroides fragilis accounts for 4–13% of the human gastrointestinal microbiota, however, it is also an important opportunistic pathogen. Human fibrinogen is a 340kDa glycoprotein involved in blood clotting. The major aims of this study are to compare the fibrinogenolytic potential of different *B. fragilis* clinical isolates and to identify the proteases responsible. Degradation of human fibrinogen *in vitro* commences in late exponential / early stationary phase. There are differences in degradation amongst isolates, with either degradation of alpha, beta and gamma chains or only the alpha and beta chains. During late exponential growth phase, fibrinogen degradation can be related to specific proteases using zymography. Mass spectrometry and inhibition studies are being used to further characterize these proteases. A putative fibrinogen binding protein has been purified by affinity chromatography. In conclusion, fibrinogen degradation may be an important virulence factor in *B. fragilis*, allowing the bacteria to slow down, or prevent clot formation, resulting in dissemination of infection.

MI 10 Towards a recombinant *Salmonella* subunit vaccine

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Salmonella is an important human and animal pathogen. Symptoms of infection range from gastroenteritis to systemic disease or death, depending on host status and the particular host:serovar combination. Infection mainly occurs via consumption of contaminated food or water. Current live vaccines offer a degree of protection but are inappropriate for use with immunocompromised individuals. Development of an efficacious subunit vaccine would circumvent this but requires knowledge of which antigens are immunodominant and, of suitable adjuvants for immune priming. In previous work in this lab, serum from hyperimmunised mice was used to identify immunodominant antigens seen during natural infection of mice with *Salmonella*. In the present study we compare the effectiveness of different adjuvant/antigen combinations for their ability to elicit an

appropriate immune response. The results indicate that the immune system can be primed to elicit an appropriate immune bias. However, effectiveness differs with adjuvant composition. The study also shows that subunit composition influences the degree of side effects observed post-challenge and the capacity to prime protective immunity.

MI 11 Effect of temperature on virulence properties of *Campylobacter* species

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Campylobacter are major international zoonotic pathogens. Their control requires a better understanding of the behaviour of the bacteria in relevant environments. Poultry are the main food animal reservoirs and the most important sources of infection for humans. Campylobacter are almost always non pathogenic in poultry whose body temperature is 42°C and pathogenic in humans who have a body temperature of 37°C. To determine if switching from commensal to pathogen is related to temperature, we examined growth, motility and invasion of T84 cells by campylobacters grown at the above temperatures. To examine species-specific differences, we used *C. jejuni, C. coli* and *C. fetus fetus*. Our results suggest that while *C. jejuni* grows similarly at both temperatures, some strains are more motile at 42°C and some more invasive at 37°C. This may account for rapid spread in poultry flocks and for infection in humans respectively. *C. coli*, which are infrequent causes of *Campylobacter* infections in humans, are less able to grow and invade at 37°C when compared to 42°C. *C. fetus fetus*, which is infrequently found in poultry, is less able to grow and invade at 42°C.

MI 12 The application of Pulsed Electric Fields to eliminate *Escherichia coli* from collagen based biomatrices

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Collagen based biomatrices have potential applications in tissue engineering and regenerative medicine. The use of Pulsed Electric Field (PEF) treatment as a sterilisation method of these matrices was examined because current sterilisation methods can damage the collagen structure. PEF, a non-thermal inactivation method, has been previously developed for use in the food industry.

The effectiveness of PEF treatment for the inactivation of *E. coli* seeded in collagen gel was investigated. Parameters such as electric field strength, microbial density and pulse number were examined. Results demonstrate that microbial susceptibility increased with greater electric field strengths. Investigations using different seeding densities found that at higher concentrations ($>10^4$ c.f.u./ml), the *E. coli* population was not completely inactivated. Results also demonstrate that PEF treatment has no gross effect on the structure and functionality of collagen. Further development of PEF treatment may offer an alternative biomatrix sterilisation method.

MI 13 Intracellular triggering of inflammation by the Gram-negative bacterium *Pseudomonas aeruginosa*

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Microbes can trigger inflammation by interaction with intracellular sensors such as the caspase-1 inflammasome and NOD family proteins, independent of interaction with Toll-like receptors. We set out to study how *Pseudomonas aeruginosa* activates these pathways, and how the host regulates this process. By a variety of methods, we have shown that infection of cells with *P. aeruginosa* does not result in pores allowing direct entry of extracellular markers or calcium. However, using a luciferase reporter gene to follow NF-κB activation, we have found that *P. aeruginosa* can activate NOD1 by a mechanism dependent on a functional bacterial type III secretion system. This suggests that peptidoglycan is introduced directly from the bacteria into the host cell through the type III secretion pore. *P. aeruginosa* can also directly activate caspase-1 following infection, although this was independent of type III secretion. Given the lack of detectable open pores following infection, the mechanism of this effect is unknown, since extracellular stimulation with ligands such as LPS alone is without effect on caspase-1 activation. Peptidoglycan delivery into the host cytoplasm by a type III secretion system is a novel means by which bacteria can trigger inflammation. Other mechanisms must also exist to deliver bacterial products into the cell.

Bacteria have a wide range of virulence factors, one of the most important being the type III secretion system (T3SS), exclusive to Gram negative bacteria. Through this system the bacteria can inject virulence proteins into the host cell, via a pore in the plasma membrane. Repair of plasma membrane damage is vital for survival of the cell and limiting infection. In *Salmonella* infection repair of the membrane has been shown to be mediated via a calcium and Synaptotagmin VII dependent lysosomal exocytosis. This has not yet been shown for an extracellular bacteria, which has been the aim for this study using *Pseudomonas aeruginosa*. Before repair of the pore ions and possibly other proteins might exit and enter the cell. To study this we have investigated whether NOD1 is activated during *P. aeruginosa* infection by studying NF-κB activation and signalling events downstream of this such as caspase-1 activation and IL-1β secretion. This has previously been shown for *Helicobacter pylori* and their type IV secretion system. These results provide further insight into the effects of an extracellular infection using a T3SS and also the mechanisms the host cell use to combat the infection.

MI 14 The diagnostic potential of miniaturised DNA microarrays

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The DNA microarray chip offers a new way for biologists to understand the complexities of a living organism. In our laboratory we have developed miniaturized microarray chips suitable for high throughput use for detection of virulence and antimicrobial resistance (AMR) genes. The virulence gene chip contains 63 oligonucleotide probes representing 7 different *Escherichia coli* pathotypes, whilst the AMR gene chip contains 54 probes representing 45 different AMR groups present in enteric bacteria. The virulence pathotype of 63 *E. coli* clinical isolates of human and animal source was established using the virulence chip. Strains of EHEC and EPEC pathotypes showed similar virulence characteristics with presence of the *stx* toxin genes being the main differential determinant. In contrast, isolates characterised as UPEC, ETEC, EIEC or EAEC tended to show distinct virulence profiles. Others harboured novel combinations of virulence determinants, the significance of which remains to be determined. For the AMR chip presence of resistance genes in a panel of 50 *E. coli* and 43 *Salmonella* clinical isolates of human and animal origin was tested. The mean number of resistant genes present in *E. coli* isolates was found to be 8 and in *Salmonella*, 5. The most common gene detected in both *E. coli* and *Salmonella* isolates was bla_{TEM}, which was present

in 90 and 56% of the isolates tested, respectively. The results demonstrate that these arrays provide an effective, economic and simple method for detection of genes in clinical isolates, which will help to understand the epidemiology of pathogens, and to detect gene linkage.

Muriel Mafura & Miranda Batchelor have contributed equally to this work.

MI 15 A comprehensive approach for the characterisation of the outer membrane proteome of *Salmonella typhimurium*

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Enteropathogenic bacteria of the genus *Salmonella* are a major cause of diarrhoea worldwide. Adhesion, host cell invasion and secretion of virulence-associated determinants are all functionalities embedded in the cell envelope. Therefore, understanding the structure, function and expression dynamics of the surface proteome of *Salmonella* are essential for the development of novel therapeutics or diagnostic targets. However, membrane proteins are notoriously difficult to characterise due to their association with the lipid bilayer and highly hydrophobic nature. Here we present four methods based on surface biotinylation or various solubilisation strategies for the extraction of the membrane proteome of *S. typhimurium*. Extracted proteins identified using gel electrophoresis and LC/MS/MS were assessed against a consensus of sub-cellular localisation prediction tools for all annotated ORFs in the *Salmonella* genome. Combining the extraction procedures described here allowed for the characterisation of large numbers of membrane proteins including host integration factors, membrane associated enzymes, and outer membrane proteins.

MI 16 Phase variation of glycosyltransferase genes in *Salmonella* Typhimurium

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Since the 1940s we have known that some *Salmonella* serovars undergo phase variation (previously referred to as form variation) of their O-antigens. This has been observed in both uninfected bacteria and lysogens of seroconverting bacteriophages such as *S.* Typhimurium phage P22. It is now known that the genes responsible for O-antigen modification are the *gtrABC* glycosyltransferases, however until now the mechanism of phase variation was unknown. The promoter sequence can often reveal clues for the mechanism; the phage P22 *gtr* promoter contains two putative OxyR binding sites overlapping four GATC sequences – the target sequence for Dam methylase. This is similar to the promoter of the putative *E.coli* virulence factor *Ag43*, which is known to phase vary in a Dam and OxyR dependant manner. This research examines the roles of Dam and OxyR in phase variation of *gtr* genes in *S.* Typhimurium LT2.

MI 17 Preliminary characterisation of a peptide antibiotic active against *Staphylococcus aureus*

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Bacteriocin-like inhibitory substances (BLISs) that are produced by various species of *S. aureus*, could have potential as topical therapeutic agents for treating highly drug-resistant staphylococcal infections. The spectrum of activity of some such agents, recently identified in our laboratory, has been characterised. This was carried out by cross-testing the producing-organisms at different times during the logerithmic growth phase and at different temperatures using the deferred antaonism method.

The biological activity of strain 487, which shows activity against Epidemic MRSA-15 and strains of MSSA, is described in detail. In comparison with 20 other isolates, BLIS from strain 487 was shown to possess a number of unique properties. The initial characterisation of this agent showed that its activity can only be detected using deferred antagonism assays on plates pre-exposed to high temperatures (70–80°C/30–40 mins). This phenomenon suggests the presence of a heat-labile immunity factor in cell exudates. In addition, the high heat resistance of BLIS-487 suggests that it is a bacteriocin in nature, possibly of Class-I.

MI 18 Intestinal microbiota modulates host susceptibility to enteric pathogens

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Microbial communities residing in the gastrointestinal tract make up the intestinal microbiota, which is critical to normal host physiology. Understanding its role in host resistance to invading pathogens can advance our understanding of host-microbe interactions. The murine intestinal microbiota was perturbed by antibiotics and resultant host susceptibility to infection with *Salmonella* Typhimurium was investigated.

Pre-treatment of mice with streptomycin and vancomycin, but not with tetracycline, promoted subsequent *Salmonella* colonization and increased the proportion of γ-Proteobacteria in the intestinal microbiota. *Salmonella* infection of mice pre-treated with all 3 antibiotics resulted in reduced levels of total intestinal bacteria, unlike infection of untreated mice or treatment with antibiotics without infection. Treatment with increasing doses of streptomycin resulted in a gradual increase in *Salmonella* colonization, the proportion of γ-Proteobacteria in the intestinal microbiota and *Salmonella*-induced typhlitis, and a gradual decrease in total numbers of intestinal bacteria.

This study suggests that microbiota plays a vital role in host resistance to enteric pathogens and is itself affected by infection, making it a potential therapeutic target.

MI 19 Identification of Stx-phage genes expressed by their lysogens

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Shigatoxigenic *Escherichia coli* (STEC) are a global health concern as they can cause disease in humans following colonisation of the intestinal tract. Symptoms range from mild to severe diarrhoea, and infection can lead to life-threatening sequelae such as haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura, both of which can be fatal. The main virulence determinant of STEC is the production of Shiga-toxin (Stx), an ability conferred on them by Stx-phages. Stx-phages possess genomes ranging in size from 50–65 kilobases of DNA, and this is up to 50% larger than the genome of the archetypal lambdoid phage, λ. A significant proportion of λ's genome encodes genes of unknown function, and to a greater extent, this is mimicked in the Stx-phage genomes. It is known that there are λ genes that aid the bacterial lysogen's ability to colonise and survive in the mammalian gut. It is therefore reasonable to assume that the Stx-phage genome will also encode genes benefiting its lysogen, except that the larger genome has the potential to encode more genes selected over time for the beneficial contribution they make to the ability of

pathogenic *E. coli* to colonise the mammalian gut or even to cause gastrointestinal disease. Phage-encoded genes expressed by the lysogen are being identified using a novel technique; change-mediated antigen technology (CMAT, iviGene Corporation). Using bioinformatic analyses, isogenic mutant analyses and expression studies, we hope to identify the role that at least some of these genes play in the life cycle of the Stx-phage lysogen.

MI 20 Evaluation of outer membrane proteins of *Pseudomonas aeruginosa* as a protective vaccine in mice model

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The crude outer membrane protein (OMP) from a strain of *Pseudomonas aeruginosa* isolated, from burn patient was purified by two different methods. One procedure involved separation of sodium dodecyle sulphale (SDS) and triton X – 100 where as the other involved using lysosyme enzyme.

The protein estimation of OMP extracted by SDS/Triton X-100 was 7 mg / ml, compare to 4 mg / ml extracted by lysosyme method. Both methods showed very similar polypeptide pattern and the major peptide with molecular weight of 37 kDa was common in both procedures.

The efficacy of immunization with OMP prepared by (Triton X-100 and SDS) in mice, and challenged with homologous strain showed very good protection compared to control mice injected with saline. The passive haemoagglutination test (PHA) in mice injected with OMP showed increased level of antibody after the second injection but stayed constant after repeated injected. The results of this study showed that purified *Pseudomonas aeruginosa* OMP induced a significant protective immunity in mice against *Pseudomonas* infection, and could be used as a vaccine candidate.

Keywords *Pseudomonas aeruginosa*, outer membrane protein, vaccine

MI 21 *Not being presented*

MI 22 *Not being presented*

MI 23 *Not being presented*

MI 24 Gut origin of sepsis: a neonatal perspective

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Background The hypothesis that the bacterial flora in the alimentary tract can act as a source of sepsis is referred to as the 'gut origin of sepsis. This phenomenon seems probable in neonates because of an immature gastrointestinal barrier and immune response that might allow the passage of bacteria across the intestinal epithelial barrier. We propose to evaluate whether there is an association between the gram-negative rods in the gut and that in the blood of neonates with sepsis.

Methods A gut derived organism in the blood was confirmed if the same organism was isolated from either the gastric aspirate or stool of the neonate.

Results A gram-negative organism was isolated from the blood of 15/104 patients. The most predominant organism isolated from blood was *Klebsiella* (8/15, 53.3%). In 11/15 cases the organism in the blood was also isolated from either the stool or gastric aspirate. A gram-negative organism in the blood with gut derived origin was observed in 10.6% cases.

Conclusion The study implicates that in neonates septicemia may be a gut derived phenomenon.

Posters

Physiology, Biochemistry & Molecular Genetics Group

PBMG 01 Comparative genomics of *Salmonella enterica* serovar Typhimurium dt104

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Salmonella enterica serovar Typhimurium definitive phage type DT104 has emerged as the predominant Typhimurium type for human infections. Its acquisition of multi drug resistance has complicated treatment.

In this study Comparative Genomic Hybridisation (CGH) DNA microarrays, consisting of over 6000 genes, were used to compare 20 DT104 strains with varying antibiotic resistance profiles and isolation dates. The CGH allowed comparison between the DT104 strains collected from between 1986 and 2005, and 7 control strains from serovars of *S.* Typhimurium, Enteritidis, Gallinarum, Typhi, and *S.* Bongori.

The results have shown that pre-epidemic 1986 DT104's lacked the *Salmonella* Genomic Island 1 (SGI1) which confers antibiotic resistance and in addition lack genes for allantoin utilization as a sole nitrogen source, present in other *S.* Typhimurium strains. The epidemic strains from 1996–2005, which showed antibiotic resistance, possess the SGI1. Comparison of resistance phenotype and genotypic data have helped identify the SGI1 variants present within the resistant strains. Antibiotic sensitive strains from 2004/2005 on the whole matched the profile of the 1986 strains, but one strain harboured the allantoin utilization genes and a region of 23 genes found in *S.* Gallinarum.

PBMG 02 DegU regulation of multicellular behaviour by *Bacillus subtilis*

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DegU functions as a co-regulator of multicellular behaviour exhibited by *B. subtilis*. We propose a model where entry into the different multicellular behaviours is triggered along a gradient of DegU phosphorylation (DegU~P). Previously it has been demonstrated that genetic competence is regulated by unphosphorylated DegU. In contrast, swarming motility is activated by very low levels of DegU~P, complex colony architecture requires low levels of DegU~P, and high levels of DegU~P inhibit the former three processes and are required for exoprotease production. This model implies that DegU(~P) regulated genes will either be activated or inhibited along the gradient of DegU~P. We aim to identify through DNA microarray analysis the categories of DegU(~P) regulated genes that control the different multicellular behaviours and identify novel target genes required for the various multicellular processes exhibited by *B. subtilis*. One such gene that we have already identified through comparative genome-wide transcriptional profile analysis is *yvcA*. Transcription of *yvcA* is activated by low levels of DegU~P and inhibited by high levels of DegU~P; correspondingly it is required for complex colony architecture.

PBMG 03 Homocysteine toxicity in *Escherichia coli* results from threonine deaminase inhibition

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Since the 1960s, the sulphur-containing amino acid Homocysteine (Hcy) has been acknowledged as harmful to humans. In an attempt to uncover the toxic effects of Hcy on the human biological system our lab is using *Escherichia coli* as a model organism. Many *E. coli* strains demonstrate growth inhibition in the presence of Hcy. To date *in vitro* studies have shown that purified threonine deaminase (TD), an enzyme involved in the deamination reaction of threonine to α-ketobutyrate during isoleucine biosynthesis, is inhibited by Hcy. Kinetic studies were undertaken to determine the mode of inhibition of this amino acid on TD. The biosynthetic enzyme appears to be inhibited by competitive inhibition, with increasing substrate providing relief from inhibition. At the genomic level, phage, gene cloning and RT-PCR analysis has allowed us to confirm that increased expression of *ilvA*, the gene encoding TD, also provides protection against Hcy inhibition. Furthermore it has been observed that valine provides relief from Hcy inhibition in cell cultures. Kinetic studies on TD in the presence of valine are being carried out. Together these data may suggest a model which accounts for the inhibitory effect of Hcy in *E. coli*.

PBMG 04 Investigation into the molecular responses to organic acids in the human pathogen Listeria monocytogenes

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Contamination of processed food by micro-organisms, such as the pathogen *Listeria monocytogenes*, is a major human health risk. Food preservation methods include the use of organic acids to reduce microbial growth. This study investigated the response of *L. monocytogenes* to organic acids. Growth of *L. monocytogenes* in response to five food-grade acids, lactic, sorbic, acetic, benzoic and citric acid, was explored. The levels of organic acid that caused 50% inhibition were established. At a given organic acid concentration, growth was more inhibited at lower pH values suggesting that it is the undissociated form of the acid that causes the inhibitory effect. Microarray analysis identified genes showing altered expression in response to 10mM (15% growth inhibition) acetic acid. The up-regulated genes included genes involved in glutamate synthesis and the down-regulated genes included those involved in cell wall biosynthesis. Currently gene expression in response to the other four food-grade acids is under investigation. A comparative analysis of the transcriptional responses to all five acids will be presented.

PBMG 05 Coupling of quorum sensing systems in *Rhizobium leguminosarum* bv. *viciae*

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Rhizobium leguminosarum bv. *viciae* 3841 has three *N*-acyl-L-homoserine lactone (AHL) quorum sensing systems (*cin*, *rhi*, and *tra*), consisting of the AHL synthases CinI, RhiI and TraI, and the associated regulators CinR, RhiR and TraR. These systems are involved in

stationary phase adaptation, legume nodulation and plasmid transfer respectively. The roles of three orphan LuxR type regulators (ExpR, AhyR and AsaR) are unknown. They may detect AHLs produced by other bacteria or they may act as independent regulators in *R. leguminosarum*.

We are investigating the interactions of the different quorum sensing systems in *R. leguminosarum*. Mutation of *cinI* or *cinR* greatly reduced *rhiI* expression, indicating that the *cin* and *rhi* regulatory systems are coupled. Mutation of the regulatory gene *expR* also reduced *rhiI* expression suggesting that, in response to CinI-made AHLs, ExpR might induce a complementary set of promoters to those regulated by CinR. However we have been unable to activate ExpR by CinI-made AHLs. We are currently examining the mechanism whereby ExpR couples the induction of the *cin* system with the *rhi* system.

PBMG 06 Artificial neural network analysis of MALDI-TOF data for the discrimination of *Citrobacter koseri* from other closely related *Enterobacteriaceae*

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Background MALDI-TOF analysis of bacteria generates large quantities of data which can be analysed using Artificial Neural Networks.

Methods Intact cell MALDI-MS (ICM-MS) was applied to 92 strains of *Enterobacteriaceae*. The positive controls were 46 strains of *C. koseri* and the negative controls were closely related *Enterobacteriaceae*. In order to identify biomarkers a computer algorithm 'artificial neural network' was utilized.

Results The model was formed by fifty random training/test/validation sub-models of the *m/z* values. The best model was obtained for the top 15 *m/z* values. This model correctly classified 98.91% of the samples. The sensitivity was 99.00% and the specificity was 98.83%. The positive predicted value was 99.00% with a negative predicted value of 98.82%. The test performance was 97.48% and the area under the curve was 0.99.

Conclusions The present study shows the application of MALDI-TOF combined with ANNs for separation of closely related species in a short period of time.

PBMG 07 Characterization of essential (photosynthesis) gene functions of cyanophage S-PM2

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Phage infection of marine unicellular cyanobacteria from the genus *Synechococcus* is an example of considering the importance of genes functions which are being transcribed during the infection course in a vast marine environment. Sequencing results have revealed lots of information about similarities and differences between the novel cyanophage S-PM2 genome and the classical *E. coli* infecting T4 model. However many ORFs of this novel exoT-evens categorized phage still have unknown role within the provisional annotation. Many genes in S-PM2 appear to have a bacterial origin rather than a viral origin, including key core photosystem proteins D1 and D2 encoded by *psbA* and *psbD*. The link between biogeochemical pathways and physiology of phage-host interaction leads us to specifically studying gene *psbA* encoding homologous D1 protein from the host Photosystem II apparatus. Constant level of expression for D1 protein suggests that phages are encoding *psbA* to ensure the safe level of expression during the infection process. In this project we prove the functionality of *psbA* gene by using site-directed mutagenesis and we will track the side-effects of knocking out such gene on phage-host metabolism and whole infection course. In this work, Amber Suppressor system will also be used in order to study the function of other essential and unique genes in S-PM2.

PBMG 08 Transgenic plants that express low levels of virus coat protein gene are resistant to virus infection

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Passion fruit woodiness virus (PWV) is currently found in all passion vines in Brazil, where it is the most important disease. The virus is transmitted in a non-persistent manner by aphid species that do not colonize passionflower. The virus causes significant loss of fruit yield and quality and reduces the productive life of orchards. A full-length coat protein (CP) gene from a severe PWV isolate was cloned into a binary vector and used to produce *Agrobacterium*-genetically transformed plants. Different numbers of insertional events were identified in 7 plants, although the 32 kDa PWV CP was detected in only 2 plants. All the transgenic plants expressed the expected transcript though in very different levels as estimated by Real Time PCR, using the elongation factor-1 alpha gene as endogenous control. The results of 3 successive mechanical inoculations against the transgenics using 3 PWV isolates showed that one transformant (T2) was immune to all isolates. This plant had low levels of the transcript suggesting that RNAi-mediated mechanism is associated with antivirus plant defense.

PBMG 09 Genetic basis of resistance to *Xanthomonas axonopodis* infection in passionflower

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Certain bacterial peptides can alter plant cell homeostasis and enable pathogens to manipulate plant responses to bring about favorable conditions to the pathogen such as the hyper-hydration seen in the wet lesions associated with *Xanthomonas axonopodis* infection. These lesions are frequently found on leaves and fruits in passionflower orchards infected with *X. axonopodis* pv. *passiflorae*, being the disease very destructive for the crop in Brazil. Mapping alleles associated with disease progression is crucial for elucidating the genetic basis of plant response. Here, a F_1 population was used to construct an AFLP-based linkage map. The same population and its parents were inoculated with two isolates of the pathogen in three independent assays. Resistance is oligogenic and there are three major quantitative resistance loci (QRL) involved in the response to bacterial infection. Our data did show that these linked AFLp-alleles should be used for assisting selection to reduce plant susceptibility. One locus located on the VIII-linkage group was validated independent of the experiment, leaf age, methodology and date of disease evaluation.

PBMG 10 *Not being presented*

Posters

Plenary

PS 01 The effects of attenuation methods on cell permeability and release of intracellular enzymes from *Lactococcus lactis* strains

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In Ireland, the cheese industry is of major economic importance and because of the extended ripening period it is the goal of researchers to accelerate this process. One possibility is the attenuation of starter cultures by various treatments to enhance intracellular enzyme accessibility. Cell pellets of three *Lactoccus lactis* cheese starter cultures grown in L-M17 broth or buffered 10% RSM were exposed to sonication, chemical (isopropyl alcohol (IPA), sodium dodecyl sulphate (SDS) and hexadecyltrimethlammonium bromide (CTAB)) and heat treatments (45°C–65°C for 10 min) to assess their effects on cell permeability and accessibility of intracellular peptidases, Pep X or Pep N. Chemical treatments, in particular CTAB, and to a lesser extent SDS positively affected the levels of accessible Pep X and Pep N. Permeability of cells was monitored using flow cytometry. Cells were stained using Live/Dead® BacLight™ reagent. Differing sub-populations were identified corresponding to intact and permeabilized cells with CTAB treatment generating a unique sub-population.

PS 02 Monitoring of the effects of varying salt levels on growth of food spoilage bacteria using plate counting and flow cytometry

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Salt has long been used in the food industry as a means of preservation by inhibiting growth of spoilage bacteria. However, food manufacturers are increasingly under pressure to reduce the sodium content in their products and hence this may impact on quality and product safety. Therefore, the objective of this study was to investigate the effects of salt levels (0–3.0%) on the growth of food spoilage bacteria including *Listeria innocua, Escherichia coli, Bacillus subtilis, Lactobacillus* and *Pseudomonas fluorescens*.. Growth was monitored in liquid media using: plate counts, OD600nm, and flow cytometry (FCM). Cells were stained for FCM analysis using Syto 9/PI or Hoechst 33342/CFDA. Overall, growth was generally unaffected over these salt levels, however final cell densities were lower at 3% added salt. FCM profiles showed different sub-populations at various added salt levels. These sub-populations also changed over incubation time. FCM, in combination with traditional methods, provided an interesting insight into the growth response of various spoilage bacteria to salt.

PS 03 Role of the glutamate decarboxylase acid resistance system in survival of *Listeria monocytogenes* LO28 in modified atmosphere packaged vegetables

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The contribution of the glutamate decarboxylase (GAD) acid resistance system to survival and growth of *Listeria monocytogenes* LO28 in modified atmosphere packaged vegetables was examined. The survival and growth of the wild-type LO28 and four GAD deletion mutants (Δ*gadA*, Δ*gadB*, Δ*gadC*, Δ*gadAB*) in packaged lettuce and dry coleslaw mix during storage at 4, 8 and 15°C were studied. Survival and growth patterns varied with strain, vegetable type, gas atmosphere and storage temperature. In both packaged vegetables at all storage temperatures, the wild-type strain survived better ($P<0.05$) than the double mutant strain Δ*gadAB*. The requirement for the individual *gad* genes varied depending on the packaged product. In the case of lettuce, *gadA* played the most important role, while the *gadB* and *gadC* genes played the greatest role in packaged coleslaw (particularly at 15°C). The work demonstrates that elements of the GAD system play significant roles in survival of *L. monocytogenes* LO28 during storage in modified atmosphere packaged vegetables.

PS 04 Tracking the *Salmonella* status of pigs through the slaughter process in Ireland

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Individual pigs from nine herds of known *Salmonella* serological status were tracked through the slaughter and dressing process in three commercial pork abattoirs. The aim of this study was to determine the correlation between the *Salmonella* status of the pigs presented for slaughter and the *Salmonella* status of the pork following slaughter and dressing operations. Each pig was examined for the presence of *Salmonella* at key stages during slaughter and dressing. The results indicate that slaughter and dressing operations have a significant effect on the transmission of *Salmonella*. When pigs presented for slaughter have caecal or rectal carriage, good slaughter practices can prevent carcass contamination. All data generated is being fed into a quantitative risk assessment model for *Salmonella* in pork on the island of Ireland.

Acknowledgement This research is funded by safefood, The Food Safety Promotion Board and the Food Institutional Research Measure administered by the Department of Agriculture and Food.

PS 05 Prevalence and characterisation of *Yersinia enterocolitica* along the Irish food chain

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A surveillance project on the prevalence of *Yersinia enterocolitica* along the Irish food chain is underway. Three pig slaughterhouses and 50 retail outlets will be visited 6 times over a 2-year period denoted cycles 1–6. Duplicate porcine tonsilar tissue, neck muscle, nasal swabs, head swabs, carcass swabs from 10 pig carcasses, four environmental samples from the slaughterhouse and 200 pork meat samples are examined microbiologically using standard methodology. Molecular confirmation and screen for virulence determinants is performed on recovered isolates using a triplex PCR, targeting 16S rRNA found in *Yersinia* spp., chromosomal *ail* genes and virulence plasmid encoded p*Yad* found in *Yersinia enterocolitica*. To date from 300 samples 12 (4%) non-virulent *Yersinia enterocolitica* have been recovered. Biotyping, serotyping, DNA fingerprinting and antibiotic resistance profiles will be performed. The presence of this emerging pathogen is a potential public health risk for Irish consumers.

PS 06 The incidence of *Salmonella*, diarrheagenic *Escherichia coli* and *Campylobacter* on Irish beef and dairy farms

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Salmonella, Diarrheagenic *E. coli* and *Campylobacter* are major foodborne pathogens in Ireland. The objective of this study was to investigate their prevalence on Irish farms.

The occurrence of *Salmonella* spp, Diarrheagenic *E. coli* spp and *Campylobacter* spp, on Irish beef and dairy farms were investigated seasonally over a 12 month period. Faecal samples were taken from food animals, domestic animals, wild birds and environmental sources, such as soil and water. Samples were subjected to both direct plating and enrichment methods of isolation.

All suspect isolates were characterised by phenotypic methods. These included antibiogram analysis, serotyping and phage typing where necessary. Genotypic studies such as multiplex PCR for virulence genes, PFGE, and PCR analysis of antibiotic resistance genes are ongoing. Preliminary results of this study suggest a low incidence of these pathogens on Irish beef and dairy farms.

PS 07 Introduction of novel compatible solute uptake systems to *Lactobacillus salivarius* to improve its physiological robustness

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Research into probiotic bacteria has increased significantly over the last decade. Certain probiotic strains have been shown to have significant health-promoting benefits in both animal and human trials. However, during their life cycle, these strains encounter and must overcome the natural physiochemical defences of the host in order to exert a beneficial effect. In some instances, certain probiotics maybe shown to have promising, clinically relevant attributes but may be unable to survive in the host for sufficient time to exert a beneficial effect or may have a poor shelf-life and be unable to survive in a commercial product for an extended period. It is therefore desirable to improve the physiological robustness of potentially clinically important strains. This study aims to introduce novel compatible uptake systems, from commensal microflora, into a well characterised probiotic strain (*Lactobacillus salivarius*) and examine its subsequent response to *in vitro* and *in vivo* stresses.

PS 08 Antibiotic resistance profiling of lactic acid bacteria isolated from pork and beef plants

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Lactic acid bacteria are common in foods and are members of the resident micro flora of the gastrointestinal tract of humans and animals. Because of their broad environmental distribution, these bacteria may serve as vectors for the dissemination of antibiotic resistance determinants via the food chain to the consumer. They are also capable of transferring resistant genes, both interspecies and to enteric pathogens. The purpose of this study was to screen lactic acid bacteria, isolated from beef and pork, for resistance to a wide range of antibiotics using a modified version of the Kirby-Bauer disc diffusion. Lactic acid bacteria species were confirmed using the API 50CH kits.

Transferability of tetracycline resistance was examined between species of lactic acid bacteria via a filter mating experiment. Donor, recipients, and transconjugants were confirmed by PCR.

PS 09 Shelf life extension of cooked ham using a bioprotective culture

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Bioprotection is the application of lactic acid bacteria to a foodstuff in order to control the microflora, without significantly altering the sensory properties. A traditional cooked ham product has a shelf life of 21–28 days. The aim of this study was to investigate the shelf life extension of cooked ham by applying a bioprotective culture of *Lactobacillus sakei* BJ–33 and assessing the effects on microbial, chemical and organoleptic properties over a 40 day shelf life. Results found the treated ham to have a slightly lower pH value and moisture content than that of the untreated ham. The treated ham contained 10^6cfu/g at point of application and increased to 10^8cfu/g throughout the remainder of the shelf life, compared with the untreated ham which initially contained 10^2cfu/g and increased to 10^8cfu/g during shelf life. Initial taste panel studies found the treated product to be acceptable in terms of appearance, flavour and texture. The development of a ham product containing bioprotective cultures with a maximum shelf life of 39 days is achievable.

PS 10 The molecular epidemiology of non-O157 verocytotoxigenic *Escherichia coli* from farm to fork

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Non-O157 verocytotoxigenic *Escherichia coli* (VTEC) are important zoonotic pathogens. However, the true incidence and the sources of infection are poorly understood. Multilocus sequence typing (MLST) schemes have been established to enhance our understanding of the molecular epidemiology of many pathogens.

MLST was applied to 183 non-O157 VTEC isolates from different sources, human infections, food and animals, 6 VTEC O157 isolates and 21 representative isolates of different groups of *E. coli* associated with diarrhoeal disease.

The majority of isolates belonged to ST-10, ST-20, ST-29 and ST-306 clonal complexes. The ST-10 complex and ST-20 complex were the most widely distributed clonal complexes and isolated from different sources whereas ST-306 complex was exclusively isolated from cattle. The non-O157 VTEC isolates belonged to distinct lineages from VTEC O157:H7 isolates and from the majority of enteropathogenic *E. coli* isolates.

MLST has defined the population structure for non-O157 VTEC informing potential zoonotic sources of VTEC infections.

PS 11 Coxsackie B virus serotypes in environmental waters; changes in prevalence over a five year period

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Enteroviruses and in particular coxsackievirus B are the most commonly isolated viruses from environmental water samples. A five year surveillance study was undertaken to examine the changing prevalence over time of coxsackievirus B serotypes in three different sample matrices; inlet sewage, sewage effluent and river water. The data obtained were also compared with reports of isolation of coxsackievirus from clinical samples. All of the most common serotypes were detected with the widest range of serotypes detected in inlet sewage samples. Coxsackievirus serotypes B4 and B5 were most

commonly isolated. Coxsackievirus B1 was only isolated in the last two years of the study and was the most common serotype isolated in the last year of the study. In two of the five years the number of isolates were highest during the summer.

In conclusion, the prevalence of the coxsackievirus serotypes changed over the five year period, seasonality of the virus was not always observed and comparison with clinical isolates showed differences over the five years.

PS 12 Nutrient supplements prevent down-regulation of intestinal defensin expression during diarrhoeal disease

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We previously demonstrated that alpha-defensin expression in small intestine is reduced in a tropical population compared to a European population. Intestinal infection is common in urban Africa.

We studied expression of HD5, HD6 and the cathelicidin LL-37 in small intestinal biopsies in 500 Zambian adults participating in a randomised cross-over controlled trial of multiple micronutrient supplement over 3.5 years. mRNA was measured by qPCR in 503 biopsies at rest, and 53 pairs of biopsies during and one month after diarrhoea.

HD5 and 6 mRNA did not differ by HIV status or treatment. But in participants with malnutrition (BMI<18.5kg/m2), HD5 was 0.8 log transcripts/µg total RNA higher (p=0.007). During diarrhoea, in placebo recipients, HD5 was lower by 0.87 logs, but in micronutrient recipients this reduction was abolished (p=0.02). LL-37 was expressed in 39% biopsies but not influenced by nutritional status, HIV or diarrhoea.

In malnutrition, micronutrient supplementation can increase defensin expression, but not LL-37. During diarrhoea, supplementation protects against down-regulation of defensins.

PS 13 Examination of the microbial safety of schoolchildren's packed lunches

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Packed lunch sandwiches, because of the way they are prepared and handled, have the potential to harbour food-borne pathogens. Previous studies have found packed lunch sandwiches are prepared on average 4–5 hours prior to consumption and stored at a variety of temperatures. In this experiment, four types of sandwich types were prepared and examined at time 0 and after 4 hours incubation at 20°C for total aerobic colony count, presence of *Listeria spp.* and *S. aureus*. Guidelines intended for retail food were used as a measure of microbiological quality. Pre-incubation, 94% of sandwiches were satisfactory for aerobic colony count (88% post incubation) but 6% of sandwiches were unsatisfactory for *Listeria* spp. (12%), and 31% for *S. aureus* (43%). There was no significant difference ($p>0.05$) in aerobic colony counts following incubation at 20°C for 4 hours. However, the high incidence of *Listeria spp.* and *S. aureus* pre and post incubation gives rise to concern over the microbiological quality of stored packed lunch sandwiches.

PS 14 The use of bergamot essential oil to limit growth of *Listeria monocytogenes* in cottage cheese

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Cases of food poisoning due to *L. monocytogenes* have risen over the last 10 years (186 cases in 2006). Concern among consumers about the use of chemical food preservatives, coupled with the increased incidence of microbial resistance towards these substances has led to interest in using natural alternatives such as essential oils.

In this study, the possible use of bergamot essential oil as an antibacterial agent against *L. monocytogenes* in cottage cheese was investigated. Three, six and nine drops of Bergamot essential oil were added to 9ml cottage cheese samples inoculated with *L. monocytogenes*. Samples were incubated for up to 48 hours with viable counts determined at time intervals.

Essential oil concentration and treatment time were found to decrease the bacterial population. Nine drops of oil was most effective, inhibiting the growth of *L. monocytogenes* to within acceptable limits for cottage cheese, but by 48 hours all concentrations significantly reduced the bacterial population ($P\leq0.05$). Bergamot essential oil could therefore be considered for use as a natural preservative in cottage cheese.

PS 15 Development of a GFP transposon mutagenesis system to investigate genes involved in the intracellular persistence of *Yersinia enterocolitica* Biotype 1A strains

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There is growing evidence that Biotype 1A *Y. enterocolitica* are potential human pathogens. BT1A isolates were shown to adhere to and invade epithelial cells in vitro, persist in macrophages in vitro (with levels of intracellular bacteria failing to significantly fall after 24 hours infection), and to be immunomodulatory, with respect to levels of cytokines secreted *in vitro* by macrophages challenged by clinical isolates. Despite further work by our group highlighting flagella as a key factor in the phenotypes mentioned, there is little known about BT1A strains with respect to their ability to survive intracellularly in the host. Previous work also suggests that BT1A strains may be as little as 42% related at the genetic level to the sequenced pathogenic *Y. enterocolitica* strain 8081. Here we describe the use of a GFP transposon to create a random mutant library in a clinical BT1A isolate, allowing investigation of genes involved in the intracellular state of this emerging zoonotic pathogen.

PS 16 Influence of raw and cooked meat juices on the attachment of *Listeria monocytogenes* to stainless steel

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Listeria can attach to and grow on a wide range of materials. *L. monocytogenes* cells attached to surfaces in processing plants can pose a significant source of contamination for products and could account for the persistence of specific *L. monocytogenes* strains in the food plant environment. The ability of one *Listeria innocua* and two *Listeria monocytogenes* strains, isolated from chicken, to form biofilms when incubated in raw and cooked meat juice was investigated. Meat juices from beef, pork, chicken, duck and lamb were prepared by pulverising meat in distilled water and autoclaving (for cooked) then filtering the resulting liquid. Stainless steel coupons in meat juice were incubated with *Listeria* at 5 and 25°C. Levels of cell attachment were obtained by viable counts of cells detached by vortexing the coupons and by epifluerescence microscopy following acridine orange staining. Incubation temperature influenced the levels of cells attached. Some differences in attachment dependent on meat juice used were evident but results were dependent on the method of assessment of attachment levels.

PS 17 Kefir made from goats' milk: are its microflora and aroma compounds affected by the length of fermentation?

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Background and objective of investigation The aim of this study was to define how the application of different fermentation lengths (24, 48 and 72 hours) affects the resulting microbial population and aroma profile of Kefir.

Methods Several batches of goats' milk were inoculated with a commercial Kefir starter culture consisting of *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* spp. lactis biovar *diacetylactis*, *Lactobacillus brevis*, *Leuconostoc* and *Saccharomyces cerevisiae* strains. From each batch three sub-batches of Kefir were produced by ending the fermentation at 24h, 48h and 72h respectively. The microbial population was counted on a selection of media. The microbial profiles were analysed using the 16S rDNA-PCR Denaturing Gradient Gel Electrophoresis (DGGE). The flavour profiles of the different batches were analysed by Atmospheric Pressure Chemical Ionisation (APCI) and Gas Chromatography – Mass Spectrometry (GC-MS).

Results By plotting in parallel the microbial counts, the pH values and the DGGE profiles, different profiles were formed referring to the three fermentation times. In addition, the flavour analysis by APCI resulted in differentiated profiles with principal component analysis showing specific groups of ions are formed around the different fermentation times. The GC-MS analysis provided information on the aroma qualitative characteristics focusing on those related to the bacterial produced acids and their metabolic products.

Conclusions The application of three different times of fermentation in Kefir results in products with qualitative and quantitative differences both in the microbial profile and the aroma and there is no linear correlation between them.

PS 18 *Salmonella* contamination of pork carcasses: nationwide baseline culture-based data determined by sponge sampling during 2006

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Pork is recognised as one of the sources of human salmonellosis. On-farm controls may reduce rather than eliminate the number of pigs carrying *Salmonella* and the slaughterhouse has been shown to be an economically advantageous site for further control. To identify appropriate locations in the slaughterline for imposition of control measures, a survey of levels of bacterial contamination throughout the process was conducted. Following a survey of practices used in UK pork slaughterhouses, four representative abattoirs were visited over one year and counts of *Salmonella*, *Escherichia coli,* aerobic bacteria and Enterobacteriaceae were generated. Results showed that *Salmonella* could be detected on 10–80% of animals at slaughter but was never detected on final carcasses. Final total aerobic counts were between $10–10^3$ cfu/cm^2 with Enterobacteriaceae and *E. coli* counts at ~1 cell /10–100 cm^2. The nature of the scald system was identified as one process which may aid contamination control.

PS 19 Acanthamoebae in soil and sewage sludge, a vector of bacterial and viral pathogens

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Acanthamoebae are ubiquitous soil protozoa, the genus contains pathogenic species and its members also act as important vectors of human bacterial pathogens. Numbers and species diversity of acathamoebae in soil and digested sewage sludge were studied using real-time PCR and 18S rDNA clone libraries. Approximately $10^3–10^4$ cells per gram were present in soil and sludge, although clone libraries revealed greater species diversity in soil. Studies using GFP reporter constructs of *S. typhimurium* were used to elucidate interactions with *A.* polyphaga. Invasion of the contractile vacuole by *S. typhimurium was* observed in a proportion of acanthamoebae and persistence observed within cysts. Population dynamics of *A. polyphaga* and *S. typhimurium* were studied in sterile and non-sterile soil microcosms at different soil moisture levels using real-time PCR. Grazing pressure was greatest in non-sterile high water content soils. An *Acanthamoeba* sp. isolate from fully digested sewage sludge contained large numbers of particles resembling the giant DNA virus known as the Microbe Mimicking virus or Mimivirus.

PS 20 Assessing the impact of farm management practices on stream FIO loads using an evidence based approach

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The introduction of a revised (EU) Bathing Waters Directive in 2006, with more stringent standards, coupled with the implementation of the (EU) Water Framework Directive by 2015 is seeing the issues of bathing, surface and ground water quality being brought to the fore. Non-compliance is occurring at designated bathing sites; in part, this can be attributed to diffuse sources contributed from agricultural land, following the recycling of livestock manures and direct faecal deposition.

We have developed an expert-weighted risk tool, to rank field and steading risk of FIO export, which coupled with detailed microbiological monitoring, has enabled the identification of risky farm practices and locations.

Results from two distinctly different farms confirm that on Farm X FIO losses from land to water are field driven. On farm Y the farmyard was identified as high risk, significantly ($P < 0.05$) increasing FIO concentrations detected in a nearby stream. The development of a relative risk tool, rather than a quantitative predictive model, will assist farmers and land owners to prioritise land that is most 'risky' in terms of contributing bacterial contamination to watercourses and will help focus mitigation efforts where they are likely to be most effective in terms of improving water quality.

PS 21 Verocytotoxigenic *Escherichia coli* serogroup O26 – the new VTEC O157?

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Escherichia coli serogroup O26 played an important part in the early work on Verocytotoxin and is an established diarrhoeal pathogen. Recently, Verocytotoxigenic *E. coli* (VTEC) O26 has been increasingly associated with diarrhoeal disease and frequently linked to outbreaks and cases of haemolytic ureamic syndrome (HUS). This review investigates the pathogenicity, geographical distribution, changing epidemiology, routes of transmission and improved detection of VTEC

O26. Laboratory data on VTEC O26 isolates and clinical data on HUS suggest a true difference in the incidence of VTEC O26 in different geographic locations. VTEC O26 is frequently found in the cattle population but rarely in food. However, the small number of outbreaks analysed to date are thought to be food borne rather than associated with direct or indirect contact with livestock or their faeces. The increase in awareness of VTEC O26 in the clinical and veterinary setting has coincided with the development of novel techniques that have improved our ability to detect and characterise this pathogen.

PS 22 The survival of FIOs in soil, following dairy cattle slurry application to land by surface broadcasting and shallow injection

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The amended Bathing Water Directive (2006/7/EC) of February 2006 saw the introduction of more stringent microbial parameters for both inland and coastal waters. Two microbial parameters are now required to be examined; intestinal enterococci and *Escherichia coli* (FIOs). Approximately ninety million tonnes of livestock manures are recycled to agricultural land in the UK annually, which is a potential source of FIO export to surface waters. The survival of FIOs within dairy cattle slurry, applied by broadcast and shallow injection was investigated at the plot scale. Soil core samples (2 cm depth) were taken and analysed for FIOs from fifteen 4 m^2 plots; 5 broadcast, 5 shallow injection, to which fresh slurry was applied at the rate of 45 m^3 per hectare and 5 controls (no slurry addition) during the summer. Application method affected the survival rate of FIOs. *E.coli*, 84 days intestinal enterococci, 111 days when applied via shallow injection as compared to 50 days and 63 days, respectfully following broadcast application. FIOs can survive for extended periods following slurry application by shallow injection.

PS 23 Investigation into the prevalence of *Legionella pneumophila* in water systems of hotels located in a coastal town in Southern England

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Hotels have been implicated in cases of legionellosis worldwide and over the past 20 years cases in the UK have doubled. The object of the study was to investigate current colonisation rates of *Legionella* in hotel water systems within a south coast town. In each hotel, water was taken from a showerhead and tested in our laboratory for the presence of *Legionella*. Out of 101 hotels sampled, 36 tested positive for *Legionella*. Of the legionella positive hotels, 69% (25 hotels) had *Legionella pneumophila* type 1, 30% (11) had *Legionella pneumophila* type 2–14 and 2% (1) had legionella species. *Legionella pneumophila* type 1 levels ranged from 20–220,000 cfu/litre. Of these 36% showed greater than 1000 cfu/litre, which under HSE guidance requires an immediate review of the control measures in place. In conclusion, this snapshot of *Legionella* colonisation demonstrates that hotels need to take greater care of their water systems to prevent colonisation and growth of *Legionella*.

PS 24 Seed transmission of cocoa swollen shoot virus

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Research was carried out to validate the assumption that cocoa swollen shoot virus (CSSV) is not seed-transmitted in an effort to improve the robustness of quarantine procedures for the international exchange and long term conservation of cocoa germplasm. Polymerase chain reaction (PCR), real-time PCR and fragment analysis assays using CSSV primers designed from the most conserved regions of the six published cocoa genome sequences, allowed the detection of CSSV in all the component parts of the cocoa seeds from CSSV-infected trees. Capillary electrophoresis further revealed the presence of the CSSV in the seedlings from seeds from CSSV-infected trees. The CSSV positive status of the pollen grains may account for the non uniform viral status of the seeds/seedlings from a single pod if pollination had been caused by a mixture of CSSV positive and negative pollen grains. The out come of this research conclude that CSSV DNA can be transmitted through cocoa seeds with the pollen gains as possible source of the virus.

PS 25 Monitoring shifts in the faecal microbiota of inbred piglets using Denaturing Gradient Gel Electrophoresis (DGGE)

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The gastrointestinal microbiota plays a key role in animal health and disease susceptibility. Pigs are particularly vulnerable to disturbances in the microbiota shortly after weaning. This predisposes them to pathogenic enteric infections compromising their health and welfare. However, little is known of factors determining the microbial diversity of the gastrointestinal tract. In the first of a series of animal trials, DGGE of amplified 16S rDNA fragments was used to monitor shifts in the faecal microbiota composition in two different genetic lines of pigs; the fully inbred Babraham and the outbred Large White line. Both lines exhibited significantly different microbial profiles between pre- and post-weaning, representing the major changes occurring during the transition from milk to solid diet. Using band numbers as an indicator of diversity, it was demonstrated that inbred piglets harbour a less diverse microbiota than outbreds. In addition, the similarity between inbred piglets was significantly higher than that between outbred, suggesting the important effect of their identical genotype. These results indicate that the inbred line can be used in the future trials to investigate the link between genetics, environment and gastrointestinal microbiota.

PS 26 Nature of the 83 kb *Salmonella enteritidis* plasmid possessed by some strains that grow rapidly in eggs

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Two *Salmonella* Enteritidis (SE) strains of phage types (PT) 4 and 1c possess an 83 kb plasmid, in addition to the 60 kb plasmid found in most Enteritidis isolates. These 2 strains exhibit the characteristic ability of *S.* Typhimurium (STm) to grow rapidly in fresh eggs, post-lay, as well as the SE-associated capability of surviving well in egg albumen.

The 83 kb plasmid was analysed by PCR, plasmid profiles, restriction enzyme fingerprinting and comparative genomic indexing. Several STm LT2 virulence plasmid genes were absent from the 83 kb plasmid. Interestingly, 65 chromosomal genes of STm SL1344 were present in the PT1c, and almost all of them were also present in the PT4 strain. Plasmid restriction profiles confirmed a difference between the STm and 83 kb plasmids.

This could have great significance, since the shelf-life regulations in the UK allow eggs to be maintained for twenty-one days at 20°C. If this type of SE began to colonise chickens and enter eggs, their rapid growth could cause a major public health problem.

PS 27 Proteomic approach to study of the cold shock response of *Salmonella typhimurium*

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Persistence of *Salmonella enterica sv.* Typhimurium through food processing and storage, places this organism as one of the major causes of food-borne disease globally. Adaptation to and multiplication at low temperature involves the multigenic cold shock response, many features of which are highly conserved across pathogens.

The CspA family of RNA binding proteins is central to the cold shock response. Since such proteins may act post-transcriptionally, aspects of the response may not be observed at the level of transcription. Proteomic analysis provides a more direct strategy for dissecting the response mechanism and underpins the basis of the current study.

Using mutants in which all of the *cspA* gene paralogues have been deleted from *S.* Typhimurium, we evaluate the cellular response to temperature downshift using 2-D gel electrophoresis and mass spectrometry analysis. Comparison is made with the isogenic parental strain and, with an *rpoS* mutant which lacks the general stress-response sigma factor. The outcome is reported in relation to the underlying processes involved in adaptation.

PS 28 *Escherichia coli* and *Salmonella enterica* serovar Typhimurium biofilms and low temperature adaptation

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Biofilms are clusters of surface-attached bacteria that are encased in a matrix of exopolysaccharides (EPS). Living within a biofilm offers greater protection from harsh environmental conditions and host immune responses, which would be advantageous to bacterial pathogens. To further understand biofilm formation and structure we are studying the closely related enteric species *E. coli* K12 and *S. enterica* serovar Typhimurium, the latter of which is a pathogen.

Enteric pathogens may encounter a number of stresses including antibiotic treatment within a host or treatment of contaminated food or surfaces under free-living conditions. One well-characterised stress response is the cold-shock response, where *cspA* homologs mediate the adaptation to growth at low temperatures, following sudden downshift. This simulates environmental conditions of bacteria within contaminated, refrigerated foods.

We aim to characterise biofilm formation under optimal conditions before exposing cells to stresses to investigate mechanisms of adaptation, using classic crystal violet staining assays and confocal microscopy of GFP-expressing stains. A number of mutants (e.g. which lack cold-shock proteins) will be used to further elucidate the mechanisms of biofilm formation and persistence.

PS 29 Cross sectional study of enteric pathogens in wildlife and cattle in Cheshire (UK)

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Food-borne infection was estimated to be responsible for 1.4M human cases of enteric disease in England and Wales during 2000. The most frequent cause was *Campylobacter*, followed by *Salmonella* and Verotoxigenic *E. coli* (VTEC).Domestic livestock are a known source of such pathogens, although their carriage in wildlife is less well understood.

The aims of the study were to determine the presence and spatial distribution of these pathogenic organisms isolated from faeces of wild mammals, birds and cattle. A cross-sectional study was conducted on 6 farms (5 dairy and 1 beef) in Cheshire, NW England, between July 2004 and May 2005. Bacteria were isolated using standard microbiological techniques: *Campylobacter* species were determined by PCR; *E. coli* isolates were screened for virulence determinants and/or confirmed as 0157 by PCR and microarrays in collaboration with the VLA; and *Salmonella* spp. were identified biochemically and serotyped.

Approximately 2600 samples were processed (40% rodents, 34% bird, 19% cattle and 7% other wildlife). *Campylobacter* spp were isolated from magpie (*Pica pica*), rabbit (*Oryctolagus cuniculus*), brown rat (*Rattus norvegicus*), bank voles (*Clethrionomys glareolus*), wood mouse (*Apodemus sylvaticus*) and cattle from six farms. There were marked differences in prevalence between species and farms. Only the beef farm was positive for *E.* coli 0157, however VTEC strains (*eae, vt1* and *vt2* genes) were isolated from all farms from cattle and wildlife. Salmonella spp., were rarely isolated except for a *Salmonella* Arizonae isolated from a wood mouse and *Salmonella* London from a badger on a farm which previously had an outbreak of this serotype.

PS 30 Norovirus gastroenteritis among children in Iraqi Kurdistan

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Introduction Diarrhoeal disease is a leading cause of morbidity and mortality among children in developing countries. Norovirus (NV) is gaining increasing recognition as an important cause of paediatric gastroenteritis, but there are few data from developing countries. We recently undertook a short study of the viral agents associated with acute gastroenteritis among children admitted to Erbil Paediatric Hospital, Iraqi Kurdistan. We now describe the prevalence and genetic diversity of NV in this population.

Materials and methods Faecal samples were collected during April and May 2005 from 260 children under 5 years of age who were hospitalised with acute diarrhoea. Following RNA extraction, NVs of genogroup I (GI) and genogroup II (GII) were identified by RT-PCR amplification followed by agarose gel electrophoresis. Nucleotide sequencing was undertaken to confirm the specificity of RT-PCR products and to enable a phylogenetic analysis to be undertaken.

Results Out of 260 samples tested, 78 (30%) were positive for NV. Among the 78 NVs identified, 18 (23%) belonged to GI, 58 (74.4%) belonged to GII and 2 (2.6%) were mixed GI and GII.

Conclusions In this population, NV was detected in nearly one-third of children with diarrhoea. These data add to the growing body of evidence that NV is emerging as an important cause of acute paediatric gastroenteritis worldwide.

PS 31 Harnessing microbes in bioremediation of cyanotoxins

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Background Safe drinking water is a global challenge specifically there is growing concern about the occurrence of toxic cyanobacterial blooms which adversely effect human health. While some advanced water treatment technologies are being explored, this level of processing is some way off for most of the world's population. One

of the most exciting areas that hold promise for a successful cost effective solution is harnessing microbes to render water safe with respects to cyanotoxins. In order to exploit these organisms in a reliable and sustainable way, characterisation of the microbial community and optimisation of degradation rates is essential.

Methods Natural water samples with populations of microbes capable of degrading cyanotoxins were identified using time course degradation studies and Biolog MT plates. Organisms were isolated and characterised using traditional microbiological and state-of-the-art molecular techniques. Degradation mechanisms were unravelled by LC-MS and MALDI-MS/MS.

Results A diverse range of degradation products were identified, including, demethylated, decarboxylated and hydrolysed peptides. Despite this diversity only a small number of degrading bacteria were isolated and characterised.

Conclusion This study has illustrated the natural diversity of micro-organisms capable of degrading cyanotoxins. However, practical challenges of harnessing this activity still remain.

PS 32 Is beta-haemolytic *Escherichia coli* a significant isolate in diarrhoeic companion animals?

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Beta-haemolytic *Escherichia coli* (BH *E. coli*) is frequently isolated from diarrhoeic companion animals; however, the clinical significance of this is unclear. Multiplex PCRs to detect the principal virulence factors associated with the *E. coli* pathotypes EPEC, VTEC, ETEC and UPEC (*eae*, VT1, VT2, LT, STI, STII, CNF1 and CNF2) were established. BH *E. coli* isolates from clinical samples submitted for routine culture to the Companion Animal Diagnostic Service at the University of Glasgow were used in this study. To date, 71 isolates have been screened; 62/71 were $CNF1^+$ and $CNF2^+$, and 3/71 were $ST1^+$. The remaining 6 isolates were negative for all virulence factors under test. All $ST1^+$ strains were isolated from dogs, and since $ST1^+$ strains have previously been associated with enteric disease in dogs, this is likely to be a clinically significant finding. However, the significance of the $CNF1^+/CNF2^+$ strains is less clear-the enteropathogenicity of such isolates has yet to be established, particularly *in vivo*. Moreover, other known pathogens were occasionally detected in $CNF1^+/CNF2^+$ samples, and $CNF1^+$ strains have been isolated from healthy dogs and cats. Clearly, the enteropathogenicity of these isolates requires further exploration.

PS 33 Microbiology of some artisanal 'farmhouse' cheeses manufactured in the United Kingdom from unpasteurised milk

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Approximately 10% (700,000 tonnes pa) of cheese produced in the EU is made from unpasteurised milk. Many of these speciality farmhouse cheeses are considered to be *haute cuisine* foods and are produced from unpasteurised milk so that the naturally occurring microbial population is able to generate specific flavour attributes. However, the microbiology of speciality cheeses produced in the UK, unlike that of their European counterparts, has not been studied in any detail, and hence the objective of this study was to investigate some aspects of the microbial diversity of specialist artisanal (farmhouse-type) cheeses that are produced in the United Kingdom from unpasteurised milk. The numbers of yeasts, coliforms and the principal bacterial groups involved in ripening (eg lactic acid bacteria, enterococci) were monitored in all cheeses examined using selective and specific differential chromogenic media. The presence of potential foodborne pathogens (eg *Listeria monocytogenes, Staph. aureus*) was confirmed in some of the cheese samples using cultural methods, phenotypic identification and other discriminatory tests. *Mycobacterium avium* subsp. *paratuberculosis*, the causative organism of Johne's disease in cattle and which may also have a role in Crohn's disease in humans, was, after immunomagnetic separation, detected unequivocally by PCR in one cheese sample and cultured from 2 (of 5) cheeses that have been tested thus far.

PS 34 Effects of surface culture on *Listeria* survival on the surface of semi-hard smear cheese

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The survival of *Listeria innocua* was investigated on Swiss raclette cheese ripened with different defined and complex surface cultures. The time of contamination and type of surface culture had a large impact on *Listeria* survival. A low *Listeria* inoculation at day 3 (3 cfu/cm^2) on cheeses treated with a defined surface culture resulted in a stable *Listeria* count for 30 days which increased thereafter. When *Listeria* contamination (15 cfu/cm^2) was done after 7 days, i.e. for a high cheese surface pH, a rapid *Listeria* growth was observed in these cheeses. Cheeses ripened with complex bacterial consortia isolated from 2 Swiss dairies processing raclette cheese and contaminated at day 7 with 15 *Listeria* $/cm^2$ showed *Listeria* counts below the detection limit after 7 or 21 day ripening. Our data indicate that complex cultures isolated from cheese environment may efficiently protect cheese against *Listeria* contamination, with strong culture effects.

PS 35 Prevalence of *Arcobacter* spp. in Bavarian food and water samples

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Arcobacter species are Gram-negative, spiral-shaped rods resembling campylobacter species. They have been associated with reproductive disorders, mastitis, gastric ulcers in livestock and gastroenteritis in man. Infection sources may be raw meat or milk from livestock or drinking water. Cultural detection involves a selective enrichment step under aerobic conditions at 30°C for 48h, growth and identification of colonies on solid blood agar plates. In this study we investigated the prevalence of *Arcobacter* spp. in raw food samples and small drinking water supplies in Bavaria with cultural and PCR methods. With a newly evaluated real time PCR we detected the genus Arcobacter in 95% of the chicken and 50% of the pork meat samples. In parallel the isolation of the bacteria was successful in 69% of the chicken and 38% of the pork samples. The most frequently isolated species was *A. butzleri*, followed by *A. cryaerophilus*, which was determined by conventional and real time multiplex PCR. Our results show a high prevalence of Arcobacter in the food chain. Now the importance of these emerging pathogens for human infections has to be investigated.

PS 36 BBQs, flies and food-borne pathogens

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The Australian summer is steeped rich in tradition, which for many involves watching the cricket and having many outdoor barbeques with family and friends. Many of these summer days attract

unwelcome company from the Australian bush fly (*Musca vetustissima*), who's lifecycle coincides with the increase in frequency of barbeques.

In this study, Australian bush flies from three different environments (Farm, Urban, and BBQ areas) were sampled. Flies were caught on days above 25°C and immediately analysed for total bacterial populations, and pathogen identification using selective media.

The total bacterial population was found to differ with regards to the environment they were caught. Those flies caught in the farm environment contained the highest bacterial population while those from urban environments had the lowest population. There was no difference in the type of presumed pathogen detected and the type of environment. However, *E. coli* was the most frequently isolated pathogen (82–96%), while *Shigella* was the least found pathogen from all fly samples (18–40%). Hence, Australian bush flies have the potential to act as vectors for food-borne pathogens.

PS 37 *Not being presented*

Posters

Systematics & Evolution

SE 01 Identification of a new phylogenetic cluster of *Propionibacterium acnes* distinct from types I and II

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Previous studies have shown that *Propionibacterium acnes* types I and II represent distinct phylogenetic groups based on *recA* gene sequencing and analysis by Random Amplification of Polymorphic DNA (RAPD). During the examination of clinical isolates of *P. acnes* by RAPD we identified five isolates with a DNA profile that was markedly different from those of type I or type II strains. Nucleotide sequencing revealed these isolates to have between 99.8% to 99.9% identity to the 16S rRNA gene (1484 bp) of ATCC 6919 (type I) and ATCC 12930 (type II). Nucleotide sequencing of the *recA* gene (1047 bp) did, however, reveal conserved polymorphisms between these isolates and those of ATCC 6919 (98.9% identity) and ATCC 12930 (99.1% identity), as well as other type I and II strains. Phylogenetic analysis demonstrated that these isolates belong to a novel *recA* cluster and, as a consequence, we propose this group as *P. acnes* type III. Isolates of *P. acnes* type III show no reaction with type I- and II-specific monoclonal antibodies, and also display differences in cellular morphology.

SE 02 Characterisation of *Bacillus* sp. based on gene targets elucidated from a metagenomic approach

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Bacillus species, due in part to their ability to sporulate, remain a major source of contamination in industrial processes. Also, as a food-borne pathogen, they are being reported increasingly in human infections. Due to the lack of reliable characters to delineate species, many taxa remain *incertae sedis*. Comparative 16S rDNA sequencing has provided a sound framework but many species cannot be defined solely on this basis. To devise a more robust system, we are exploring two major approaches; one based on MLST and a second strategy based upon a predictive approach to identify more phylogenetically-informative genes from metagenomic analysis. The results of the latter have highlighted an entirely new set of potential targets. Interestingly, 16S rDNA does not feature a major target but three ribosomal genes, rplF, rpsN and rpsC are within the 10 most prominent genes. Current studies are aimed at characterising more isolates and field testing these methods.

SE 03 Evolutionary history of the genomic region (Region XII) of *Vibrio vulnificus* present in clinical isolates

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Vibrio vulnificus is an opportunistic human pathogen that causes septicaemia after ingestion of raw or undercook seafood and wound infections exposed to contaminated water. *V. vulnificus* is prevalent in warm waters and is abundant in oyster farms, leading to significant economic losses.

Multilocus sequence typing (MLST) data using six housekeeping genes divided the isolates in to the two main lineages previously noted for this species.

We identified a 33-Kb genomic island named Region XII present in most clinical isolates and associated with one of the lineages identified. This region contains an arylsulfatase gene cluster which potentially plays a role in host-pathogen interactions, indicating a role of Region XII in the emergence of virulence in *V. vulnificus*. Phylogenetic analysis based on the Open Reading Frame (ORF) VVA1632 encoding for arylsulfatase A suggests that this region is stable may be involved in the adaptation of this pathogen to the human host.

Authors

Authors

Authors

Plenary session

Food, fluids, fingers, faeces and flies: food- and water-borne pathogens

Functional genomics to enhance the effectiveness of food preservatives; mode of action of sorbic acid, a case study

Stanley Brul

University of Amsterdam, The Netherlands

The talk will address the options that state of the art functional genomics technologies offer to make an inventory of gene-expression events upon sorbic acid stress as well as to provide a physiological context to the data obtained. The experimental part will focus on yeast and *Bacillus subtilis* as models for fungi and bacterial spore formers.

Similar concepts can be extended to physical phenomena to which foods and the micro-organisms contained in them may be exposed.

Hot topic symposium

Post-genomic analysis of microbial function in the environment

Metagenomic and functional analysis of the hindgut bacteria of a wood-feeding higher termite

Falk Warnecke[1], Peter Luginbühl[2], Natalia Ivanova[1], Majid Ghassemian[2], Toby H.Richardson[2,9], Justin T. Stege[2], Michelle Cayouette[8], Gordana Djordjevic[2], Nahla Aboushadi[2], Rotem Sorek[1], Susannah G. Tringe[1], Mircea Podar[3], Hector Garcia Martin[1], Victor Kunin[1], Daniel Dalevi[1], Julita Madejska[1], Edward Kirton[1], Darren Platt[1], Ernest Szeto[1], Asaf Salamov[1], Kerrie Barry[1], Natalia Mikhailova[1], Nikos C. Kyrpides[1], Eric G. Matson[4], Elizabeth A. Ottesen[5], Xinning Zhang[4], Alice C. McHardy[6], Myriam Hernández[7], Catalina Murillo[7], Luis G. Acosta[7], Isidore Rigoutsos[6], Giselle Tamayo[7], Brian Green[2], Cathy Chang[2,9], Edward M. Rubin[1], Eric J. Mathur[10], Dan E. Robertson[2], **Philip Hugenholtz**[1] & Jared R. Leadbetter[4]

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Termites, with their complex hindgut microbiota, play significant global roles in the conversion of wood lignocellulose into CO_2 and CH_4. We performed a metagenomic analysis of the microbial community resident in the luminal contents of the major hindgut paunch of a wood-feeding Nasutitermes species. Gut spirochetes and fibrobacters were discovered to encode a large and diverse set of genes associated with cellulose and xylan decomposition. Many of these genes were expressed *in vivo* and had cellulase activity *in vitro*, further implicating the bacterial community in lignocellulose degradation. Novel insights into other important symbiotic functions including H_2 metabolism, CO_2-reductive acetogenesis, and N_2 fixation were also provided by this first system-wide gene analysis of a microbial community specialized towards plant lignocellulose degradation.

Methodological advances in proteomics-based analysis of environmental microbes

Phillip C. Wright

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Bottom-up, or trypsin-based proteomics relies on relatively short amino acid sequences for both protein identification and quantification. Identical peptides in different proteins are referred to as 'shared peptides'. Protein identification and quantification become complicated when these proteins are encoded in the same organism, with this being known as the 'protein inference' problem. However, as we discuss in this paper, shared peptides however offer many opportunities for cross-species quantitation, if these peptides are encoded in distinct species. An *in silico* study was performed on the occurrence of shared peptides with intra- and inter-species peptide redundancy investigated across the domains of life. The protein inference problem is most significant in *Homo sapiens,* since ca. 40% of all peptides with between 6 and 15 residues are shared. Interestingly mouse and human proteomes share 30–50% of all peptides of between 6 and 15 residues. Furthermore, almost 33% of all proteins shared over 40% of their peptides with at least one other protein in a related species, thus offering strong potential for cross-species protein quantification. We use ^{15}N metabolic labelling to experimentally demonstrate this principle in the archaea *Sulfolobus tokodaii* and *Sulfolobus solfataricus*, and also for environmental applications in the cyanobacteria *Synechocystis* sp. PCC6803 and *Euhalothece* sp. Opportunities for environmental and medical applications of this approach are discussed.

Applications of Raman Spectroscopy in environmental microbiology

Wei Huang[1], Killian Stoecker[2], Lyndsay Newbold[1], Rob Griffiths[1], Holger Daims[2], Michael Wagner[2] & **Andrew Whiteley**[1]

[1]Biodiversity & Ecosystem Function Group, CEH Oxford; [2]Dept Microbial Ecology, University of Vienna, Austria (Email aswhi@ceh.ac.uk)

Raman microscopy is a longstanding spectroscopy based technology which has recently shown a high degree of promise for determining the functional phylogeny of microbial cells. Recent developments in instrumentation, such as confocal capabilities and sub-micron analysis regions allow analyses of biological cells right down to the individual bacterial cell. Here, we show the power of Raman spectroscopy for differentiating different species of bacteria, their physiological states and the use of Raman when coupled to fluorescent *in situ* hybridisation and ^{13}C based process tracers. Finally, we will present new developments in Raman spectroscopy, such as high density Raman mapping and single cell sorting using laser based 'Raman tweezers'.

Multigene analysis of acetogenic spirochetes with microfluidic digital PCR

Elizabeth A Ottesen, Jong Wook Hong, Stephen R Quake & Jared R. Leadbetter

California Institute of Technology, USA

PCR-based gene inventory techniques have greatly expanded our understanding of microbial diversity in the environment, and metagenomic techniques have allowed rapid exploration of the potential physiologies present within microbial communities. However, it remains a major challenge to discover which organisms within a community encode particular genes of interest. As a result, it is often difficult to connect key microbially-catalysed processes to the bacterial species that carry them out in nature. We have developed the use of microfluidic digital PCR to simultaneously amplify multiple genes from individual bacterial cells within environmental samples. This allows retrieval and sequence analysis of multiple genes associated with a single genome. We are using this technique to identify (based on 16S rRNA sequence) bacteria that bear key genes involved in homoacetogenic C1 metabolism in the hindgut of wood-feeding termites. We are also engaged in optimizing this method to allow easy application to different gene choices and template types. We believe that this ability to co-localize multiple genes to individual genomes in complex ecosystems will have broad application in microbial ecology and other studies of the environment.

The CAMERA project (environmental metagenomics and meta-analysis)

P. Gilna

University of California at San Diego, USA

Marine microbial metagenomics is becoming a focus for innovation at the interface of genomics and information technology. The pace of development and the power of genomics for biological discovery are increasing rapidly with the application of both conventional sanger –based and pyrosequencing-baed shotgun sequencing to entire microbial communities. New sequences, genes, and gene families, together with associated environmental data, offer tremendous potential to understand better the functioning of natural ecosystems: Scientists will be able to consider each sequence in the context of its ecology: the composition of the rest of the community, the environmental conditions in which it is found, and its relationships with other species with which it is found at other times and places.

CAMERA project: To push development of this discipline, the Gordon and Betty Moore Foundation awarded, in January 2006, a 7-year, $24.5-million research grant to the CAMERA project, a state-of-the-art genomics data, analysis, and synthesis center using the most advanced computational data grid, optical networking, and computing technologies available.

Cyberinfrastructure, metagenomic data, and analysis tools: CAMERA, which stands for the Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis, is based on an innovative cyberinfrastructure leveraging emerging concepts in data storage, access, analysis, and synthesis not available in current gene sequence resources and a database of new genomic sequences being collected.

Earlier this year, CAMERA made available approximately 8 million sequence reads collected by the J. Craig Venter Institute as part of the Global Ocean Sampling expedition, plus ~90 new full genome maps of ocean microbes. This resource includes the metadata associated with the collection of the samples: the precise location, date and time of collection, the chemical and physical conditions where the sample was taken, and a measure of its living environment, i.e., all the other sequences found in the same sample. CAMERA will grow in value to include new sequences, genes, and gene families, together with their annotations and associated environmental metadata. In addition, a suite of tools is being developed to enable scientists to analyse the massive data sets.

Partners: CAMERA brings together leaders in the new technologies of high-throughput DNA sequencing and metagenomic analysis tools on the one hand, and cyberinfrastructure innovations in optically coupled computing, emerging Grid middleware, and user workspaces on the other. The project is led by UCSD/Calit2 in partnership with the J. Craig Venter Institute, the Center for Earth Observations and Applications, and the Scripps Genome Center (the latter two are based at the Scripps Institution of Oceanography).

Group sessions

Eukaryotic Microbiology Group

Investigating the biology of plant infection by the rice blast fungus *Magnaporthe grisea*

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The rice blast fungus *Magnaporthe grisea* causes one of the most serious diseases of cultivated rice. The availability of full genome sequences for *M. grisea* and its host has allowed the first opportunity to define the gene inventory associated with a fungal phytopathogen and has provided insights into both the fungal-plant interaction and the evolution of fungal pathogenicity. During plant infection, the rice blast fungus elaborates a specialised infection structure known as an appressorium. This unicellular, dome-shaped structure generates turgor that is translated into mechanical force to allow rupture of the rice cuticle and entry into plant tissue. We set out to explore whether the development of a functional appressorium was linked to the control of cell division. This was based on the observation that following germination of a conidium on the rice leaf surface, a single round of mitosis always occurs during germ tube elongation, prior to the formation of an appressorium. We found that blocking completion of mitosis by generation of a temperature-sensitive *MgnimA* mutant prevented appressorium morphogenesis. Furthermore, we found that following mitosis, conidia always undergo cell collapse and cell death, which appears to be a programmed, autophagic process. Deletion of *MgATG8* prevented autophagy in *M. grisea* and rendered the fungus non-pathogenic. Taken together, our results indicate that appressorium morphogenesis requires genetic control by completion of mitosis and autophagic cell death of the conidium. Once the *M. grisea* appressorium has formed, cellular turgor is generated by accumulation of osmotically-compatible solutes, notably glycerol. We have used genetic, biochemical, proteomic and, most recently, metabolomic analysis to investigate how turgor is generated and to define the key genetic determinants of appressorium function. Of particular interest is the central role of trehalose metabolim to the genetic control of fungal virulence, sugar signaling and nitrogen source utilization, and the role of peroxisomal fatty acid beta-oxidation to appressorium physiology. The appressorium brings about plant infection by elaborating a penetration hypha that differentiates further into invasive hyphae which grow rapidly within the host plant cells. One of the key challenges in understanding rice blast disease is to determine how fungal proteins are delivered to the host during plant infection and to define the mechanisms by which the fungus proliferates biotrophically within the rice leaf. Progress towards determining the secretory processes necessary for *M. grisea* plant tissue colonization will also be presented.

Fermentation & Bioprocessing Group

The bioprocess defines the product – what you should measure and when

D. Simpson

Eden Biodesign, National Biomanufacturing Centre, Liverpool

To minimise potential risks involved in developing novel biotherapeutics it is essential for both upstream and downstream processes to be considered as early as possible in the product development cycle. Our experience with assisting UK universities and start up biotech companies with product development suggests that the regulatory implications and scalability of the current expression systems and developed processes are often over looked.

Typically, proof of concept studies undertaken will have used a proprietary expression system for reasons of availability, ease of use and attainment of the desired product quantity. However, consideration of regulatory applicability and the impact of licence or royalty fees during current and future development (and potential outlicence) are often disregarded.

To successfully develop a biological product to phase I areas including vectorology, strain selection and basic fermentation development focussed on increasing biomass are commonly investigated. However, product quality is often sacrificed at early stage development in favour of increased total product yield, a premise often flawed for further clinical development. Here we will examine the concept of “bug to drug” and address poignant issues in developing microbial expression systems and upstream processes.